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Dipartimento di BIOSCIENZE E TECNOLOGIE AGRO-ALIMENTARI E AMBIENTALI

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GSICA

Italian Network of the PhD Courses in Food Science Technology & Biotechnology

29th Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology

10th – 12th September 2025 | University of Teramo



Editors

Prof. Paola Pittia
Dr. Marco Faieta
Dr. Roberta Prete

Department of Bioscience and Technology for Food, Agriculture and Environment
University of Teramo

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Preface

This book collects the oral and poster presentations from the 29th Workshop on the Developments in the Italian PhD Research on Food Science, Technology, and Biotechnology, held at the University of Teramo from September 10th to 12th, 2025.

The Workshop is organized by the National Coordination of PhD Programs in Food Science, Technology, and Biotechnology, established in 1995. This unique initiative—unparalleled in both Italy and Europe—currently brings together 31 PhD programs from across Italian universities in this scientific sector. The inaugural edition in 1996 launched a long-standing tradition of annual workshops, each hosted by a different university.

Today, the event offers a premier opportunity to share field research, assess the progress and quality of doctoral projects, and foster deep interaction among the participating universities. It has proven crucial for the cultural and scientific growth of Italian PhD students, providing them with a platform for broader discussion and insight into diverse educational environments. Furthermore, it serves as the primary benchmark for evaluating the state-of-the-art of Italian research in food and related sciences, technologies, and biotechnologies—a field that has progressively expanded its multidisciplinary approach within a modern food "system" framework.

The successful 2025 edition, organized by the PhD program in Food Sciences at the University of Teramo, brought together 270 doctoral students who presented their ongoing research. They were joined by 110 tutors and professors, alongside over 30 national and international invited experts and food industry representatives. Notably, this edition was officially included in the "Month of Research" initiative organized by the Doctoral School of the University of Teramo.

We express our sincere gratitude to all the PhD students, tutors, and colleagues, as well as the endorsing scientific societies (SISTAL, SIMTREA, GSICA, SISS), the Municipality of Teramo, and the sponsors who contributed to the success of this workshop. Special thanks are extended to the members of the Scientific and Organizing Committee of the Department of Bioscience and Technology for Food, Agriculture, and Environment. Finally, we deeply appreciate the students, post-docs, collaborators, and staff of the University of Teramo, whose dedication made this workshop possible.

Paola Pittia, University of Teramo
President *pro-tempore* (October 2024- September 2025) of the National Coordination of PhD Programs in Food Science, Technology, and Biotechnology and Chair of the Organising Committee of the 29th Workshop, University of Teramo

Antonio Piga, University of Sassari
Secretary of the National Coordination of PhD Programs in Food Science, Technology, and Biotechnology

Acknowledgments

The Organizing Committee express sincere gratitude to Agata Di Rico and Davide Parente for their essential help in compiling and editing these proceedings.

The Organizing Committee gratefully acknowledges the support of the Municipality of Teramo through its sponsorship and patronage of the event.


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Model: simplification from a certain point of view
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29th WORKSHOP Developments in the Italian PhD Research on Food Science Technology and Biotechnology

Scientific Programme

WEDSNEDAY, 10th SEPTEMBER 2025 — DAY 1

09:00-13:30 REGISTRATION

13:30 OPENING SESSION AND INSTITUTIONAL GREETINGS

AULA MAGNA

14:00 — KEYNOTE LECTURE
Designing Future Food Research for a Sustainable Planet
Prof. Milena Corredig, Aarhus University, Denmark

14:30 — KEYNOTE LECTURE
Unraveling the Role of Foods and Recipes as Modulator of Inflammatory and Oxidative Response in "Real Life" Nutrition
Prof. Mauro Serafini, University of Teramo, Italy

	AULA MAGNA Chair: Sonia Calligaris (UNIUD), Giorgia Spigno (UNICATT)	AULA TESI Chair: Fabio Licciardello (UNIMORE), Eleonora Carini (UNIPR)	AULA 7 Chair: Clara Comuzzi (UNIUD), Prospero Di Pierro (UNINA)
	PARALLEL SESSION 1 **Processing-Structure-Function Relationships in Food Design**	**PARALLEL SESSION 2** **Smart Packaging and Green Technologies for Food Preservation**	**PARALLEL SESSION 3** **Innovative and Green Approaches for Food Safety and Sustainability**
15:15	Application of innovative technologies for the functionalisation of alternative proteins and the associated functional and rheological characterisation, **Federico Drudi**, UNIBO	Microbial Biopolymers for Potential Applications in Food Packaging, **Marianna Ciccone**, UNIBO	Use of Non-Thermal Treatments to Improve the Quality, Safety, and Shelf-Life of Raw Pork Sausages, **Chiara Angelucci**, UNIBO
15:30	Impact of process factors and agri-food by-products on the oxidative and physico-chemical stability of oleogels, **Giulia Salvatori**, UNIBO	Biodegradable Innovative Packaging: A Sustainable Solutions for Food Shelf Life Extension, **Corinne Giacondino**, UNIRC	Nature-inspired compounds: synthesis and interaction with molecular targets for the application in plant protection, **Francesca Sacchi**, UNIMI
15:45	Development of Plant-Based Cheese Analogues: The Role of Ingredients and Processes, **Irene Fenga**, UNIPR	Sustainability and innovation of spray packaging applied to food and nutraceutical products: development of innovative sprayable solutions and study of their application in bakery and confectionery, **Chiara Rossetti**, UNICATT	Biomimetic synthesis of phytoalexins as environmentally friendly antimicrobials, **Denise Dozio**, UNIMI

16:00-16:45 COFFEE BREAK AND POSTER SESSION

16:45	Exploring the physical, structural and technological properties of ball-milled starches from different origins from granules to porous gels, **Reindorf Boateng**, UNITE	Free and microencapsulated olive pomace extracts to improve the phenolic content and shelf-life of fresh pasta, **Mariana Miccolis**, UNIBA	Dietary Exposure to Chemicals and Implications for Consumers Health, **Giovanni Tommaso Lanza**, UNIPR

17:00-17:30 POSTER SESSION

	Aula Magna Chair: Simone Giacosa (UNITO), Fabio Chinnici (UNIBO)	Aula Tesi Chair: Giovanna Ferrentino (UNIBZ), Giacomo Squeo (UNIBA)	Aula 7 Chair: Donato Angelino (UNITE), Luca Cocolin (UNITO)
	PARALLEL SESSION 4A **Advanced Approaches in Wine Science**	**PARALLEL SESSION 5A** **Functional Ingredients from By-Products**	**PARALLEL SESSION 6A** **Food, Microbiota and Health**
17:30	Optimizing Negroamaro Wine Quality: The Effect of Withering Techniques and Defoliation the Apulian Landscape, **Ilaria Prezioso**, UNISALENTO	Fostering Sustainability in the Olive Oil Supply Chain: Valorization of Typical Extra Virgin Olive Oils and Olive Mill By-products, **Sofia Zantedeschi**, UNIBO	Ultra-Processed foods: from the nutritional characteristics to the impact on human health, **Beatrice Pellegrini**, UNIMI
17:45	Assessment of quercetin precipitation risk in Italian red wines, **Alessandra Luciano**, UNINA	Development of functional ingredients using byproducts from agro-industrial process, **Benedetta Fanesi**, UNIVPM	In Vitro Evaluation of Probiotic, Enzymatic, and Botanical Supplements on Gut Microbiota and Digestion, **Robin Duncan**, UNIMI
18:00	Wild yeasts as source for designing specific "synthetics microbiota" to be used for wine fermentations, **Ernesto Petruziello**, UNINA	Technological treatments to obtain high-quality vegetable products, **Iolanda Cilea**, UNIRC	Genomic characterization of Lactic Acid Bacteria strains for the development of a novel psychobiotic product, **Chiara Maria Calvanese**, UNINA

18:15-19.00 POSTER SESSION

19:00 WELCOME COCKTAIL

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TERAMO (TE)



29th WORKSHOP Developments in the Italian PhD Research on Food Science Technology and Biotechnology

Scientific Programme

THURSDAY, 11th SEPTEMBER 2025 — DAY 2

KEYNOTE LECTURE

09:00 *Computational Food Science: making sense of food data*
Prof. József Baranyi, ERA-Chair Holder, foodIT Computational Food Science Centre, Aristotle University of Thessaloniki, Greece

	Aula Magna Chair: Simone Giacosa (UNITO), Fabio Chinnici (UNIBO)	Aula Tesi Chair: Giovanna Ferrentino (UNIBZ), Giacomo Squeo (UNIBA)	Aula 7 Chair: Donato Angelino (UNITE), Luca Cocolin (UNITO)
	PARALLEL SESSION 4B **Advanced Approaches in Wine Science**	**PARALLEL SESSION 5B** **Functional Ingredients from By-Products**	**PARALLEL SESSION 6B** **Food, Microbiota and Health**
09:45	Yeast Derivatives for Precision Oenology: Emerging and Sustainable Application for Wine Production (WInnY), **Alessio Altomare**, UNIMI	Integrated strategies to overcome lignocellulosic recalcitrance for the recovery, fractionation and characterization of functional fibers, **Pio Viscusi**, UNIPR	Study of the Effects of Plant-Derived MicroRNAs on the Human Gut Microbiota, **Hatice Hazal Okur,** UNIBZ
10:00	Study of combined stress factors on shelf-life of rosé wines: interaction of oxygen, light and temperature on color and aroma evolution, **Leonardo Vanzo**, UNIVR	Sustainable valorisation of high value added compounds from secondary raw materials of the milling industry, **Claudio Cinquanta**, UNIMOL	Influence of food matrix on metabolic fate of polyphenols: role of phenol-nutrient interactions, **Manuela Flavia Chiacchio**, UNINA
10:15	Oenological characterization of ancient Sicilian cultivars and grape heterogeneity assessment using technological parameters and phenolic markers, **Clara Vitaggio**, UNIPA		Methodologies to guide consumers towards a healthy and sustainable Mediterranean Diet, **Perla Degli Innocenti**, UNIPR

10:30-11:15 COFFEE BREAK AND POSTER SESSION

	Aula Magna Chair: Roberto Moscetti (UNITUS), Cristina Alamprese (UNIMI)	Aula Tesi Chair: Amalia R.M. Piscopo (UNIRC), Massimiliano Rinaldi (UNIPR)	Aula 7 Chair: Diego Mora (UNIMI), Teresa Zotta (UNIBAS)
	PARALLEL SESSION 7 **Smart and Sustainable Food Processing**	**PARALLEL SESSION 8** **Processing and Reformulation Strategies for Product Innovation**	**PARALLEL SESSION 9** **Food Microbiology and biotechnology (1)**
11:15	Innovative Technologies and Processes for Sustainable Frozen Foods, **Bilge Ece Ozel**, UNITE	Technological and Nutritional Properties of Pigmented Whole Wheat Foods: The Role of Innovative Technologies, **Ilenia Gaeta**, UNIMOL	Innovative Approaches in the Development of Novel Organic Food Products by Applying Careful Processing, **Yasmin Muhammed**, UNIBA
11:30	Integrating automation in food processing operations, **Eleonora Di Palma**, UNIFG	Valorization of dairy production in inland areas: product and process innovation, **Caroline Vitone**, UNIMOL	Microbial Collections as Biorepositories for Sourdough Microbiomes: Microbiological Assessment, Metagenomic Analysis, and Nutritional Profiling, **Roberta Coronas**, UNISS
11:45	Integrating food reformulation strategies and emerging technologies to promote healthy diet, **Grazia Marinaro**, UNIFG	Application of Liquid Nitrogen for Pistachio Skin Removal, **Fabiola Pesce**, UNICT	Lactic acid bacteria as a tool to reduce the allergenicity of Arginine Kinase and Tropomyosin from the edible insect *Hermetia illucens*, **Claudia Dellapina**, UNIPR
12:00	Application of IoT and Machine Learning Technologies for Monitoring Hop Cone Ripening, **Martina Galaverni**, UNIPR	Effect of durum wheat meal in low-moisture texturized vegetable proteins and its application in filled pasta, **Vittoria Latrofa**, UNIBA	Genomic and functional characterization of a novel BSH-positive *Lacticaseibacillus rhamnosus* probiotic candidate with potential hypocholesterolemic effect, **Gianluigi Agolino**, UNICT
12:15	Innovative approaches for shelf-life extension: chemical, physical and microbiological strategies, **Giulio Scappaticci**, UNIPI/BARILLA	Plant-based analogues: a sensory-based and consumer-led approach to enhance product acceptance and innovation, **Marta Appiani**, UNIMI	Selection of lactic acid bacteria to improve the technological, nutritional and functional value of products and by-products in the agri-food chain, **Saverio Monica**, UNIPR
12:30	Applications of MOX Sensors for Volatile Organic Compound Detection in Agri-Food sector, **Elisabetta Poeta**, UNIMORE	Bioactive-Rich Mushrooms for Food Reformulation (BIOMUSH-FOOD), **Giulia Bearzi**, UNIMI	Exploitation of the functional potential of autochthonous microorganisms from fermented foods, **Anna Rossi**, UNIUD

12:45-14:00 LUNCH AND POSTER SESSION

29th WORKSHOP Developments in the Italian PhD Research on Food Science Technology and Biotechnology

Scientific Programme

THURSDAY, 11th SEPTEMBER 2025 — **DAY 2**

AULA MAGNA

KEYNOTE LECTURE

14:00 *Consumer-led Product Ecodevelopment: Can We Truly Shape the Future of Sustainable Food Using Consumers, Sensory Sciences, and Life Cycle Analysis?*
Prof. Ronan Symoneaux, Ecole Supérieure des Agricultures (ESA), France

14:30 *Flow Cytometry Meets Food Science: Exploring Applications from Food Processing to Human Health*
Dr. Marina Ramal Sanchez, UNITE — ***sponsored by Beckman Life Sciences***

	Aula Magna **Chair: Alessandra Fratianni (UNIMOL), Michele Paradiso (UNISALENTO)**	**Aula Tesi** **Chair: Cristina Restuccia (UNICT), Giampiero Sacchetti (UNITE)**	**Aula 13** **Chair: Fabio Minervini (UNIBA), Annalisa Serio (UNITE)**
	PARALLEL SESSION 10 **Emerging Analytical Tools for Food Quality and Safety**	**PARALLEL SESSION 11** **Genomics and Microbiomes for Food Systems and Environment**	**PARALLEL SESSION 12** **Food Microbiology and biotechnology (2)**
15:00	Combining Instrumental and Sensory Methods to Assess the Quality and Authenticity of Foods of Animal Origin, **Mara Antonia Gagliano**, UNIBO	Development of a comprehensive cheese metagenome catalogue reveals potential markers of origin and quality, **Raffaele Magliulo**, UNINA	Innovative bioprocesses and extraction techniques of high value molecules and products from agricultural biomasses, **Luca Fontechiari**, UNIPR
15:15	Advanced chemical characterization and functional assessment of hemp flour-enriched bread: opportunity and challenges in valorizing hemp chain by-products, **Lorenzo Del Vecchio**, UNIPR	Closing the loop in agriculture: effects of a fermented tomato-based biostimulant on tomato growth and fruit quality, **Anna Agosti**, UNIPR	Effect of GABA production on biomass yield and stress robustness of *Levilactobacillus brevis*, **Emanuela Lavanga**, UNIBAS
15.30	Electrochemical and Machine Learning Approaches for the Classification and Functional Assessment of Oenological Tannins During Wine Ageing, **Rosario Pascale**, UNIVR	Effect Of Biochar and Wood Distillate Application on Vegeto-Productive Performances of Strawberry Plants (cv. Charlotte), Under Controlled Conditions, **Samreen Nazeer**, UNIPR	Valorisation of Alternative Protein Sources by Tailored Biotechnological Processes and Non-Thermal Technologies to Obtain New Ingredients to Be Used in the Formulation of Innovative Foods, **Solidea Amadei**, UNIBO
15:45-16:30	**COFFEE BREAK AND POSTER SESSION**		
16:30	Analysis of volatilome of virgin olive oils and flavored oils: quality grade evaluation and study of modification during storage, **Rosalba Tucci**, UNIBO	Genomic and physiological insights into bacterial heavy metal resistance for environment decontamination, **Alice Melzi**, UNIMI	Quorum Sensing Inhibition as a Strategy for Control of Proteolytic Activity by *Pseudomonas gessardii*, **Mohammed Salman**, UNINA
16:45	Identification and quantification of microplastics in the clam *Chamelea gallina* using different analytical techniques, **Rosaria Aloia**, UNITE	Microbiome Mapping in Meat Food Chain from Farm to Fork, **Asim Ur Rahman**, UNINA	Optimization of Fermentation Parameters for Biomass Production of *Streptococcus thermophilus* BT01 with Low or High Urease Activity, **Ylenia Zanchetta**, UNIMI
17:00	Designing advanced materials and self contained platforms for analytical applications in food analysis, **Davide Paolini**, UNITE		
17:00-18.30	**POSTER SESSION**		
20:30-23:30	**WORKSHOP GALA DINNER**		

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Roseto degli Abruzzi (TE)



29th WORKSHOP Developments in the Italian PhD Research on Food Science Technology and Biotechnology

Scientific Programme

FRIDAY, 12th SEPTEMBER 2025	DAY 3

09:15	**OPENING SESSION**

AULA MAGNA

Chair: Prof. Dario Compagnone, Prof.ssa Daniela Mancini (UNITE)

09:15	**KEYNOTE LECTURE** *Title in progress,* **Maarten Van Der Kamp** (EITFood, BE)
09:45	**KEYNOTE LECTURE** *From Research to Market: First Steps to Build a Startup* **Dr. Danilo Mazzara** (SkillforEquity, IT)

Chair: Prof. Luca Simone Cocolin (UNITO), Prof.ssa Paola Pittia (UNITE)

10:15	**Interactive session: My PhD skills: which, what for, and in my future I will..**
10:45-11.15	**COFFEE BREAK**

Chair: Prof. Marco Dalla Rosa (UNIBO), Prof. Antonello Piga (UNISS)

11:15	**Round Table: Research and PhD programs for the food systems transition** Lorenzo Cerretani (Vice-President Order of Food Technology, Industry consultant) Lorenzo De Berardinis (Process and Product development Perfetti van Melle) Maria Cristina Di Domizio (Federalimentare) Fabio D'Elia (Food Hub) Rosalba Lanciotti (SIMTREA,University Alma Mater Bologna) Daniele Rossi (Vice-President Cluster CLAN)

Chair: Prof. Dino Mastrocola (SISTAL, UNITE)

12:15	**AWARDS SESSION: What For (CLAN), SISS, Anton Paar, Barilla**
13:00	**CLOSING SESSION**

29th WORKSHOP Developments in the Italian PhD Research on Food Science Technology and Biotechnology

Side events and meetings

WEDSNEDAY, 10th SEPTEMBER 2025		DAY 1
	Food Science Laboratories	
10:00-12:30	INTENSIVE TRAINING *sponsored by ANTON PAAR* Gluten-free products: conventional and innovative methodologies for rheology evaluation	
	AULA 8 - AULA 10	
08:30-13:00	Global Food Venture Programme Pre-Event *organized by EIT Food*	
	SALA RIUNIONI DIP. BIOSCIENZE	
14:30-18:30	ASN MEETING	
	AULA 10	
ALL DAY	OPEN SPACE FOR NETWORKING	

THURSDAY, 11th SEPTEMBER 2025		DAY 2
	AULA 8	
from 12:00	COSTAL MEETING	
	SALA RIUNIONI DIP. BIOSCIENZE	
14:30-18:30	ASN MEETING	
	AULA 8	
16:30-18:30	ITALIAN NETWORK PHD COURSES IN FOOD SCIENCE AND TECHONOLOGY	
	AULA 10	
ALL DAY	OPEN SPACE FOR NETWORKING	

FRIDAY, 12th SEPTEMBER 2025		DAY 3
	SALA RIUNIONI DIP. BIOSCIENZE	
09:00-10:30	WHAT FOR COMMITTEE	
	AULA 10	
ALL DAY	OPEN SPACE FOR NETWORKING	

29th WORKSHOP Developments in the Italian PhD Research on Food Science Technology and Biotechnology

Poster Presentation Tableau

POSTER SESSION	Number	Year	Name	Surname	SSD	TUTOR	AFFILIATION	POSTER TITLE	REVISORI	
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-1**	I	Yana	Madonna	AGRI-07/A	Giorgia Spigno/Andrea Bassani	Università Cattolica del Sacro Cuore	Food Contact Compatibility and Shelf-life Effects of New Packaging Materials	**Stefano Farris (UNIMI)**	**Paola Pittia (UNITE)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-2**	I	Sofia	Panzani	AGRI-07/A	Francesca Venturi/Chiara Sanmartin	Università di Pisa	Quality evolution during storage of Spices and Herbs: understanding organoleptic and chemical (VOCs and main bioactive compounds) decay during Primary and Secondary Shelf Life	**Stefano Farris (UNIMI)**	**Paola Pittia (UNITE)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-3**	I	Asja	Brovedani	AGRI-07/A AGRI-08/A	Comuzzi Clara	Università di Udine	Development of novel photodynamic materials to reduce microbial contamination in foods	**Stefano Farris (UNIMI)**	**Paola Pittia (UNITE)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-4**	I	Chiara	Pisana	AGRI-08/A	Cinzia Caggia	Università di Catania	Cheese Food Safety: biocontrol systems through bacteriophages and active compounds	**Stefano Farris (UNIMI)**	**Paola Pittia (UNITE)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-1**	II	Andrea	Marianelli	AGRI-07/A	Angela Zinnai/Monica Macaluso	Università di Pisa	Innovative methods in breadmaking, packaging and distribution able to increase nutraceutical value, extend shelf-life and reduce waste of bakery products	**Stefano Farris (UNIMI)**	**Paola Pittia (UNITE)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-5**	I	Davide	Falotico	AGRI/07-A	Francesco Caponio	Università di Bari	Technological Strategies for Developing Sustainable and Functional Plant-based Ingredients and their Applications in Innovative Food Products	**Donatella Albanese (UNISA)**	**Maria Alessandra Paissoni (UniTO)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-2**	II	Andrea	Fachin	AGRI-07/A	Stefano Farris	Università di Milano/Università di Torino	Valorization of Food Waste for New Packaging Solutions	**Donatella Albanese (UNISA)**	**Maria Alessandra Paissoni (UniTO)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-3**	II	Vittorio	Bassi	AGRI-07/A	Sara Limbo	Università di Milano	Safety Assessment of Recyclable Coffee Packaging: Screening NIAS and Quantifying Primary Aromatic Amines in Polyolefin-Based Materials	**Donatella Albanese (UNISA)**	**Maria Alessandra Paissoni (UniTO)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-4**	II	Matteo	Gennaro	CHEM-07/B	Sabrina Dallavalle	Università di Milano	Functionalization of biopolymers via chemical methods for food packaging applications.	**Donatella Albanese (UNISA)**	**Maria Alessandra Paissoni (UniTO)**

DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-5**	II	Chiara	Vatieri	MEDS-24/B	Cirillo Teresa/Esposito Francesca	Università di Napoli	Valorisation of Food Waste for the Development of Film Coatings for Food Packaging Applications	**Donatella Albanese (UNISA)**	**Maria Alessandra Paissoni (UniTO)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-6**	I	Rui	Song	AGRI-07/A	Pietro Rocculli	Università di Bologna	Effect of emerging technology on different techno-functional properties in rice	**Concetta Condurso (UniME)**	**Marcello Alinovi (UniPR)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-7**	I	Ezeh	Chinedu	AGRI-07/A	Pittia, Rastelli, Faieta	Università di Teramo	Optimization of Candy Processing Conditions for Sustainable Confectionery Products with Improved Sensory Properties	**Concetta Condurso (UniME)**	**Marcello Alinovi (UniPR)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-8**	I	Santhoshkumar	Paramasivam	AGRI07-A	Antonio Derossi	Università di Foggia	Use of Robotic Applications for Elevating the Quality and Sustainability of Food Products	**Concetta Condurso (UniME)**	**Marcello Alinovi (UniPR)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-6**	II	Irene	Locatelli	AGRI-07/A	Silvia Grassi	Universitàdi Milano	Advancing in Process Analytical Technology: e-sensing for food cold chain	**Concetta Condurso (UniME)**	**Marcello Alinovi (UniPR)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-7**	II	Marta	Baviera	AGRI-07/A	Daniela Fracassetti	Università di Milano	Optimizing Red Straw Wine Production: A Multi-Faceted Approach	**Concetta Condurso (UniME)**	**Marcello Alinovi (UniPR)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-8**	II	Annachiara	Ferraioli	AGRI-07/A	Silvana Cavella	Università di Napoli	Valorization of food by-products: technological strategies for tailored food ingredients	**Lilia Neri (UniTE)**	**Alessio Cimini (UniTUS)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-9**	II	Busra	Oktar	AGRI-07/A	Pietro Rocculli	Università di Bologna	Emerging Processing Technologies for the Sustainable Production of Innovative Foods	**Lilia Neri (UniTE)**	**Alessio Cimini (UniTUS)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-10**	II	Giulia	Basile	AGRI-07/A	Romano Raffaele/Aponte Maria/De Luca Lucia	Università di Napoli	Bioactive compounds extracted through green technologies from lemon peels, fennel and tomato waste: their potential use as antioxidant	**Lilia Neri (UniTE)**	**Alessio Cimini (UniTUS)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-11**	II	Angela	Lomonaco	AGRI-07/A	Fernanda Galgano	Università della Basilicata	Characterization of doughs and flours for leavened bakery products: correlation with process parameters and finished product quality for continuous improvement	**Lilia Neri (UniTE)**	**Alessio Cimini (UniTUS)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-12**	II	Burcu	Aykanat	AGR-09	Francesco Genovese	Università della Basilicata	Impact of Supplier Variability and Storage Time on the Hydration and Mechanical Behavior of Durum Wheat Semolina	**Lilia Neri (UniTE)**	**Alessio Cimini (UniTUS)**

DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-13**	II	Niccolò	Pilla	AGRI-07/A	Emanuele Marconi	Università Campus Bio-Medico Di Roma	Charcaterization of prtoeins from barley malting by-products for food applications	**Alessandra Marti (UniMI)**	**Francesco Caponio (UniBA)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-14**	II	Marco	Torre	AGRI-07/A AGRI-02/A	Antonella Verzera, Fabio Gresta	Università di Messina	Physicochemical and Techno-functional Characterization of Flours from Neglected Sicilian Black Chickpea Varieties	**Alessandra Marti (UniMI)**	**Francesco Caponio (UniBA)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-9**	I	Emanuele	Greco	AGRI-07/A	Francesca Bot	Università di Parma	Upcycling pea waste side streams for developing future food ingredients (poster)	**Alessandra Marti (UniMI)**	**Francesco Caponio (UniBA)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-15**	II	Francesco	Iervese	AGRI-07/A	Carla Di Mattia, Paola Pittia	Università di Teramo/Università di Torino	Valorization of Grass Pea (*Lathyrus sativus L.*) Proteins through Microgels: Impact of pH on Structural Properties	**Alessandra Marti (UniMI)**	**Francesco Caponio (UniBA)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-10**	I	Elisa	Bonanno	AGRI-02/A AGRI-07/A	Fabio Gresta, Maria Merlino	Università di Messina	Agronomic and technological strategies to produce Mediterranean cereal and legume-based snacks for sustainable healthy nutrition	**Alessandra Marti (UniMI)**	**Francesco Caponio (UniBA)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-11**	I	Ruiqi	Hu	AGRI-07/A	Fabio Chinnici	Università di Bologna	Study on the effect and mechanism of action of chitosan on fixed compounds, polyphenolics, volatiles substances and color of red wines and beers	**Simona Pizzi (UniMI)**	**Francesca Venturi (UniPI)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-12**	I	Ana Catarina	Cabral Vale da Cunha	AGRI-07/A	Simone Giacosa	Università di Torino	Composizione fenolica e tipicità in vini Nebbiolo: Influenza del terroir, annata e tecniche di vinificazione	**Simona Pizzi (UniMI)**	**Francesca Venturi (UniPI)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-13**	I	Anastasiia	Kasianova	AGRI-07/A	Maria Alessandra Paissoni Luca Giorgio Carlo Rolle	Università di Torino	New Sensory and Instrumental-Chemical Approaches for Defining Wine Typicity: Exploring the Diversity of Monovarietal White Wines	**Simona Pizzi (UniMI)**	**Francesca Venturi (UniPI)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-14**	I	Opeyemi Folashade	Adesuyi	AGRI-08/A	Rosanna Tofalo	Università di Teramo	Oenological functions of Metschnikowia pulcherrima in co-culture with Saccharomyces cerevisiae to improve wine production	**Simona Pizzi (UniMI)**	**Francesca Venturi (UniPI)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-16**	II	Beatrice	Cordero	AGRI-07/A	Susana Río Segade	Università di Torino	Modulating White Wine Quality: The Effects of Ozone Treatments During Production	**Margherita Modesti (UniTUS)**	**Giorgio Gargari (UniMI)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-15**	I	Marco	Manta	AGRI-05/A	Alberto Alma/Elena Gonella	Università di Torino	Environmental sustainability and impact of grapevine pest control on wine growing	**Margherita Modesti (UniTUS)**	**Giorgio Gargari (UniMI)**

DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-16**	I	Cecilia Akotowaa	Offei	AGRI-07/A	Angela Zinnai	Università di Pisa/Università di Torino	Use of by-products from the olive oil and wine making industries to increase their sustainability and obtain high quality and healthy products	**Margherita Modesti (UniTUS)**	**Giorgio Gargari (UniMI)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-17**	I	Virginia	Gaiato	AGRI-07/A	Luca Giorgio Carlo Rolle	Università di Torino	Innovative oenological practices to adapt the winemaking and wine preservation process to the different grape composition resulting from climate change	**Margherita Modesti (UniTUS)**	**Giorgio Gargari (UniMI)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-17**	II	Marco	Lagori	AGRI-07/A	Luca Giorgio Carlo Rolle, Antonella Bosso	Università di Torino	Exploring the behavior of alternatives to montmorillonite clays in white wine protein stabilization	**Margherita Modesti (UniTUS)**	**Giorgio Gargari (UniMI)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-18**	I	Manuel	Chinello	AGRI-08/A	Alessio Giacomini	Università di Padova	Grape-based fermented beverages distinct from wine	**Luca Rolle (UniTO)**	**Giorgia Perpetuini (UniTE)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-19**	I	Ilenia	Pellicano	AGRI-07/A	Andrea Bellincontro - Francesca Luziatelli	Università della Tuscia	Biotechnological solutions for limiting climate change consequences, and maintaining high-quality standards in wine production	**Luca Rolle (UniTO)**	**Giorgia Perpetuini (UniTE)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-18**	II	Giulia	Bertazzoli	AGRI-08/A	Giovanna Felis	Università di Verona	Valorization of wine-related microbial bioresources for sustainability and innovation: case studies in the agri-food chain (poster communication)	**Luca Rolle (UniTO)**	**Giorgia Perpetuini (UniTE)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-19**	II	Margherita	Chiarini	AGRI-08/A	Maria De Angelis	Università di Bari	Techniques for Wine Dealcoholization and Microbiota Characterization (poster)	**Luca Rolle (UniTO)**	**Giorgia Perpetuini (UniTE)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-20**	I	Gabriele	Fioschi	AGRI-07/A	Vito Michele Paradiso	Università del Salento	Innovative approaches to wine health and sustainability in hot climates	**Luca Rolle (UniTO)**	**Giorgia Perpetuini (UniTE)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-21**	I	Michele Arcangelo	Dicorato	AGRI-07/A	Ombretta Marconi	Università di Perugia/Università di Torino	Valorization of Brewing By-Products as an Alternative Protein Source	**Aldo Todaro (UniCT)**	**Nadia Innocente (UniUD)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-22**	I	Mariacristina	D'Ascoli	BIOS-07/A	Di Pierro Prospero	Università di Napoli	Valorization of algal biomass through enzymatic biotechnology for the development of new food products	**Aldo Todaro (UniCT)**	**Nadia Innocente (UniUD)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-20**	II	Arianna	Bonucci	AGRI-07/A	Agnese Taticchi, Sonia Esposto	Università di Perugia/Università di Torino	From by-products to bioactive ingredients: the development of a functional snack using olive pâté and brewing spent grain	**Aldo Todaro (UniCT)**	**Nadia Innocente (UniUD)**

DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-21**	II	Marianna	Sala	AGRI-07/A	Carola Cappa	Università di Milano	Food System Innovation and Sustainability: Legume Cooking Water	**Aldo Todaro (UniCT)**	**Nadia Innocente (UniUD)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-23**	I	DAVIDE	RITORTO	AGRI-07/A	Marco Poiana	Università Mediterranea di Reggio Calabria	Valorization of bioactive compounds from Citrus by-products for food applications	**Aldo Todaro (UniCT)**	**Nadia Innocente (UniUD)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-24**	I	Marcello	Gigliotti	AGR-07/A BIO/10	Eleonora Carini, Claudia Folli	Università di Parma	Fava bean (*Vicia faba L.*) proteins: allergens identification, extraction methods, and technological modulation of biological and techno-functional properties	**Roberta Dordoni (UniCATT)**	**Antonio Derossi (UniFG)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-25**	I	Roberta	Pratolino	AGRI-07/A	Calligaris Sonia	Università di Udine	Designing next-generation hybrid foods by structuring biopolymers from food waste	**Roberta Dordoni (UniCATT)**	**Antonio Derossi (UniFG)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-22**	II	Simone	Musollini	AGRI-07/A	Fabio Favati	Università di Verona	Insights into the impact of ultra-high pressure treated rice husk microfibrillar cellulose on the viscoelastic and thermomechanical properties of model ice cream mixes	**Roberta Dordoni (UniCATT)**	**Antonio Derossi (UniFG)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-23**	II	Silvia	Gianfagna	AGRI-07/A	Maria Cristina Messia, Francesca Cuomo	Università del Molise	Process and Product Innovation for the Valorization and Sustainability of Confectionary Productions	**Roberta Dordoni (UniCATT)**	**Antonio Derossi (UniFG)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-26**	I	Alessandro	Rosso	AGR/02	Massimo Blandino	Università di Torino	Agronomic and technological solutions for achieving innovative food ingredients from legume crops	**Roberta Dordoni (UniCATT)**	**Antonio Derossi (UniFG)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-24**	II	Claudia	Troisi	AGRI-07/A	Maria Teresa Rodriguez Estrada	Università di Bologna	Innovative approaches for the development and performance evaluation of new frying mixtures through physical, chemical and sensory analyses	**Nicoletta MIELE (UniNA)**	**Marco Faieta (UniTE)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-27**	I	Ann Maria	Don	AGR-07/A	Matteo Scampicchio	Libera Università di Bolzano	Effect of soilless culture on the physiochemical and sensory attributes of strawberry	**Nicoletta MIELE (UniNA)**	**Marco Faieta (UniTE)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-28**	I	Erica	Bensmail	AGRI-07/A	Tullia Gallina Toschi	Università di Bologna	Ingredients and foods containing alternative protein sources: evaluation of sensory quality and acceptability	**Nicoletta MIELE (UniNA)**	**Marco Faieta (UniTE)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-25**	II	Pierina	Díaz-Guerrero	AGRI-07/A	Francesca Venturi, Isabella Taglieri	Università di Pisa	Sensory analysis and emotional profile as a tool for the valorisation of sustainable and innovative food chain	**Nicoletta MIELE (UniNA)**	**Marco Faieta (UniTE)**

DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-26**	II	Ejona	Boci	AGRI-07/A	Sara Spinelli	Università di Firenze	Attitude to sustainability and health and acceptability of baked goods by consumers	**Nicoletta MIELE (UniNA)**	**Marco Faieta (UniTE)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-29**	I	Anna	Lazaridou	AGRI-08/A	Vasileios Englezos, Simone Giacosa	Università di Torino	The role of grape microbiome in wine origin traceability	**Chiara Sanmartin (UniPI)**	**Luciano Cinquanta (UniPA)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-30**	I	Emiliano	Nardin	AGRI-07/A	Battistutta Franco	Università di Udine	The selection of wood for the production of quality wines	**Chiara Sanmartin (UniPI)**	**Luciano Cinquanta (UniPA)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-31**	I	Akash Kalyanrao	Jadhav	AGRI-07/A	Ombretta Marconi	Università di Perugia	Development and Optimization of Malt Extracts for Beer Production	**Chiara Sanmartin (UniPI)**	**Luciano Cinquanta (UniPA)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-32**	I	Shazina	Jabeen	AGRI-08/A	Daniele Daffonchio	Università di Torino	The Contribution of Soil Microbiome to Grapevine Resilience Against Drought, Fruit Quality and Terroir and Carbon Stock in the Vineyard	**Chiara Sanmartin (UniPI)**	**Luciano Cinquanta (UniPA)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-33**	I	Martina	Tarditi	BIO-04 AGR-03	Claudio Lovisolo/Alessandra Ferrandino	Università di Torino	Study of the effects of climate change combined with biostimulant application on grapevine physiology and the consequences for nutraceutical quality of grapes	**Chiara Sanmartin (UniPI)**	**Luciano Cinquanta (UniPA)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-34**	I	Nadia	Ciotola	AGRI-07/A	Ferranti Pasquale	Università di Napoli	Development of an innovative omic-based method to monitor Alternaria mycotoxins in tomato and assess their persistence	**Michele Del Carlo (UniTE)**	**Alessandra Bendini (UniBO)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-35**	I	Giulia	Gula	CHIM/10	Augusta Caligiani	Università di Parma	Food Chemistry: Development and application of untargeted methods and omics approaches for the characterization of raw materials and semi-finished products in the confectionery industry	**Michele Del Carlo (UniTE)**	**Alessandra Bendini (UniBO)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-27**	II	Daniele	Catanzaro	AGRI-07/A	Biagio Fallico	Università di Catania/Università di Torino	Development and Validation of an Analytical Method for the Identification and Quantification of Heterocyclic Amines in Foods	**Michele Del Carlo (UniTE)**	**Alessandra Bendini (UniBO)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-28**	II	Lorenzo	Oliverio	AGRI-07/A	Francesco Capozzi	Università di Bologna	Application of high-resolution nuclear magnetic resonance (HR-NMR) to evaluate the antioxidant properties of natural compounds used to extend the shelf life of food products rich in unsaturated lipid components	**Michele Del Carlo (UniTE)**	**Alessandra Bendini (UniBO)**
DAY 1 Wedsneday 10 September 13:30-19:00	**P-II-29**	II	Ludovica	Milzi	AGRI-07/A	Panunzi Barbara, Ferranti Pasquale	Università di Napoli	A smart hydrogel sensor for food spoilage detection: visual pH monitoring in milk and chicken samples	**Michele Del Carlo (UniTE)**	**Alessandra Bendini (UniBO)**

DAY 1 Wedsneday 10 September 13:30-19:00	**P-I-36**	I	Giorgio	Lupi	CHIM/10	Augusta Caligiani	Università di Parma	Bridging Analytical Chemistry and Regulation in Dietary Fiber Research: From Molecular Characterization to Novel Food Authorization	**Michele Del Carlo (UniTE)**	**Alessandra Bendini (UniBO)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-37**	I	Milad	Daneshniya	AGR-07/A	Matteo Scampicchio	Libera Università di Bolzano	Isolation and Characterization of Antioxidant Compounds from Maillard Reaction Products in Cooked Foods Using Advanced Spectroscopic Techniques	**Alessandra De Bruno (UNIROMA5)**	**Fabio Favati (UniVR)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-30**	II	Claudia	Lombroni	AGRI-07/A	Matteo Scampicchio	Libera Università di Bolzano/Università di Torino	Study of anthocyanins from red cabbage as potential natural alternatives to synthetic antioxidants	**Alessandra De Bruno (UNIROMA5)**	**Fabio Favati (UniVR)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-31**	II	Valentina	Antonioni	AGRI-07/A	Maria Fiorenza Caboni	Università di Bologna	Characterization of performances of various natural antioxidants in the formulation of food, feed and pet food, to identify the best processing conditions	**Alessandra De Bruno (UNIROMA5)**	**Fabio Favati (UniVR)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-32**	II	Pamela	Laera	AGRI-07/A	Francesco Caponio	Università di Bari	Can blueberry extract improve the oxidative stability of sunflower oil during a cooking process?	**Alessandra De Bruno (UNIROMA5)**	**Fabio Favati (UniVR)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-38**	I	ELNAZ	BAVEL	AGR-07/A	Matteo Scampicchio, Ksenia Morozova	Libera Università di Bolzano	Enhancing Antioxidant Activity in Breadcrumbs Through Controlled Maillard Reaction	**Alessandra De Bruno (UNIROMA5)**	**Fabio Favati (UniVR)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-39**	I	Alexandra-Mihaela	Ailoaiei	AGRI/07-A	Antonella Pasqualone	Università di Bari	Use of innovative plant-derived ingredients in the production of functional foods for the enhancement of marginal areas	**Annalisa Romano (UniNA)**	**Marco Dalla Rosa (UniBO)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-33**	II	Rossella Francesca	Lanza	AGRI-07/A	Donatella Albanese	Università di Salerno	Development and optimization of sustainable protein-source-based food	**Annalisa Romano (UniNA)**	**Marco Dalla Rosa (UniBO)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-40**	I	Letizia	Crema	AGR-07/A BIO/9	Emma Chiavaro, Francesca Scazzina	Università di Parma	Sustainability evaluation of plant-based protein sources by integrating nutritional, environmental and food safety dimensions	**Annalisa Romano (UniNA)**	**Marco Dalla Rosa (UniBO)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-41**	I	Maurizio	Asfalto	AGRI-07/A	Paola Pittia	Università di Teramo/Università di Torino	Development of Innovative Processes to produce micro- and nano-sized Extracellular Vesicles from Plant matrices intended for Nutraceuticals and Food applications	**Annalisa Romano (UniNA)**	**Marco Dalla Rosa (UniBO)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-42**	I	Maryam	Kazempour Sammak	AGRI-07/A	Fabio Favati	Università di Verona	Innovative food formulations with plant protein - based bigels	**Annalisa Romano (UniNA)**	**Marco Dalla Rosa (UniBO)**

DAY 2 Thursday 11 September 09:00-13:30	**P-I-43**	I	Muhammad Junaid	Arshad	AGRI-08/A	Elisa Salvetti	Università di Verona	Development of a body of knowledge of selected microbial bioresources for the production of an innovative kombucha	**Francesca Patrignani (UniBO)**	**Pasquale Massimiliano Falcone (UnivPM)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-34**	II	Tommaso	Ugolini	AGRI-07/A	Lorenzo Cecchi	Università di Firenze	Improvement of the monovarietal EVOO production chain: phenolic and volatile profiling for oil authentication and by-products exploitation	**Francesca Patrignani (UniBO)**	**Pasquale Massimiliano Falcone (UnivPM)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-35**	II	Giulia	Tapparo	AGRI-07/A	Marta Bertolino	Università di Torino	Chemical characterization of *Ocimum Basilicum L* . for pesto sauce production	**Francesca Patrignani (UniBO)**	**Pasquale Massimiliano Falcone (UnivPM)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-44**	I	Paolo	Cerruti	AGRI-07/A	Alberto Acquadro, Matteo Martina	Università di Torino	Parthenocarpy for enhanced food quality in eggplant: An approach to breeding seedless fruits	**Francesca Patrignani (UniBO)**	**Pasquale Massimiliano Falcone (UnivPM)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-36**	II	Alberto	Akiki	AGRI-07/A	Giampiero Sacchetti	Università di Teramo	Central Italy Emmer wheat types: healthy foods from sustainable agriculture	**Francesca Patrignani (UniBO)**	**Pasquale Massimiliano Falcone (UnivPM)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-37**	II	Mirella	Luciani	VET/04	Maria Schirone, Luigi Iannetti	Università di Teramo	Chicken Muscle Proteome as a Marker of Farming Practices: Implications forStress response and Meat Quality	**Fernanda Galgano (UniBAS)**	**Marilena Marino (UniUD)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-38**	II	Giulio	Giannini	AGRI-07/A	Marco Dalla Rosa	Università di Bologna	Application of chitosan coating and gaseous ozone to improve quality of fresh-cut apples	**Fernanda Galgano (UniBAS)**	**Marilena Marino (UniUD)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-45**	I	Matteo	Carletta	AGR01 AGR-07/A	Maria Martuscelli, Emilio Chiodo	Università di Teramo	Tackling Sustainability and Quality in Beef Production: a Consumer-Driven Approach	**Fernanda Galgano (UniBAS)**	**Marilena Marino (UniUD)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-39**	II	Sona	Hajiyeva	AGRI-08/A	Francesca Patrignani	Università di Bologna	Sustainable Production of Novel Natural Food Colorants by Filamentous Fungi and Yeasts through Carbon and Nitrogen Source Diversification and C/N Ratio Optimization	**Fernanda Galgano (UniBAS)**	**Marilena Marino (UniUD)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-46**	I	Alessia	Iannone	CHEM-01/A CHEM-01/B	Pasquale Avino	Università del Molise	Application of pre-labelled ACF filters for micropollutants extraction in foods and beverages	**Fernanda Galgano (UniBAS)**	**Marilena Marino (UniUD)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-47**	I	Giacomo	Pedron	CHEM-07/B	Jaouhari Yassine, Bordiga Matteo	Università del Piemonte Orientale	Roasting Effects on the Nutritional and Flavor-Related Biochemical Composition of *Pistacia vera L.*	**Fernanda Galgano (UniBAS)**	**Marilena Marino (UniUD)**

DAY 2 Thursday 11 September 09:00-13:30	**P-I-48**	I	Roberto	Senatore	AGR/19	Paolo Cornale, Davide Biagini	Università di Torino	Strategies for Reducing Emissions and Enhancing Sustainability in Livestock Systems	**Maria Concetta Tenuta (UniBZ)**	**Erica Pontonio (UniMI)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-49**	I	Matteo	Profeti	CHIM/10	Augusta Caligiani	Università di Parma	Extraction and characterisation of biomolecules from insects reared on food by-products for food and feed applications	**Maria Concetta Tenuta (UniBZ)**	**Erica Pontonio (UniMI)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-50**	I	Gianfranco	Desideri	AGR-07/A	Maria Martuscelli	Università di Teramo	Quality and sustainability of Pasta as social integration tool	**Maria Concetta Tenuta (UniBZ)**	**Erica Pontonio (UniMI)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-40**	II	Tanveer	Ahmad	MEDS-24/B	Teresa Cirillo, Francesco Esposito	Università di Napoli	From Waste to Taste: Formulation of Fiber-Rich Bakery Products from Coffee Silver Skin	**Maria Concetta Tenuta (UniBZ)**	**Erica Pontonio (UniMI)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-51**	I	Arjun	MOHANAKUMAR	AGR/03,AGR/15	Tommaso Ganino, Massimiliano Rinaldi	Università di Parma	Processing and valorisation of agri-food by-products and their use as elicitors in agriculture	**Maria Concetta Tenuta (UniBZ)**	**Erica Pontonio (UniMI)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-52**	I	Claudia	Siclari	AGRI-07/A AGRI-03	Tommaso Ganino, Massimiliano Rinaldi	Università di Parma	Valorization of agri-food by-products through innovative technologies: circular economy in the brewing supply chain	**Carola Cappa (UniMI)**	**Lucia Parafati (UniCT)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-53**	I	Giovanni	Ciano	AGRI-04/B	Roberto Moscetti	Università della Tuscia	Characterization and smart par-drying of potatoes belonging to PGI “Patata dell’Alto Viterbese”	**Carola Cappa (UniMI)**	**Lucia Parafati (UniCT)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-54**	I	Mattea Maria Antonietta	Lauceri	AGRI-07/A	Concetta Condurso, Fabrizio Cincotta	Università di Messina	Vegetable Coagulants and New Dairy Technologies for the Innovation and Diversification of Goat's Milk Dairy Production	**Carola Cappa (UniMI)**	**Lucia Parafati (UniCT)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-55**	I	Salvatore	Chiarenza	AGRI-07/A	Elena Arena	Università di Catania	Recovery of bioactive compounds and fiber from citrus wastes	**Carola Cappa (UniMI)**	**Lucia Parafati (UniCT)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-56**	I	Francesca	Colicigno	AGRI-07/A	Donatella Albanese	Università di Salerno	Sustainability Aspects in the Reformulation of Products for the Confectionery Industry: A Case Study on Gummy Candies	**Carola Cappa (UniMI)**	**Lucia Parafati (UniCT)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-41**	II	Anna	Bortolini	AGRI-07/A	Piergiorgio Comuzzo	Università di Udine	Effect of closure system on the evolution of Pinot gris and Pinot noir wines	**Rosanna Tofalo (uniTE)**	**Vasileios Englezos (UniTO)**

DAY 2 Thursday 11 September 09:00-13:30	**P-II-42**	II	Maria Carla	Cossu	AGRI-08/A	Giacomo Zara	Università di Sassari	Development of a Synthetic Microbial Community for Producing A Sardinian Functional Acid-Alcoholic Dairy Beverage	**Rosanna Tofalo (uniTE)**	**Vasileios Englezos (UniTO)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-43**	II	Giorgia	La Rocca	AGRI-08/A	Angela Capece	Università della Basilicata	Development of biological and monitoring tools for improving energy efficiency and sustainability in winemaking	**Rosanna Tofalo (uniTE)**	**Vasileios Englezos (UniTO)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-44**	II	Leonardo	D'Intino	AGRI-07/A	Mario Gabrielli	Università Cattolica del Sacro Cuore	Modelling of time and temperature effects for the identification of ageing trends in volatiles composition of Malvasia di Candia Aromatica wines	**Rosanna Tofalo (uniTE)**	**Vasileios Englezos (UniTO)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-45**	II	Eleonora	Muccio	AGRI-07/A	Donatella Albanese	Università di Salerno	High Value Food Products from Microalgae	**Francesca Malvano (UniSA)**	**Silvana Cavella (UniNA)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-46**	II	Federica	Torregrossa	AGRI-07/A	Luciano Cinquanta, Diego Planeta	Università di Palermo	Application of high pressure treatment (HPP) to extend shelf life of liquid artisanal ice cream bases	**Francesca Malvano (UniSA)**	**Silvana Cavella (UniNA)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-47**	II	Shahzal	Babar	AGRI-07/A	Alessio Cimini	Università della Tuscia	Development and Optimization of Instant Fresh Egg Pasta Enhancing Rehydration Efficiency, Structural Integrity, and Nutraceutical Potential	**Francesca Malvano (UniSA)**	**Silvana Cavella (UniNA)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-48**	II	Emanuele	Tomassini	AGRI-07/A	Antonio Piga	Università di Sassari	Salt reduction strategies in bread making: exploring food-grade seawater	**Francesca Malvano (UniSA)**	**Silvana Cavella (UniNA)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-49**	II	Gloria	Sciuto	AGRI-08/A	Cinzia Caggia	Università di Catania	Valorization of Second Cheese Whey through microalgal cultivation for high-value biomass production	**Francesca Malvano (UniSA)**	**Silvana Cavella (UniNA)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-50**	II	Roberta	Montebello	AGRI07-A	Antonio Derossi	Università di Foggia	Understanding the effects of heat treatment conditions on the physicochemical and technological properties of mycelium-based meat substitute	**Rosalba Lanciotti (UniBO)**	**Isabella Taglieri (UniPI)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-57**	I	Michele	Isotti	AGR-08/A	Pasquale Russo, Diego Mora	Università di Milano	From food biofortification to probiotic: exploring biotechnological applications of B2-Overproducing LAB	**Rosalba Lanciotti (UniBO)**	**Isabella Taglieri (UniPI)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-58**	I	Evangelia	Tsara	AGR-08/A	Rantsiou Kalliopi	Università di Torino	Design of bioprotective microbial consortia to improve the shelf life and overall quality of meat products	**Rosalba Lanciotti (UniBO)**	**Isabella Taglieri (UniPI)**

DAY 2 Thursday 11 September 09:00-13:30	**P-I-59**	I	Nataliya	Baschinski	AGR-08/A	Luca Simone Cocolin	Università di Torino	Coffee bean microbiome as a suitable source for new microbial starter cultures	**Rosalba Lanciotti (UniBO)**	**Isabella Taglieri (UniPI)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-60**	I	Martina	Nironi	AGR/03 AGR/16 CHIM/10	Benedetta Chiancone, Martina Cirlini, Valentina Bernini	Università di Parma	A green approach to the synthesis and extraction of bioactive compounds from vitro-derived hop plants	**Rosalba Lanciotti (UniBO)**	**Isabella Taglieri (UniPI)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-61**	I	Noemi	Martinengo	AGR-08/A	Monica Gatti	Università di Parma	Application of targeted lactic acid bacteria for the revalorization of food matrices and by-product	**Antonello Paparella (UniTE)**	**Giacomo Zara (UniSS)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-62**	I	Mubshra	Naseer	AGR-08/A	Severino Zara, Francesco Fancello	Università di Sassari/Università di Torino	Study of the Antimicrobial Activity of Natural Extracts Obtained from Olive Trees on Food-Borne and Spoilage Bacteria	**Antonello Paparella (UniTE)**	**Giacomo Zara (UniSS)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-63**	I	Martina	Filippini	AGRI 08/A	Giulia Tabanelli	Università di Bologna	Microbial biodiversity as a sustainable strategy for innovative plant-based fermented products	**Antonello Paparella (UniTE)**	**Giacomo Zara (UniSS)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-64**	I	kiana	Aghaei	AGRI-07/A	Marcello Fidaleo	Università della Tuscia	Bio-Based Surfactants for a Greener Future: Synthesis of Alkyl Polyglucoside from Renewable Resources.	**Antonello Paparella (UniTE)**	**Giacomo Zara (UniSS)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-65**	I	Gabriele	Capriglia	AGRI-08/A	Sara Borin	Università di Milano	Exploring plant-microbiome synergy for hydrocarbon bioremediation of polluted soils	**Antonello Paparella (UniTE)**	**Giacomo Zara (UniSS)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-51**	II	Gaia	Vanzetti	AGRI-08/A	Ilario Ferrocino	Università di Torino	Optimizing Taggiasca table olives fermentation: development of autochthonous microbial starter culture	**Aldo Corsetti (UniTE)**	**Severino Zara (UniSS)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-52**	II	Emanuele	Della Monica	AGRI-08/A	Alessia Levante, Camilla Lazzi	Università di Parma	Interspecies interactions in cheese microbiota: metabolic synergies among lactic acid bacteria species	**Aldo Corsetti (UniTE)**	**Severino Zara (UniSS)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-53**	II	Caterina	Nicolotti	AGRI-08/A	Benedetta Bottari	Università di Parma	Fermented foods: from raw material fermentation to microbiota and related post-digestion bioactive compounds	**Aldo Corsetti (UniTE)**	**Severino Zara (UniSS)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-54**	II	Irene Maria	Zingale	AGRI-08/A	Cinzia Caggia	Università di Catania	Biovalorization of Pâtè Olive Cake (POC) for food applications	**Aldo Corsetti (UniTE)**	**Severino Zara (UniSS)**

DAY 2 Thursday 11 September 09:00-13:30	**P-II-55**	II	Andrea	Torreggiani	AGRI/08-A	Carlo Giuseppe Rizzello	Università di Bari	Valorization of Agrifood Waste for Bioplastic Production via Microbial Fermentation Using Cupriavidus necator	**Aldo Corsetti (UniTE)**	**Severino Zara (UniSS)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-56**	II	Polina	Makarycheva	BIO/19	Stefania Silvi	Università di Camerino	Evaluation of the ability to induce well-being and health by plant extracts and their constituents	**Aldo Corsetti (UniTE)**	**Severino Zara (UniSS)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-66**	I	Mariasimona	Cavaliere	AGRI/08-A	Maria De Angelis	Università di Bari	Development of sustainable strategies for effective delivery of probiotics and bioactive substances	**Lucia Vannini (UniBO)**	**Maria Martuscelli (UniTE)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-67**	I	Simona	Carbone	AGRI/08-A	Erica Pontonio	Università di Bari	Functional foods: set-up of biotechnology processes, design and market strategies	**Lucia Vannini (UniBO)**	**Maria Martuscelli (UniTE)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-68**	I	Viola	Termine	AGRI-08/A	Diego Mora	Università di Milano	Beyond Probiotics: Unravelling the Secret Weapons of VSL#3 as a Postbiotic	**Lucia Vannini (UniBO)**	**Maria Martuscelli (UniTE)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-69**	I	Elisabetta	Cutecchia	AGRI/08-A	Giuseppe Celano	Università di Bari	Biotechnological innovations for the reduction of microbiological risk in the dairy supply chain	**Lucia Vannini (UniBO)**	**Maria Martuscelli (UniTE)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-70**	I	Aziza	Caccia	CHEM-05	Sabrina Dallavalle	Università di Milano	Hybrid nature-inspired metal chelators as antifungal agents for crop protection	**Maria Teresa Rodriguez Estrada (UniBO)**	**Elena Bancalari (UniPR)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-71**	I	Matteo	Corti	CHEM-07/B	Martina Letizia Contente	Università di Milano	Enzymatic prenylation of waste-derived polyphenols to enhance their biological activity and/or physico/chemical properties	**Maria Teresa Rodriguez Estrada (UniBO)**	**Elena Bancalari (UniPR)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-72**	I	Sara	Foiadelli	CHEM-07/C	Francesco Enzo Molinari	Università di Milano	Sustainable bioprocesses for plant secondary metabolite biotransformation	**Maria Teresa Rodriguez Estrada (UniBO)**	**Elena Bancalari (UniPR)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-73**	I	Elif	Kartal	AGRI-07/A	Deborah Pacetti, Massimiliano Gasparini	Università Politecnica delle Marche	Chemical and biological characterization of pomegranate seed oil: a novel functional ingredient	**Maria Teresa Rodriguez Estrada (UniBO)**	**Elena Bancalari (UniPR)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-58**	II	Francesco	Regi	AGRI-07/A	Paola Pittia	Università di Teramo	Exploring Vegetal Sources of Extracellular Vesicles: A Step Toward Functional Food Ingredients	**Maria Teresa Rodriguez Estrada (UniBO)**	**Elena Bancalari (UniPR)**

DAY 2 Thursday 11 September 09:00-13:30	**P-II-59**	II	Susanna	Lelli	AGRI-07/A	Emanuele Marconi	Univerità Campus Bio-Medico di Roma	Characterization of Pea Proteins, Soft Wheat Cultivars, Pomegranate and Derivatives to Design Products for Preconceptional and Pregnant Women	**Rosa Palmeri (UniCT)**	**Marco Poiana (UniRC)**
DAY 2 Thursday 11 September 09:00-13:30	**P-II-60**	II	Ilaria	Proetto	AGRI-07/A	Biagio Fallico	Università di Catania	Valorisation of Brewer's Spent Grain as a Functional Ingredient to Improve Nutritional and Health Quality in Dry Pasta Formulation	**Marco Faieta(UniTE)**	**Marco Poiana (UniRC)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-74**	I	Elisa	Canazza	AGRI-07/A	Anna Lante	Università di Padova	Bioactive compounds in convenience foods and valorisation of their processing by-products and waste	**Rosa Palmeri (UniCT)**	**Marco Poiana (UniRC)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-75**	I	Mario	Guida	AGRI-07/A	Enrico Valli	Università di Bologna	Innovative and sustainable methodologies for the extraction and characterization of bioactive and high value-added compounds obtained from agri-food supply chain by-products.	**Rosa Palmeri (UniCT)**	**Marco Poiana (UniRC)**
DAY 2 Thursday 11 September 09:00-13:30	**P-I-76**	I	Emanuele	Marchesi	AGRI-07/A	Fabio Favati	Università di Verona	Valorization of animal by-products: industrial production of hydrolyzed animal collagen and evaluation of its technological and nutraceutical properties	**Rosa Palmeri (UniCT)**	**Marco Poiana (UniRC)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-61**	II	Davide	Addazii	AGRI-07/A	Andrea Gianotti	Università di Bologna	Modulatory Effects of Nutritional Supplements on Gut Microbiota: An In vitro Study on Crohn's Disease and Healthy Donors	**Robereta Prete (UniTE)**	**Benedetta Bottari (UniPR)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-62**	II	Ananya	Gupta	AGRI-08/A	Luca Cocolin	Università di Torino	Harnessing microbial resources for innovations in cocoa fermentation	**Robereta Prete (UniTE)**	**Benedetta Bottari (UniPR)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-63**	II	Martina	Parziale	AGRI-08/A	Francesca De Filippis	Università di Napoli	Development of probiotics and post-biotics for the modulation of skin microbiome	**Robereta Prete (UniTE)**	**Benedetta Bottari (UniPR)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-64**	II	Sara	Basiglio	AGRI-08/A	Elena Crotti	Università di Milano	Impact of chemical pollutants on the gut microbiome of pollinators	**Robereta Prete (UniTE)**	**Benedetta Bottari (UniPR)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-65**	II	Alberto	Binello	AGRI-08/A	Kalliopi Rantsiou	Università di Torino	Human and food microbiome: interactions explored in vitro	**Robereta Prete (UniTE)**	**Benedetta Bottari (UniPR)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-66**	II	Beatrice Maria	Stella	MEDS-08/C	Simona Bo	Università di Torino	Ultra-processed food intake and its impact on public health in older adults	**Mauro Serafini(UniTE)**	**Simone Perna (UniMI)**

DAY 2 Thursday 11 September 14.00/18.30	**P-II-67**	II	Arianna	Gallina	MEDS-08/C	Letizia Bresciani	Università di Parma	Application of a new semi-quantitative food frequency questionnaire for evaluating seasonal (poly)phenol intake	**Mauro Serafini (UniTE)**	**Simone Perna (UniMI)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-68**	II	Silvia	Callegaro	MEDS-08/C	Alice Rosi	Università di Parma	Woman's nutrition: eating habits, nutritional status and main sex-related risk factors in the physiological phases of female life	**Mauro Serafini(UniTE)**	**Simone Perna (UniMI)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-77**	I	Ellis	Bianchi	MEDS-08/C	Patrizia Riso	Università di Milano	Valorization of Fish in Healthy and Sustainable Dietary Patterns	**Mauro Serafini (UniTE)**	**Simone Perna (UniMI)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-78**	I	Francesco Matteo	Curatolo	MEDS-08/C	Margherita Dall'Asta	Università Cattolica del Sacro Cuore, Piacenza	Discovering the role of SEASOnal dietary choices on Mediterranean diet adherence and effect on gut microbiota (4-SEASOMed)	**Mauro Serafini (UniTE)**	**Simone Perna (UniMI)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-69**	II	Cristiano	Negro	MEDS-08/C	Pedro Mena	Università di Parma	Mapping the complexity of the diet: circulating metabolites of plant bioactives and food-gut microbiome-host interactions	**Mauro Serafini (UniTE)**	**Simone Perna (UniMI)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-70**	II	Lara Naim	Chehade	MEDS-08/C	Daniela Martini	Università di Milano	Nutritional Quality and Environmental Impact of Menus in the Italian Institutional Catering Sector: Potential for Optimization and Sustainability	**Mauro Serafini (UniTE)**	**Donato Angelino (UniTE)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-71**	II	Domenico	Nuzzolese	AGRI/08-A	Fabio Minervini	Università di Bari	Valorization of Apulian black chickpea and Senatore Cappelli durum wheat for the production of foods with high nutritional and functional value	**Stefania Silvi (UniCAM)**	**Giulia Tabanelli (UniBO)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-72**	II	Federica	Montagano	AGRI/08-A	Aldo Corsetti, Roberta Prete	Università di Teramo	Selection of *Lactiplantibacillus plantarum* strains for g-amino butyric acid (GABA) production in a legume-based fermented beverage	**Stefania Silvi (UniCAM)**	**Giulia Tabanelli (UniBO)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-73**	II	Giulia	Brioschi	CHEM-07/B	Gigliola Borgonovo	Università di Milano	Sustainable chemical processes for the valorization of agri-food residues	**Stefania Silvi (UniCAM)**	**Giulia Tabanelli (UniBO)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-74**	II	Antonella	Damiano	MVET-04/A	Michele Amorena, Carmine Merola, Antonio Francioso	Università di Teramo	Toxicological profile and preliminary therapeutic effects of a green extra-virgin olive oil extract in the zebrafish model	**Stefania Silvi (UniCAM)**	**Giulia Tabanelli (UniBO)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-75**	II	Shazia	Akram	AGR-08/A	Clemencia Chaves Lopez	Università di Teramo	Antifungal Effects of Essential Oils (EOs) and Pulsed Light (PL)on Cheese Spoilage Fungi: Inhibition, Gene Expression, and Cellular Disruption	**Stefania Silvi (UniCAM)**	**Giulia Tabanelli (UniBO)**

DAY 2 Thursday 11 September 14.00/18.30	**P-I-79**	I	Riccardo	Riggi	AGRI-07/A	Andrea Bellincontro, Margherita Modesti	Università della Tuscia	No-alcohol, Low-alcohol (NoLo) and wines produced by sustainable and safe protocols: technological strategies and operative opportunities for new-frontier oenological productions	**Rosanna Tofalo (UNITE)**	**Piergiorgio Comuzzo (UniUD)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-76**	II	Silvia	Arduini	AGRI-07/A	Fabio Chinnici	Università di Bologna	Characterization and Authentication of Alcoholic Products with Geographical Indications	**Rosanna Tofalo (UNITE)**	**Piergiorgio Comuzzo (UniUD)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-80**	I	Sara	Zanella	AGRI-07/A	Matteo Marangon, Andrea Curioni	Università di Padova	Exploring the nature and mechanism of formation of red wine colloids: implications for stability and quality	**Rosanna Tofalo (UNITE)**	**Piergiorgio Comuzzo (UniUD)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-77**	II	Roberto Salvatore	Di Fede	AGRI-07/A	Piombino Paola	Università di Napoli	Release and perception of γ-nonalactone and massoia lactone in the red wine matrix: impact of ethanol and acidity	**Rosanna Tofalo (UNITE)**	**Piergiorgio Comuzzo (UniUD)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-81**	I	Thomas	Croci	AGRI-07/A	Alessandra Marti	Università di Milano	Bridging Agriculture to Food Technology for Sustainable Bakery Products	**Antonella Verzera (UniME)**	**Matteo Scampicchio (UniBZ)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-82**	I	Alberto	Saitta	AGRI-07/A	Manzocco Lara	Università di Udine	Integral Conversion of Industrial Fruit and Vegetable Waste into Extracts and Aerogels with Enhanced Functionality	**Antonella Verzera (UniME)**	**Matteo Scampicchio (UniBZ)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-83**	I	Marco	Cardillo	STAT-01/A	Piscitelli Alfonso	Università di Napoli	Characterization and evaluation of food products using advanced statistical methods and models	**Antonella Verzera (UniME)**	**Matteo Scampicchio (UniBZ)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-84**	I	Martina	Gattuso	AGRI-07/A	Pasquale Crupi, Matteo Pollon	Università di Palermo/Università di Torino	Formulation of new confectionery products based on functional compounds deriving from plant by-products	**Antonella Verzera (UniME)**	**Matteo Scampicchio (UniBZ)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-85**	I	Davide	Fascioli	AGRI-07/A	Pasquale Massimiliano Falcone	Università Politecnica delle Marche	AI and emerging technologies driving innovation in functional and health- promoting biopeptides for enhanced development and efficacy of plant- based foods	**Antonella Verzera (UniME)**	**Matteo Scampicchio (UniBZ)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-78**	II	Francesca	Trevisiol	AGRI-07/A	Calligaris Sonia, Innocente Nadia, Comuzzi Clara	Università di Udine	Valorization of Polysaccharides and Oligosaccharides from Food Waste (Poster)	**Tullia Gallina Toschi (UniBO)**	**Silvia Grassi (UniMI)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-79**	II	Silvia	Gattucci	AGR-08/A	Maurizio Ciani	Università Politecnica delle Marche	Development of new functional foods derived from the honeybees' world	**Tullia Gallina Toschi (UniBO)**	**Silvia Grassi (UniMI)**

DAY 2 Thursday 11 September 14.00/18.30	**P-II-80**	II	Helen Stephanie Ofei	Darko	AGRI-07/A	Paolo Lucci, Deborah Pacetti	Universita Politecnica delle Marche	Alternative Food Sources as Functional Ingredients for Food Applications	**Tullia Gallina Toschi (UniBO)**	**Silvia Grassi (UniMI)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-86**	I	Stefano	Sallustio	AGRI-07/A	Vitaglione Paola	Università di Napoli	Valorization of Food By-products for the Development of Innovative Functional Snacks	**Tullia Gallina Toschi (UniBO)**	**Silvia Grassi (UniMI)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-87**	I	Federica	Zannini	AGR-08/A	Francesca Comitini, Maria Elena Lionetti	Università Politecnica delle Marche	Regenerative recycling of Agro food waste for enhancing the Mediterranean food system's circularity	**Tullia Gallina Toschi (UniBO)**	**Silvia Grassi (UniMI)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-88**	I	Michele	Napolitano	AGRI-07/A	Maria Cristina Messia; Francesca Cuomo	Università del Molise	Evaluation of emerging risks in food system, and development of strategies to guarantee the safety and the quality of traditional and innovative foods.	**Maria Schirone (UniTE)**	**Francesco Esposito (UniNa)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-82**	II	Melisa	Jaramillo Zapata	AGRI-08/A	Luca Cocolin	Università di Torino	Microbiological food safety: risk mitigation of foodborne pathogens by applying omics approaches within the food system	**Maria Schirone (UniTE)**	**Francesco Esposito (UniNa)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-83**	II	Ivan	Ciliberti	AGRI-08/A	Aponte Maria	Università di Napoli	BALOs as biocontrol agent in bivalve shellfish depuration	**Maria Schirone (UniTE)**	**Francesco Esposito (UniNa)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-89**	I	Seyedeh Farnaz	Sadeghian	AGRI-07/A	Moret Sabrina	Università di Udine	Characterization of mineral oil contamination in vegetable oils and fats and study of innovative and sustainable approaches for their mitigation, with a focus on the most toxic aromatic components	**Sonia Esposto (UniPG)**	**Carla Di Mattia (UniTE)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-90**	I	Gianfilippo	Nigro	AGRI-07/A	Giacomo Squeo, Giusy Rita Caponio	Università di Bari/Università di Torino	Design and quality assessment of improved nutritional value food products using by-products and minor crops as ingredients	**Sonia Esposto (UniPG)**	**Carla Di Mattia (UniTE)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-91**	I	Cosimo Damiano	Dellisanti	AGRI/04-A	Alessandro Leone	Università di Bari	Application of vacuum prior to centrifugal separation of oils from olives, avocados, and other agri-food processes	**Sonia Esposto (UniPG)**	**Carla Di Mattia (UniTE)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-92**	I	Aparnadevi	Premkartikkumar Geethalakshmi	AGR-07/A	Giovanna Ferrentino	Libera Università di Bolzano	Supercritical CO_2 as a Green Technology for the Extraction and Characterization of Bioactives from Alpine Botanicals: Application in Oleolites	**Sonia Esposto (UniPG)**	**Carla Di Mattia (UniTE)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-93**	I	Sofia	Trentanove	AGRI-07/A	Caterina Dinnella	Università di Firenze	Home sensory dietary patterns and the acceptability of school meals: the role of parental and child individual variables	**Sonia Esposto (UniPG)**	**Carla Di Mattia (UniTE)**

DAY 2 Thursday 11 September 14.00/18.30	**P-I-94**	I	Chiara Alessandra Carmen	Rutigliano	AGRI-07/A	Aldo Todaro	Università di Catania	Innovative strategies for oxidation protection in pistachio products	**Antonio PiGA (UniSS)**	**Agnese Taticchi (UniPG)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-84**	II	Giorgio Innocenzo	Ascrizzi	AGRI-07/A	Laura Piazza	Università di Milano	Mucoadhesive and Mechanical Properties of Prototype Soft-solid Foods Designed for Dysphagia Management	**Antonio PiGA (UniSS)**	**Agnese Taticchi (UniPG)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-85**	II	Antonella	Manzi	AGRI07-A	Antonio Derossi	Università di Foggia	Optimizing the dehydration of tuna by-products as a strategy to develop innovative nutritious fish powder	**Antonio PiGA (UniSS)**	**Agnese Taticchi (UniPG)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-95**	I	Rubab	Fatima	AGRI-07/A	Fabio Favati	Università di Verona	Transition from gas to electrical ovens at industrial level: optimization of the cooking profile and product formulation	**Antonio PiGA (UniSS)**	**Agnese Taticchi (UniPG)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-86**	II	Monica	Di Maria	AGRI-07/A	Diego Planeta, Pasquale Crupi, Pellegrino Conte	Università di Palermo	Enhancement and stability of Sicilian olive oils: accelerated shelf life study	**Antonio PiGA (UniSS)**	**Agnese Taticchi (UniPG)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-96**	I	Filippo	Bibi	MEDS-08/C	Francesca Scazzina	Università di Parma	Athlete hydration and development of a validated questionnaire for monitoring hydration status in different sports disciplines	**Daniela MARTINI (UniMI)**	**Donato Angelino (UniTE)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-97**	I	Federica	Bergamo	MEDS-08/C	Alice Rosi, Pedro Mena	Università di Parma	Nutritional needs of lung cancer patients: assessment of nutritional status for the development of new personalized nutrition interventions	**Daniela MARTINI (UniMI)**	**Donato Angelino (UniTE)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-87**	II	Joana Maria	Santos Rebelo	MEDS-08/C	Pedro Mena	Università di Parma	Study of the association between phenolic metabotypes and cardiometabolic health in response to chronic intake of (poly)phenols	**Daniela MARTINI (UniMI)**	**Donato Angelino (UniTE)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-98**	I	Federica	Grant	MEDS-08/C	Maria Cristina Messia, Laura Rossi	Università del Molise	Food systems transformation for healthy and sustainable diets	**Daniela MARTINI (UniMI)**	**Donato Angelino (UniTE)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-99**	I	Lucia	Ghiretti	MEDS-08/C	Pedro Mena	Università di Parma	Datasets integration for the development of dietary strategies aimed at promoting cardiometabolic health through plant-based diets	**Daniela MARTINI (UniMI)**	**Donato Angelino (UniTE)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-88**	II	Maria Sole	Morandini	MEDS-08/C	Pedro Mena	Università di Parma	Predictive models for the effect of foods containing (poly)phenols: a personalized nutritional approach	**Daniela MARTINI (UniMI)**	**Donato Angelino (UniTE)**

DAY 2 Thursday 11 September 14.00/18.30	**P-I-100**	I	Asmita Mukundrao	Acharya	MEDS-08/C	Simone Perna	Università di Milano	Investigating the citrus fruit polyphenols and Their Metabolites on Immunoaging, Neurodegeneration, and Skeletal Muscle	**Daniela MARTINI (UniMI)**	**Donato Angelino (UniTE)**
DAY 2 Thursday 11 September 14.00/18.30	**P-II-89**	II	Chiara	Bravo Trippetta	MEDS-08/C	Donato Angelino	Università di Teramo	Development of the Nutritional Inflammatory Index: cell culture- and artificial intelligence-based methods to evaluate the Inflammatory Modulation of Foods and Recipes	**Simone PERNA (UniMI)**	**Daniela MARTINI (UniMI)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-101**	I	Elena	Corvaglia	MEDS-08/C	Mauro Serafini	Università di Teramo	Nutritional and functional sustainability for human and planetary health: the study of the dietary habits and metabolic profile of centenarians from Abruzzo	**Simone PERNA (UniMI)**	**Daniela MARTINI (UniMI)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-102**	I	Raffaella	Bravi	MEDS-08/C	Mauro Serafini	Università di Teramo	Sustainable Diets for Health and Equity: Advancing Food Policies in Urban Environments	**Simone PERNA (UniMI)**	**Daniela MARTINI (UniMI)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-103**	I	Bianca	Romanelli	MEDS-08/C	Pedro Mena, Letizia Bresciani	Università di Parma	Study of flavan-3-ols metabolised and non-metabolised at colonic level through the use of several chromatographic techniques	**Mauro SERAFINI (UniTE)**	**Daniela MARTINI (UniMI)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-104**	I	Federica	Volpe	MEDS-08/C	Dall'Asta Margherita, Giuberti Gianluca	Università Cattolica del Sacro Cuore, Piacenza	Nutritional and technological strategies to study and develop cereal-based products for different population targets	**Donato Angelino (UniTE)**	**Daniela MARTINI (UniMI)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-105**	I	Georgiana	Bosco	AGRI-08/A	Cinzia Lucia Randazzo	Università di Catania	Set up of a probiotic and germinated brown rice (GBR) formulation for health benefits	**Francesca Bot (UniPR)**	**Pasquale Filannino (UniBA)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-106**	I	Alessandra	De Vivo	AGRI-08/A	Francesca De Filippis	Università di Napoli	Exploiting the microbiome of traditional and sustainable plant-based fermented foods with pro- and post-biotic properties	**Francesca Bot (UniPR)**	**Pasquale Filannino (UniBA)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-107**	I	Federica Violetta	Conti	AGRI-08/A	Carlo Giuseppe Rizzello	La Sapienza Università di Roma	Study of sourdoughs microbial communities to develop novel biotechnological strategies for the food industry	**Francesca Bot (UniPR)**	**Pasquale Filannino (UniBA)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-108**	I	Maria Veronica	Faulisi	AGRI-08/A	Cristina Restuccia	Università di Catania	Pomegranate (*Punica granatum L*.) by-products extracts with high antioxidant and antimicrobial action as food additives in a circular economy and environmental sustainability perspective – "From waste to wealth" objective	**Francesca Bot (UniPR)**	**Pasquale Filannino (UniBA)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-109**	I	Irene	Gandolfi	AGRI-08/A	Francesca Patrignani	Università di Bologna	Biotechnological valorization of legumes for the development of high-value-added ingredients to be used in plant-based food formulations	**Francesca Bot (UniPR)**	**Pasquale Filannino (UniBA)**

DAY 2 Thursday 11 September 14.00/18.30	**P-I-110**	I	Martina	Calabrese	AGRI-07/A	Romano Raffaele, Aponte Maria	Università di Napoli	Development of Functional Dairy Products made from Buffalo Milk: The role of Probiotics, Prebiotics and Production Technology	**Cristina Messia (UniMOL)**	**Lara Manzocco (UniUD)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-111**	I	Lia	Mansueto	AGRI/07-A	Graziana Difonzo	Università di Bari	Baked goods with a high nutritional profile: from their design using alternative ingredients to the health-related properties	**Cristina Messia (UniMOL)**	**Lara Manzocco (UniUD)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-112**	I	Gaia	Gadaleta-Caldarola	AGRI/07-A	Francesco Caponio	Università di Bari	Study for the formulation of innovative durum wheat bakery products and extension of their shelf-life (poster)	**Cristina Messia (UniMOL)**	**Lara Manzocco (UniUD)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-113**	I	Michela	Tramontini	CHEM-01/A	Paola Calza	Università di Torino	Chemical characterization, photochemical behaviour and environmental fate of agricultural technological products	**Cristina Messia (UniMOL)**	**Lara Manzocco (UniUD)**
DAY 2 Thursday 11 September 14.00/18.30	**P-I-114**	I	Paolo	Rolando	CHIM/06 AGR/15	Tullia Tedeschi, Marcello Alinovi	Università di Parma	Animal-derived proteins in the confectionery industry: Knowledge, Applications, and Alternatives	**Cristina Messia (UniMOL)**	**Lara Manzocco (UniUD)**

PhD Student Dissertation projects
3rd year

PARALLEL SESSION 1
Processing-Structure-Function Relationships in Food Design

Application of innovative technologies for the functionalisation of alternative proteins and the associated functional and rheological characterisation

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The growing market for plant-based products requires specific ingredients to meet new technological challenges. Non-thermal technologies, including cold plasma (CP), pulsed electric field (PEF) and high-pressure processing (HPP), could offer sustainable and efficient approaches for product functionalization. This PhD project aims to evaluate the use of these innovative treatments on legume flours, studying their effects on functional and rheological properties. The modified flour and their potential practical application have been assessed. The research contributes to understanding the complex changes taking place when such modification techniques are applied to a complex matrix such as flour.

Applicazione di tecnologie innovative per la funzionalizzazione di proteine alternative e relativa caratterizzazione funzionale e reologica

Il crescente mercato dei prodotti a base vegetale richiede ingredienti specifici per affrontare le nuove sfide tecnologiche. Tecnologie innovative, tra cui il plasma freddo, i campi elettrici pulsati e le alte pressioni di omogenizzazione, potrebbero offrire approcci efficienti per la funzionalizzazione di sfarinati. Questo progetto di dottorato mira a valutare l'uso di questi trattamenti sulle farine di legumi, studiandone gli effetti sulle proprietà funzionali e reologiche. Si sono valutate le farine modificate e la loro potenziale applicazione pratica, contribuendo alla comprensione dei complessi cambiamenti che si verificano quando tali tecniche di modifica vengono applicate a matrici complesse come le farine.

Key words: Legumes flour; Pulsed electric fields, high pressure homogenization, chickpeas

1. Introduction

In accordance with the PhD thesis project previously described, this oral communication reports the main results of the following activities directed to:

A3) Apply and assess the use of high-pressure homogenization (HPH) for the functionalization of chickpea flour

A4 & A5) Assess the use of pulsed electric field (PEF) for the functionalization and subsequent production of end product from chickpea flour

2. Materials and Methods

Chickpea flour was treated using two different technologies as follows:

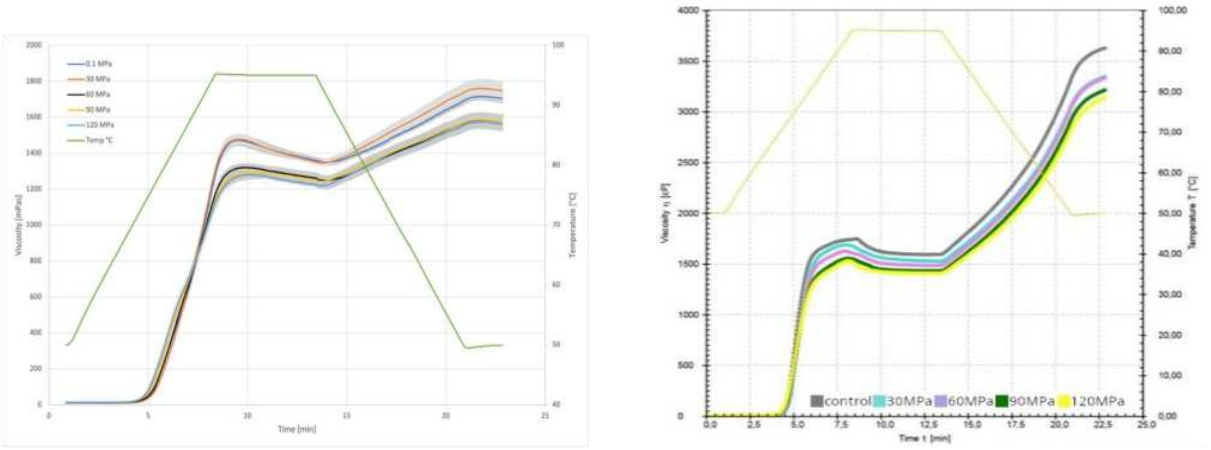
A3) The HPH treatments were performed on freshly prepared mixtures of flour and water at room temperature with native pH (6.55). To prepare the mixtures, 100 g (dry bases - db) of flour and 300 ml of distilled water were mixed for 1 minute at 8000 rpm using a homogenizer (T18 Digital Ultra Turrax, IKA, Germany) to ensure that no lumps were present. This high concentration was selected in order to operate with conditions closer to industrial applications (Zhu, 2021). The HPH treatment was performed with a single-pass at 30, 60, 90 and 120 MPa using a high-pressure homogenizer (PandaPLUS 2000, GEA Nitro Soavi, Italy). The tested conditions were selected as previous studies observed functional changes on similar products with pressures not exceeding 120 MPa (Huang *et al*., 2023; Ma *et al.*, 2023).
Afte the treatment samples were collected in a beaker kept in ice in order to minimize the thermal effects (exit temperature never exceeded 45°C), while the untreated dispersion was used as a control.

A4 & A5) Chickpea flour and distilled water were weighed (PBC 3500, Kern, Albstadt, Germany) in individual screw cups to obtain 100 g slurry with a flour concentration of 10±0.01% (wet basis, wb) and electrical conductivity of 2.15±0.08 mS/cm. To ensure flour hydration, all slurries were left to hydrate at 3.1±0.6 °C for 16 h prior to PEF treatment. PEF treatments were performed using a pilot scale PEF system (ELCRACK-HVP 5, DIL, Quakenbrueck, Germany) operated in batch mode using a chamber (10 cm length and 5 cm depth) with an 8 cm stainless-steel electrode gap. The system delivered bipolar square pulses with a constant pulse duration of 30 µs and frequency of 200 Hz, monitored through an oscilloscope (UT2025C, Uni-Trend, Dongguan, China). For each treatment, the slurry was gently shaken in the screw cup and transferred into the chamber (average treatment mass 98.41±0.22 g). Based on preliminary trials, two electric field strengths (1 and 2 kV/cm) were selected, based on the conductivity of the sample and the maximum current (100 A) that could be applied using said PEF generator. The application of PEF at specific energies exceeding 500 kJ/kg resulted in an in-chamber starch gelatinisation,

producing strong gels that could not be poured and formed undesirable clumps.
Conversely, PEF application at energies below 410 kJ/kg failed to form gels, with sedimentation observed over time, similar to what was observed in control samples. Thus, the following energies were selected for treatment: 410, 425, 450, 465, 485 and 500 kJ/kg. upon treatment gels were let to set in containers at 4°C for 24h before further assessment (Drudi *et al.*, 2025).
Assessments of the treated product were carried out in terms of functional, rheological and pasting properties. Moreover, specific physicochemical properties were also assessed on specific products such as FTIR, DSC, sulphydryl content, reducing sugar and total starch for flour treated with HPH and textural, while for product treated with PEF DSC, FTIR, *in-vitro* digestion and gel syneresis were also studied.

3. Results and Discussion

Regarding section A3, the initial treatment trials focused on understanding the feasibility of high-pressure homogenization (HPH) for legume flour modification. Specifically, a chickpea flour-water (1:2 ratio) slurry was prepared and treated at different pressures, namely 0, 30, 60, 90 and 120 MPa for one cycle. The results (Table 1) showed that some functional properties were significantly improved, namely solubility, WHC and OHC as observed also by other authors (Ma *et al.*, 2023). The viscosity profile was also affected (Fig. 1a), with an overall decrease in viscosity and a strong decrease in breakthrough viscosity. This result could mainly be explained by three phenomena: reduction in particle size, partial protein denaturation and partial starch damage. However, when evaluating the starch damage on the treated flours, the low values found (never more than 2%) indicate that the effects on the functional properties are likely not due to starch modifications due to the overall minimal degree of starch damage (below 5%). Protein denaturation, on the other hand, could be possibly one of the reasons behind the changes, since an increment of solubility and the changes in disulphyde bridges/free sulfuryls were observed, especially at a treatment pressure of 30-60 MPa. Nevertheless, other possible causes for the observed changes could be linked also to interaction between these components and fibers or fats, since flour is to be considered complex matrices comprising different macromolecules (Liu *et al.*, 2016). The results of this research are the subject of a manuscript currently submitted to a scientific indexed journal.



(a) ***(b)***

Figure 1 *Pasting profile of whole chickpea flour treated with HPH (a) and pasting profile of the extracted starch upon HPH pressure (b). Assessments were carried out at the same concentration, and 1 cP equals 1 mPas.*

Following this initial test, a more in-depth study was carried out. Specifically, aiming at isolating the components likely linked to the observed changes. For this purpose, the protein and starch fractions were extracted from the chickpea flour and then treated separately with HPH using the same parameters as in the previous trial. The control and treated samples were analyzed for the following properties: WHC, OHC, WAI, WSI and foam stability, pasting and rheological characterization. Unfortunately, it seems that the changes occurring on the protein isolation step limit the ability to detect a change after the HPH treatment, probably as a result of the denaturation induced by isoelectric precipitation. The effects on the starch fraction, on the other hand, are clearly visible in the pasting profiles (Fig. 1b). In fact, although maintaining it characteristic shape, all viscosity values are lowering proportionally to the pressure applied. This contrasts with what it is possible to see for the whole flour that experience an increase upon 30-60 MPa treatment.

Table 1 *Selected functional properties evaluated for the HPH treated chickpea flour.*

Sample	Control	30 MPa	60 MPa	90 MPa	120 MPa
WHC [g/g db]	1.11 ± 0.02 ^{bc}	1.06 ± 0.04 ^{c}	1.11 ± 0.01 ^{bc}	1.18 ± 0.04 ^{a}	1.35 ± 0.02 ^{a}
OHC [g/g db]	0.89 ± 0.02 ^{c}	1.09 ± 0.06 ^{a}	1.06 ± 0.06 ^{ab}	0.98 ± 0.02 ^{bc}	0.99 ± 0.01 ^{ab}
WAI [g/g db]	5.47 ± 0.07 ^{d}	5.59 ± 0.18 ^{cd}	5.83 ± 0.14 ^{bc}	6.14 ± 0.11 ^{ab}	6.46 ± 0.06 ^{a}
WSI [g/100g db]	39.95 ± 1.07 ^{b}	43.50 ± 0.38 ^{a}	42.70 ± 0.50 ^{a}	40.07 ± 0.07 ^{b}	39.44 ± 0.15 ^{b}
Foam capacity [%]	19.33 ± 1.15 ^{d}	26.00 ± 0.00 ^{c}	29.33 ± 2.13 ^{ab}	28.00 2.00 ^{bc}	32.00 ± 0.00 ^{ab}
Foam stability after 30 min [%]	86.30 ± 5.48 ^{a}	69.23 ± 7.69 ^{b}	63.69 ± 7.22 ^{b}	66.89 ± 6.06 ^{b}	68.75 ± 0.00 ^{b}

Different letters in each row indicate statistically significant ($p<0.05$) differences between samples.

Thus, it is likely that the improvement of viscosity (especially peak viscosity) is due to the effect of other components present in the flour, possibly proteins or the fibrous fraction that are also affected by the high-pressure treatment. Nevertheless, it is not possible to exclude possible interaction between components resulting in synergistic/antagonistic effects.

Finally, studies regarding section A4 and A5 were carried out during my period abroad at the University of Otago (New Zealand), where the feasibility of using pulsed electric field (PEF) as a technology to improve functional properties of chickpea flour was investigated. Upon few trials it seemed clear that due to the high conductivity of the mixture the PEF treatment inevitably induced sufficient heating in the mixture to induce starch gelation and possibly protein denaturation. Thus, being unfeasible to obtain a powdered product after treatment the interest shifted on producing soft gels with varying consistencies that could be suitable for dysphagia treatment, thus obtaining a final product to meet objective 5A.

Obtained results (Fig. 2) highlight that chickpea flour slurry (10% w/w) formed gels upon treatment with 1-2 kV/cm and specific energies above 410 kJ/kg. As specific energy increased so did hardness (0.35±0.01 to 2.94±0.19 kPa) and rheological properties such as storage modulus (195±42 to 2350±466 Pa) and yield stress (5.7±5.6 to 423.4±135.2 Pa) of the gels, obtaining products with textures spanning a range of International Dysphagia Diet Standardization Initiative levels (IDDSI), a commonly use standard to evaluate product applicability for dysphagia diet treatment. To better understand the gel structure and formation, Fourier transform infrared spectra (FTIR), light microscopy and differential scanning calorimetry (DSC) were used. Results showed that gel formation was mainly attributed to starch gelatinization (FTIR 1047/1021 ratio decreasing from 0.759±0.007 to 0.699±0.002) plausibly caused by a temperature increase due to the Joule effect during PEF, as suggested also by the absence of statistically significant differences between the 2 electric field tested. Protein denaturation and aggregation became more important in PEF-treated chickpea slurries above 450 kJ/kg, as those samples presented improved gelling strength although the DSC thermogram already showed a fully gelatinized starch.

Interestingly, although some literature works suggest that even at low electric fields (below 5 kV/cm) PEF treatment are able to induce some degree of structural changes in protein and starch molecules (Giteru *et al.*, 2018; Milanezzi and Silva, 2025), mainly as a result of molecule polarization and/or electrochemical reactions, results from the present work offer unclear answers. On one side, no clear significant differences in textural or rheological parameters were observed between the two field strengths (only specific energy was responsible for the modification observed, thus suggesting that the effect was mainly driven by Joule heating), while on the other, gels formed following treatment at 2 kV/cm led to an increase in readily digestible starch (from 16.28±0.15 to 89.06±1.78 %) and a faster intestinal protein digestion rate (from 1.16±0.20 $\times 10^{-2}$ to 1.65±0.04 $\times 10^{-2}$ min^{-1}) during simulated gastrointestinal digestion, with a clear effect of the electric in contrast with what was observed for rheological and textural properties. This different effect of the electric filed could be explained by the fact that the gel structure and hardness, although arising from the formation of a 3D network (comprising starch and protein molecules) is not affected by the possibly minor structural modification induced by the electric field, while digestibility properties might be more susceptible to such changes.

Overall, this study shows that PEF treatment could be used as a rapid method (44.4-227.4 ms) to produce gels with varying textural and rheological consistency using a single ingredient, e.g. chickpea flour, that could find an application in the development of dysphagia friendly products. Nevertheless, it is important to state the current needs of further studies especially regarding the applicability in the dysphagia sector as the IDDSI method used for the classification lacks objective parameters and needs to be coupled with more advanced techniques such as videofluoroscopic shallowing studies (VFSS). The result of this study is currently undergoing the review process for publication in an indexed journal.

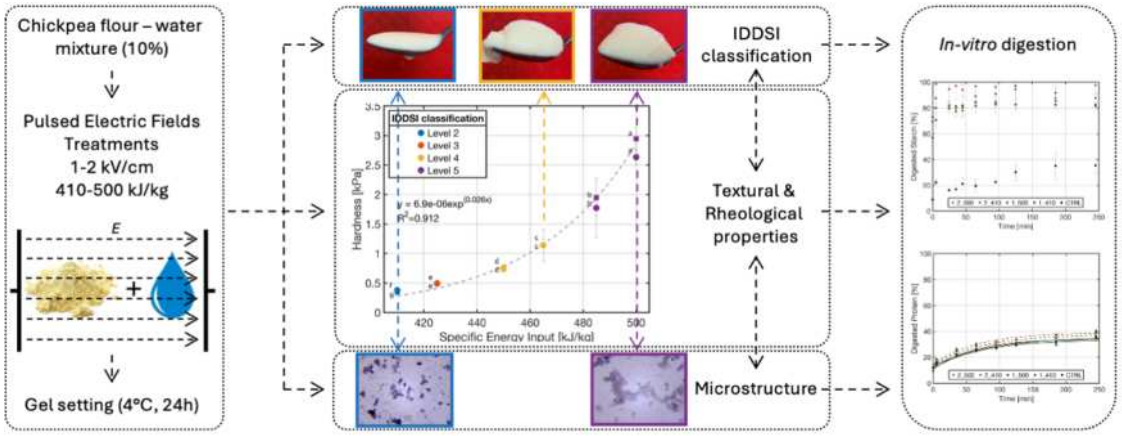


Figure 2 *Graphical abstract of the trial carried out using PEF. From left it can be seen the condition tested, the main structural properties of the obtained gels and finally the resulting effects on the in-vitro digestibility.*

4. Conclusions and Future Perspectives

Generally speaking, from all trials it emerges that the effects of the application of the studied treatments induce macroscopic effects on the flour that are the results of how different components (starch, proteins, fibers) are affected by the treatment.

As it was the case for HPH treatment, two general trends were observed, with some properties changing linearly as a function of pressure (such as WHC, WAI, FC, disuphide bonds, gelatinization enthalpy and damaged starch), while others peaked at about 30-60 MPa and then decreased at higher pressures (90-120MPa), such as OHC, WSI, solubility and pasting properties. The underlying mechanism behind these effects remains elusive, especially due to the presence of many different macromolecules in the flour, such as starch, proteins, fibers and lipids that can be affected by the treatment, inducing also eventual interaction. Generally, starch was found to be proportionally affected by pressure (decreasing gelatinization enthalpy and increasing damaged starch), resulting in a better interaction with water, which could result in an improvement of WHC and WAI proportional to the applied pressure. On the contrary, protein showed no changes in primary structure (SDS-PAGE) but possibly underwent partial unfolding and structural rearrangement (change in sulfhydryl groups and disulfide bonds), which allowed it to peak in OHC and pasting at a pressure of about 30 MPa.

Similarly, upon PEF treatment, the gel formation can plausibly be attributed to a combination of mechanisms: primary thermal effects induced by Joule heating, which led to starch gelatinisation, and structural modifications caused by PEF on starch and proteins. Joule heating, on one hand, facilitated rapid temperature increases that caused granule swelling and amylose leaching, forming the gel network (as suggested by the clear specific energy effect (e.g. higher energies increased starch gelatinisation and reduced the 1047/1022 ratio, thus improving the overall textural and rheological properties of the gel). On the other hand, electrochemical effects, such as molecular polarisation, likely contributed to enhanced macromolecular interactions and structural rearrangements between starch, protein and other macromolecules in the chickpea flour.

Next steps and future perspective will likely need to focus, for both technologies used, on the understanding if eventual synergistic or antagonistic interaction are present between the components upon the treatment. Specifically for PEF it will be interesting to understand the precise role of electrochemical effects, including protein denaturation and starch chain rearrangements on the functional properties of the gels. Particularly given that digestibility differences observed in chickpea gels between PEF treatments applied at different electric fields were not reflected in the rheological or textural properties of the gels.

Regarding HPH, further studies are still needed to fully understand the effects of HPH on the specific fraction of flour and their possible interactions. As an example, FC increased linearly with pressure, although protein solubility peaked at 30 MPa. Foaming capacity is often associated with the amphipathic nature of proteins, but the results obtained here suggest that other mechanisms also play a role in foam formation. Finally, the effects of incorporating modified ingredients into real food formulation need to be investigated to understand whether the observed modifications can translate into appreciable changes in the end consumer experience of some products.

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Impact of process factors and agri-food by-products on the oxidative and physico-chemical stability of oleogels

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This PhD thesis was focused on the development of oleogels with the aim of assessing the combined impact of process parameters on the physico-chemical and oxidative stability of oleogels (OGs), as well as the effect of using agri-food by-products (plant fibers or by-products as such) on the structural properties and oil loss (OL%) of final gels.

Impatto dei fattori di processo e dei sottoprodotti agro-alimentari sulla stabilità ossidativa e chimico-fisica di oleogels

Questa tesi di dottorato ha riguardato lo sviluppo di oleogels con l'obiettivo di valutare l'impatto dei parametri di processo sulla stabilità chimico-fisica e ossidativa degli OGs, nonché l'effetto dell'utilizzo di sottoprodotti agro-alimentari (fibre vegetali pure o sottoprodotti tal quali) sulle loro proprietà strutturali e di perdita di olio (OL%) dei gel finali.

Key words: oleogels, circular economy, up-cycling, sustainability, innovation, food formulation, by-products, vegetable fibers, brewer's spent grain.

1. Introduction

In accordance with the PhD thesis project previously described (Salvatori *et al*., 2024), this oral communication reports the main results of the following activities:

A1) investigation of the influence of processing parameters (different time-temperature ratios) on the OL% and oxidative stability of gels
A2) characterization of the oil-holding capacity (OHC) of bamboo and citrus fibers (BF and CF) and brewers' spent grain (BSG), and preliminary development of OGs (RBW-based) with plant fibers
A3) characterization of rheological, thermal and microstructural properties of OGs formulated with different concentrations of RBW and glycerol monostearate (GMS)
A4) evaluation of the effects of fiber addition on the following mixtures: RBW:fibers, GMS:fibers and three-component systems (RBW-GMS-FIBERS).

2. Current and potential oleogels applications in food industry

Oleogels (OG) are a class of gels that can be defined as bi-continuous systems composed of a vegetable oil immobilized and trapped within a thermo-reversible three-dimensional network of non-triglyceridic structuring agents (or oleogelators) held together by non-covalent interactions (*e.g*., hydrogen bonding, electrostatic and Van der Waals interactions) (Wijarnprecha *et al*., 2019). Nowadays, OGs represent one of the most studied and promising alternatives for replacing solid fats or "hard fats" usually rich in saturated and/or *trans* fatty acids.

3. Preliminary considerations

To design stable oleogels, it is important to choose the right oleogelator. Waxes are considered very promising as they possess a high structuring capacity, are low cost, and can be easily employed with direct dispersion approaches. However, high wax levels can impair sensory quality; the main design priority is therefore to improve the mechanical properties while minimizing the use of oleogelators. In this regard, dietary fibers are well known to be outstanding thickening agents in water-based systems. However, there are still few studies on their use in OG formulation, especially by direct dispersion. Regarding process parameters, the literature mainly focuses on cooling modes (slow or fast, static or dynamic), while the effects of temperature and holding time on OL% and oxidation remain largely unexplored. Consequently, reduced texture properties, oil release over time and limited oxidative stability among others, represent some of the main challenges still hindering a larger use of OGs in the food industry.

4. Experimental Procedure

In this PhD thesis, a three-step experimental procedure was set up by performing in sequence the following independent tests: i) preliminary assessments: investigation of the impact of different time-temperature conditions during the OG production on OL% and oxidation parameters such as peroxide value (PV), conjugated dienes and trienes (K_{232} and K_{268}), volatile organic compounds (VOCs) and oxidative stability index (OSI); ii) characterization

of the oil-holding capacity (OHC) of bamboo fibers (BF), citrus fibers (CF) and brewers' spent grains (BSG) with different particle sizes (as such, 125, 90 and 63 µm); preliminary formulation of OGs (RBW-based) with vegetable fibers (BF, CF and BSG) at three different concentrations (1, 3 and 6%) and four different particle sizes; iii) rheological, thermal and microstructural analyses of different OGs formulated with RBW and GMS, at different concentrations (4.5%, 6% and 10%), individually and together; iv) evaluation of the effects of fiber addition: analysis of RBW:fibers and GMS:fibers mixture, testing three concentrations of structuring agents (4.5%, 6% and 10%) with 1.5% fibers (BF90, BSG90 and CF125); analysis of three-component mixtures (RBW-GMS-FIBERS).

5. Materials and Methods

OGs were prepared by direct dispersion using a heating plate (HP) with a magnetic stirrer and equipped with a thermoregulator. After production, samples were placed in 20 mL vials, cooled to room temperature and then placed at 4°C for 24 h before further analysis. The oil binding capacity (OBC) of the gels (expressed as oil loss, OL%) was assessed by centrifugation of at 14000 rpm for 30 min (Blake and Marangoni, 2015). VOCs were analyzed by GC-MS (Tura *et al*., 2022). PV, K_{232} and K_{268} (methods NGD C35 and C40, respectively), and the OSI time (AOCS Official Method Cd 12b-92) were also determined. The rheological thermal and microstructural analysis were performed according to Basso *et al*. (2024) with slight modifications, using a controlled stress rheometer (Haake Rheostress 6000, Thermo Scientific, Karlsruhe, Germany) with a 35-mm parallel-plate geometry system, DSC-3 Star^e system (Mettler-Toledo, Greifensee, Switzerland) and a polarized light microscopy (PLM). Two independent batches were prepared for all the tested OG formulations. The results are reported as averages of 2-3 independent replicates for each batch. All the data were subjected to analysis of variance (one- or two-way ANOVA, depending on the experiment). In order to identify significant differences among samples, the Tukey HSD post-hoc test was applied (p<0.05).

5. Results and Discussion

5.1 Preliminary assessments: evaluation of the impact of process conditions on OL% and oxidation parameters

Different OGs formulations containing both RBW (4.5%) and GMS (1.5%) were prepared by applying 4 different time/temperature conditions: for low temperature coupled to short/long time (LS and LL) samples were prepared at 80°C for 10 and 30 min of holding time, respectively; for high temperature and short/ long time (HS and HL), samples were prepared at 90°C for 10 and 30 min of holding time. The gels were left cooling at room temperature for 1 h and then left at 4°C for 24 h before further analysis. The OL% and oxidative parameters were evaluated right after their production. The results of the average trend of the oxidation parameters and OL% for each treatment are reported in Figure 1.

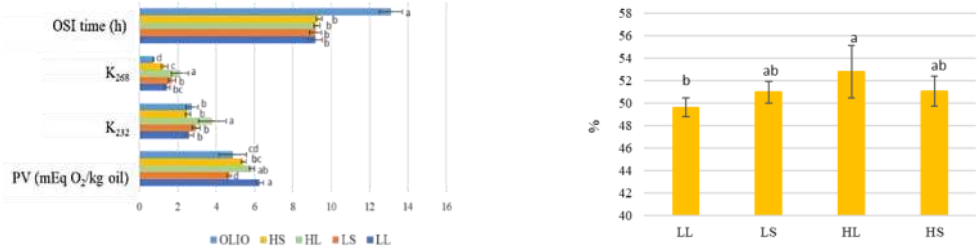


Figure 1 *Average trend for oxidation parameters and OL% based on the treatment (LL, LS, HL, HS).*

The LL treatment showed the highest PV (≈6 mEq O_2/kg), whereas LS displayed the lowest one (≈5 mEq O_2/kg). This could be due to the fact that, at lower temperatures and extended heating, the accumulation of hydroperoxides (ROOH) tends to be favored, whereas at higher temperatures their decomposition is promoted, yielding to secondary oxidation products (Choe and Min 2006). Accordingly, HL displayed the highest conjugated trienes (K_{268}) value, suggesting an accelerated ROOH breakdown. Moreover, also K_{232}, a primary-oxidation index that reflects conjugated-diene formation in the earliest oxidation stage, was significantly higher in the HL samples. This confirms that high temperatures and prolonged exposure times accelerate the onset of lipid-oxidation induction. Regarding OSI time, it was significantly lower in all OGs compared to the starting oil, with an average reduction of ≈ 4 h. VOCs analysis revealed carboxylic acids as the predominant class, followed by aldehydes, alcohols, ketones and hydrocarbons.

5.2 Characterization of the OHC of three vegetable fibers and preliminary OG-formulation tests (RBW-based)

All the OGs formulated in these preliminary tests included only RBW as structuring agent. Figure 2 shows the average OHC of each fiber with various particle sizes. From these results, it seemed that BF was the most performant fiber in "holding" oil. However, the effect of fiber addition on OL% of OGs were strictly linked to the

fibers' concentration and particle size. In the formulation of OGs, BSG and BF were the most effective at 1%, while BF and CF performed better at higher concentrations (3%). In general, at 6%, CF and BF generated slight improvements in OL%, while BSG's performance worsened considerably. Figure 3 shows the optimal particle size for each fiber: at 1% of fiber concentration the best were BSG90, BF90 and CF125, whereas at 3% were BSG90, BF63 and CF125.

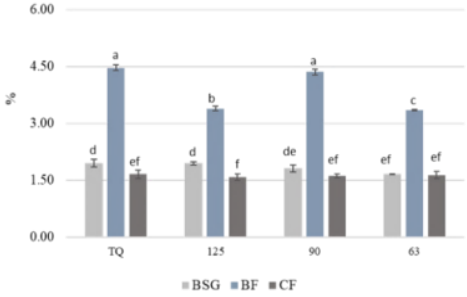


Figure 2 *OHC (%) for each fiber and grain size.*

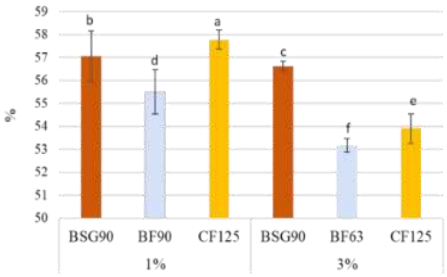


Figure 3 *OL (%) of OGs based on the best particle size.*

5.3 Rheological, thermal and microstructural analyses of OGs with different RBW and GMS concentration

Figure 4 shows the results of thermal (melting enthalpy or ΔH, transition peak and onset temperatures or T_{peak} and T_{on}), rheological (storage modulus or G') and OL% properties of the analyzed OGs. Overall, GMS performed better than RBW for OGs formation. In fact, 4.5% GMS-based OGs showed a G' of 10.3 kPa, ΔH of 8.0 J/g and an OL% of ≈28%, which could be due to the crystal morphology as explained below. On the other hand, RBW-based OGs, at the same concentration, exhibited limited stability over time, with structural failure phenomena, thus making it impossible to perform rheological analysis; in addition, they had a lower ΔH (4.7 J/g) and a higher OL% (≈50%). When increasing the dose at 6%, RBW managed to form a stable gel, but showed elastic and thermal properties even lower than GMS (G'=1.9, *vs* 14.5 kPa; ΔH= 7.9, *vs* 9.5 J/g), thus suggesting greater amounts and order of crystals in the network formed by GMS. When the wax concentration was 10%, a reversal trend was observed, where OGs with RBW and GMS reached a G' of 64.8 kPa and 51.2 kPa, respectively. However, despite melting 6-8°C earlier (T_{peak} ≈ 63°C) than the RBW network, the GMS network exhibited higher ΔH (17.7 J/g), thus further confirming the greater organization of the crystalline network, as evidenced by its greater stability and lower OL% (< 1 %). These results are also supported by microstructural analysis, where PLM images showed a platelet-like morphology of the crystals (RBW), with star/rosette lamellar aggregates. In addition, the crystals were more widely spaced and less homogeneously dispersed, resulting in greater network porosity and lower OHC over time (Blake and Marangoni, 2015a and 2015b). In contrast, GMS showed self-organized crystals in an inverted lamellar phase, constituting a continuous network of micro-needles grown from a nucleation center, densely intertwined, and uniformly distributed. This structure had very small black spaces between bundles, indicating a compact three-dimensional network with a high specific surface area (Prakansamut *et al*., 2024).

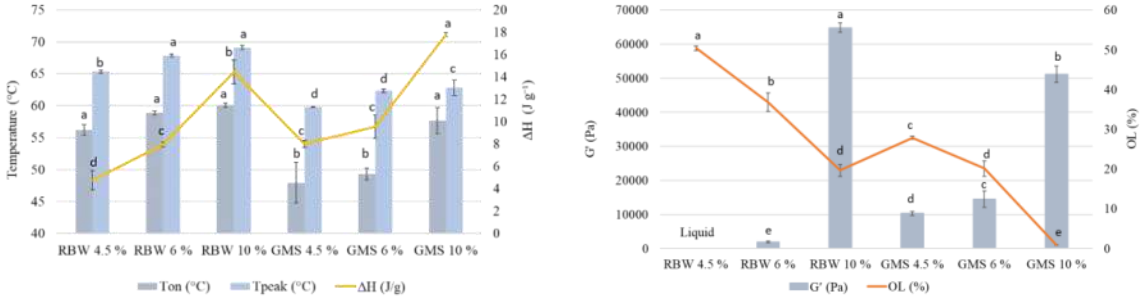


Figure 4 *Rheological, thermal and OL% analysis of OGs with 4.5, 6 and 10% of RBW and GMS.*

When mixed, the GMS behavior seemed to be strongly affected by the presence of RBW. In fact, systems with equal concentration of both components (2.25:2.25) showed that a low presence of GMS (2.25%) favored system structuring, with a G' of 2.4 kPa, higher ΔH values and significantly lower OL% than the 4.5% RBW-based OGs. Even in systems consisting of 6% of total oleogelators, OGs with RBW:GMS (1:3 ratio) showed a drop in G' to 8.3 kPa compared with 6% GMS-based OGs (G'=14.5 kPa). This could be due to the lamellar morphology of RBW crystals, which might have acted as break points in the dense network of needle-like crystals of GMS, thus reducing overall network strength. However, the presence of GMS in the blend resulted in a higher ΔH of melting and a significantly lower OL% than the 6% RBW-based system, confirming the higher oil structuring efficiency of GMS in the present work. The 1:3 (RBW:GMS) blend showed G' and OL% similar to the control (GMS only),

whereas the 3:3 blend decreased G' and increased OL%, further demonstrating the negative effect of RBW on the GMS network. Even at 10% of total oleogelators, the 5:5 blend resulted in a significantly lower G' than both 10% GMS-based OGs and 10% RBW-based OGs; however, it displayed a higher ΔH and a lower OL% than the 10% RBW-based systems, but an opposite trend than the 10% GMS-based OGs. These behaviors confirmed the better overall structuring efficiency of GMS.

5.4 Effects of fibers addition (BSG90, BF90, CF125)

Figure 5 reports the results of the addition of 1.5% fibers (BF and CF) or BSG to OG formulations containing 4.5, 6 or 10% total structuring agent (RBW, GMS, or tri-component RBW-GMS-fiber mix). Fiber addition to the 4.5% RBW-based OGs improved their structure, since rheological tests revealed a stronger physical network (especially with BF), which raised G' and lowered OL%. In contrast, when GMS was used, fibers appeared to have the opposite effect, as they reduced G' and markedly increased OL% compared with the control (4.5% GMS-based OGs). Overall, the thermal properties remained essentially unchanged in all formulations containing fibers or BSG, since the fibers themselves did not directly induce structuring or crystallization but simply provided mechanical support. The only exception was the slight variation in ΔH recorded for the 4.5% RBW:1.5% fibers OGs compared with the control. This deviation could arise from their higher wax-to-fiber mass ratio (0.33 g of fiber/g of oleogelator) with respect to other systems (0.25 g/g and 0.15 g/g for 6% and 10% RBW-1.5% fibers-based OGs, respectively), in the presence of the minimum concentration required to RBW to obtain an OG (4.5%). Therefore, under these conditions, the effect of fiber dilution (ΔH decrease) or mechanical anchorage points for crystals under formation (ΔH increase) could be more easily detected by DSC analysis.

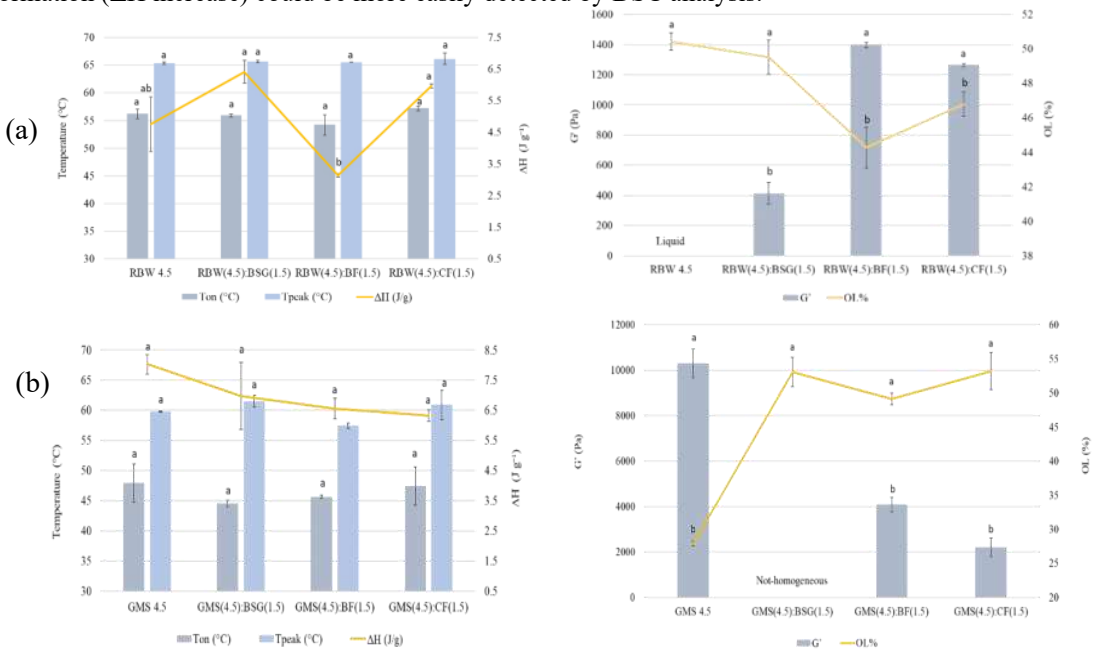


Figure 5 *Rheological, thermal and OL% analysis of OGs with fibers (BF, CF, BSG) and 4.5% of total RBW (a) or GMS (b).*

In the case of OG with 6% oleogelators (Figure 6), the addition of fibers confirmed a significant positive effect on RBW-containing formulations, with the best effect on G' recorded by BSG, which increased from 1.9 kPa (control) to 10.1 kPa. In the case of GMS, the addition of BSG and CF had a worsening effect on G' compared to 6% GMS-based OGs (control), while it showed a significant positive effect in the presence of BF, doubling G' and significantly reducing OL%. These differences could be due to the different concentration of soluble fiber present in the fibers used; in particular, the polar fractions (free hydroxyl groups) of GMS could create hydrogen bonding with hydroxyl, carboxyl or carboxylate groups of CF and BSG soluble fractions. Considering that BSG contains about 20% of protein, the side chains of some amino acids could expose their hydroxyl groups, which could potentially interact with GMS. These interactions could reduce the amount of structuring agent involved in the formation of the crystal network, thus resulting in a weakened network, decreased G' and increased OL%. In contrast, BF is known to possess more than 90% insoluble fiber and only ≈2% soluble fiber (Principato *et al.*, 2021), so it would act exclusively as a support for nucleation and crystal accretion of GMS, with positive effects on G' and OL%. In fact, even in the PLM images, it could be observed that the GMS crystals anchored themselves around the BF fibers, keeping them interconnected. When the oleogelators reached 10%, a reversed trend was observed, with positive effects on GMS-based OGs and negative or no effects on RBW-based OGs. BSG and BF significantly increased G' (doubling and tripling it, respectively) of GMS-based OGs, also maintaining positive effects on OL%. In contrast, the addition of CF penalized both G' and OL %. This is probably due to a potentially

high presence of pectin; in fact, the numerous -COOH/-COO- groups make the surface of the fibers more hydrophilic, thus hindering its oil wettability and thus its capillary absorption. In parallel, an adsorption of the glyceryl heads of the GMS could occur, resulting in a reduction of the crystallization and density of the GMS crystal lattice. In the three-component mixes, the presence of fibers had worsening effects at 4.5% and 6%, while no effect or slightly improving effects were noted at 10%. In the former case (crystalline network not densely structured due to the co-existence of GMS and RBW), the presence of fibers might have generated a prevalence of negative effects due to dilution factors and physical disruption of the network. In contrast, with increasing concentrations of oleogelators, the fibers appeared to have a potentially "filling" effect, with no or slightly enhancing effects on G' and OL%.

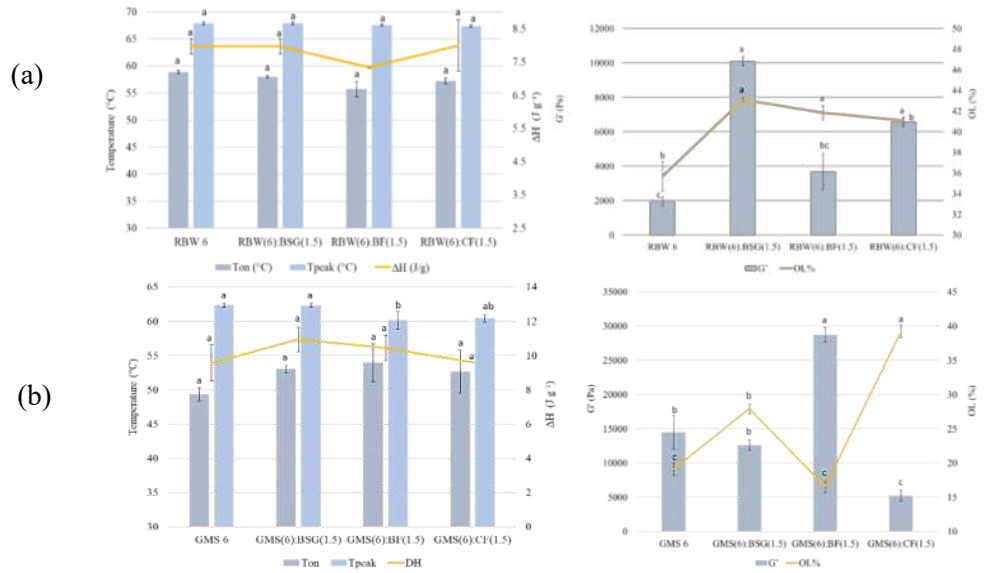


Figure 6 *Rheological, thermal and OL% analysis of OGs with fibers (BF, CF, BSG) and 6% of total RBW (a) or GMS (b)*

6. Conclusions and Future Perspectives

The present study suggested that process variables strongly influence both physico-chemical and oxidative stability of OGs and that vegetable fibers can be promising ingredients to improve the rheological performance and the OBC of the final gels. However, their benefits depend on the botanical origin and granulometry of the vegetable fibers, the type/level of oleogelator and the presence of more structuring agents in the oil phase. To foster the maturation of OG technology and provide a scalable alternative to saturated or hydrogenated fats, future research should: (i) elucidate fiber-lipid interactions; (ii) assess mechanical stability; (iii) evaluate the sensory, thermal and rheological behavior of the best prototypes in real food matrices; (iv) perform oxidative and microbiological shelf-life tests.

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Development of Plant-Based Cheese Analogues: The Role of Ingredients and Processes

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This PhD project contributes to the understanding and improvement of plant-based food alternatives. It begins by examining existing plant-based cheese analogues on the Italian market, assessing how well they compare to dairy cheese. The study then investigates key ingredients, looking at how lactic acid fermentation affects the functional properties of plant proteins and how different starches influence the texture and melting behavior of formulations. This work helps identify current challenges and provides practical information to guide the development of better plant-based cheese alternatives.

Sviluppo di Analoghi Vegetali del Formaggio: Il Ruolo di Ingredienti e Processi

Questo progetto di dottorato contribuisce alla comprensione e al miglioramento delle alternative alimentari a base vegetale. Inizia esaminando gli attuali analoghi del formaggio a base vegetale presenti sul mercato italiano, valutandone il confronto con il formaggio tradizionale. Lo studio prosegue indagando ingredienti chiave, analizzando come la fermentazione lattica influenzi le proprietà funzionali delle proteine vegetali e come diversi amidi incidano sulla consistenza e sul comportamento di fusione delle formulazioni. Questo lavoro aiuta a identificare le sfide attuali e fornisce informazioni pratiche per guidare lo sviluppo di migliori alternative vegetali al formaggio.

Key words: plant-based; Cheese-analogues; alternative proteins; food formulation; lactic acid fermentation

1. Introduction

In accordance with the PhD thesis project previously described (Fenga, 2023), this oral communication reports the main results of the following activities directed to:

A1) Physico-chemical, Functional and Sensory characterization of plant-based cheese analogues present on the Italian market
A2 Effect of lactic acid fermentation on the techno-functional properties of plant-based proteins
A3) Effect of different starches on the properties of starch-based gels and plan-based cheese analogues

2. Physico-chemical, Functional and Sensory characterization of plant-based cheese analogues present on the Italian market

2.1 State of the art

The growing demand for plant-based alternatives to animal-derived foods has led to an increase in the availability of cheese analogues, particularly those mimicking mozzarella. However, these products often fail to replicate the functional and sensory properties of dairy cheese. Previous studies have highlighted poor melting, stretchability and sensory acceptance in plant-based cheese analogues (Grasso et al., 2021; Falkeisen et al., 2022). However, there is a lack of work that combines functional, structural and sensory evaluation in mozzarella-like products. This study aimed to address this gap by analyzing products currently available on the Italian market in comparison with two dairy references to identify critical weaknesses and support future formulation improvements

2.2 Material and Methods

Physicochemical and structural analyses included moisture content, pH, oscillatory rheology, differential scanning calorimetry (DSC), low-field ^{1}H NMR and texture profile analysis. Meltability, oiling-off, and stretchability tests were also conducted to assess their functionality during cooking. Sensory acceptability and Just-About-Right (JAR) attributes were combined on the samples melted on pizza to identify their penalizing attributes. The study evaluated five commercial pizza products samples available on the Italian market, including two dairy references (D1, D2) and three plant-based mozzarella cheese analogues (PB1, PB2, PB3). All samples were analyzed in triplicate. Moisture content was determined by oven drying, and pH was measured using a digital pH meter. Differential Scanning Calorimetry (DSC) was used to investigate thermal transitions during heating. Structural characteristics and water distribution were assessed through low-field ^{1}H Nuclear Magnetic Resonance (NMR) using CPMG sequence. Rheological behavior was studied by small amplitude oscillatory rheology analyzing the samples in their raw samples and during a temperature program. Textural properties were assessed through Texture Profile Analysis (TPA). Meltability, oiling-off, and stretchability were also evaluated. Color before and after cooking was acquired according to the CIELAB color space and browning was expressed as total color difference caused by cooking ($\Delta E_{browning}$). Sensory evaluation involved 50 untrained consumers who assessed melted samples on pizza using a 9-point hedonic scale for overall liking. Specific attributes such as stretchability, flavor, and browning were evaluated using Just-About-Right (JAR) scales, and penalty analysis was applied to identify the main penalizing attributes. All the analyses, except for those that require baking or temperature ramp, were

conducted at +25°C. Statistical analysis was conducted using one-way ANOVA with Tukey's post-hoc test for multiple comparisons ($p < 0.05$).

2.3 Results

The results highlighted notable differences between plant-based and dairy cheese for all the investigated properties.

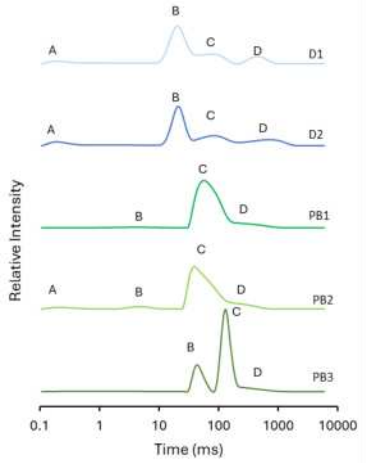


Figure SEQ Figure * ARABIC 1 *^1H T1 NMR relaxation curves of commercial*

D1 and D2, the two dairy samples, exhibited similar NMR relaxation curves (Figure 1), showing four distinct proton populations, in line with previous findings on mozzarella-type cheeses (Alinovi et al., 2020; Gianferri et al.). Their DSC profiles displayed broad endothermic peaks between 20 and 50 °C, mainly attributed to the melting of lipid fractions. Rheological tests confirmed a thermo-reversible viscoelastic transition with a crossover in tanδ values around 75 °C, associated with casein network melting (Lucey et al., 2003). In sensory analysis, both samples achieved high overall acceptability. PB1 showed a dominant NMR population (~40 ms), indicating a matrix with immobilized water associated with biopolymers. Its DSC thermogram displayed a low-temperature endothermic peak (26–29 °C), likely related to lipid melting, and a second event at higher temperature, possibly due to agar transitions (Khoobbakht et al., 2024). The rheological profile was stable upon heating, with no crossover observed and only partial recovery after cooling. PB1, rich in hydrocolloids, had the lowest hardness and the highest meltability among plant-based samples, but was associated with low stretchability. PB2, based on chickpeas and high amylopectin starches, exhibited a more complex structure. Its NMR profile showed an additional short T_{2A} component (~2 ms), possibly indicating tightly bound water (Luo et al., 2020). DSC analysis revealed two overlapping peaks between 53 and 72 °C, potentially linked to starch retrogradation peak (Yuan & Thompson, 1998). PB2 showed the highest hardness at 25 °C, but an increase in tanδ during heating (Figure 2), with no recovery upon cooling, probably caused by the melting of retrograded amylopectin (Yuan & Thompson, 1998).

The sample PB3, formulated with cashew and almond preparation, showed in NMR a T_{2D} population at higher relaxation time (>200 ms) compared with the other plant-based samples, indicating the presence of more mobile water. The DSC curve displayed a single low-temperature peak (26–29 °C), corresponding to lipid melting. From a rheological point of view PB3 maintained its structure through heating and cooling, without relevant variation in G′ and G. Among the PB samples, it had intermediate hardness and moderate stretchability.

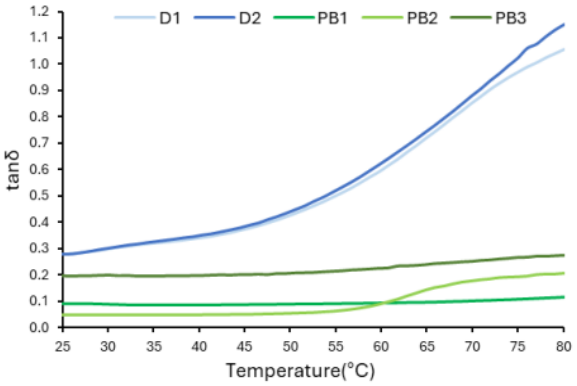


Figure SEQ Figure * ARABIC 2 *Temperature dependance of tanδ for the commercial mozzarella-like products samples*

Sensory evaluation revealed a clear preference for dairy samples (≈6.9), while plant-based analogues scored below 5.0, confirming previous findings on consumer rejection of plant-based cheese alternatives (Falkeisen et al., 2022). PB3 was the most accepted among the plant-based samples. For plant-based samples more attributes were reported on non-JAR levels with many identified as penalizing, with too low level of perceived stretchability identified as penalizing for all the plant-based products.

Table SEQ Table * ARABIC 1 *Oiling-off, meltability, stretchability, texture parameters and browning of commercial*

	Oiling-off [%]	Meltability [%]	Stretchability [mm]	Hardness [N]	Cohesiveness	Springiness [%]	$\Delta E_{browning}$
D1	181.68±27.21 c	190.45±10.02 bc	148.82±1.00 a	25.59±2.98 b	0.53±0.04 a	78.45±8.95 a	26.45±3.00 a
D2	254.57±22.63 b	167.26±23.35 c	148.68±1.84 a	11.10±1.61 c	0.52±0.03 a	74.77±5.75 a	15.90±3.05 c
PB1	0.06±0.05 d	252.41±14.65 a	7.71±1.21 c	5.97±0.22 e	0.20±0.02 b	41.67±5.46 bc	9.24±0.69 d
PB2	32.15±12.23 d	154.13±11.54 c	67.24±10.39 b	30.36±3.63 a	0.11±0.03 c	51.70±11.97 b	22.31±2.01 b
PB3	459.71±43.42 a	224.53±31.86 ab	74.52±21.42 b	7.47±0.62 d	0.24±0.01 b	28.52±2.39 c	17.00±8.15 c

3. Effect of lactic acid fermentation on the techno-functional properties of plant-based proteins

3.1 State of the art

Utilization of plant proteins is not always straightforward; some strategies have been studied over time to modify their sensory, nutritional, and technological properties, including fermentation (Nasrabadi et al., 2021).

The aim of this work was to investigate the techno-functional properties of selected plant-based proteins and lactic

acid fermentation effect on their modification.

3.2 Material and Methods

Pea protein isolates (80% protein) were of commercial origin while pumpkin proteins (74% protein) were extracted by alkaline extraction preceded by ultrasound pre-treatment, provided by a partner of the project. Fermentation was performed at 37°C for 18h with a strain of Lactobacillus delbrueckii subsp. bulgaricus (2214). It was selected on the base of a preliminary screening (data not shown) of the growing performances and ability to modify the sample viscosity. The fermentation was conducted on protein dispersion concentration of 20%w/w (pumpkin protein), or 10%w/w (pea proteins) added with 2%w/w saccharose. Techno-functional properties were investigated two different pH on dried powder. Solubility was measured on protein dispersions (2%w/w), expressed as percentage of total solid weight in the supernatant on the initial weight of the powder. WAC and OAC were expressed as weight (g) of absorbed water (or oil) to the weight (g) of powder. Emulsifying capacity was expressed as EAI and ESI, measured according to Tang et al. (2023) with slight modifications. Foaming was analyzed on 1%w/w protein dispersions, expressed as Foam Capacity (Percentage of difference between foam volume and initial volume on the initial volume) and Foam stability (ratio of volume of foam after 30 min to the one of fresh foam). Rheological behavior was also investigated by small amplitude oscillatory rheology.

3.3 Results

The results showed that solubility was generally lower for all protein samples at pH 4.5, regardless of the source. Fermentation did not appear to have any effect on this parameter.

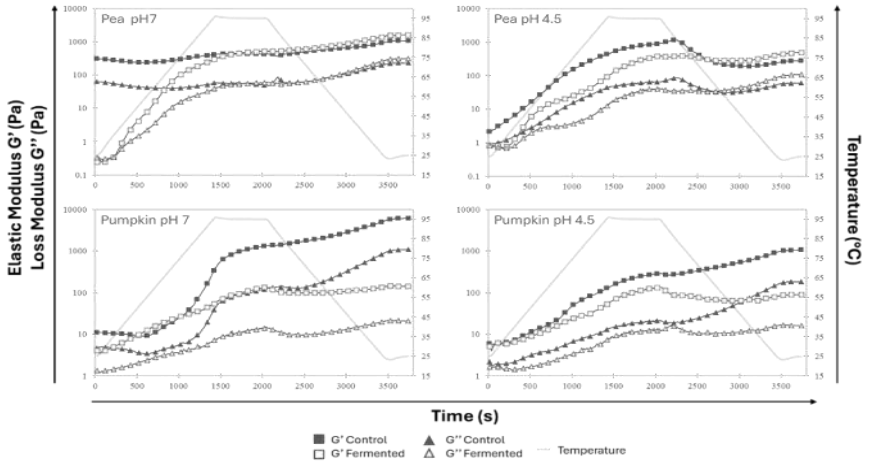
Table 2 Effect of pH and of Fermentation treatment on tehcnofunctional properties of pea proteins

Treatment	pH	Solubility (%)	EAI (m^2/g)	ESI (%)	FC (%)	FS (%)	WAC(g/g)	OAC(g/g)
Control	4.5	22.1±0.6 bc	12.4±1.0 a	52.3±1.7 a	64.0±6.9 a	14.8±4.6 b	2.3±0,09 a	1.43±0,04 a
Control	7.0	26.8±1.7 a	13.6±1.5 a	86.3±16.5 a	60.0±6.9 a	44.7±4.7 a		
Fermented	4.5	19.9±1.4 c	6.7±1.7 b	59.7±30.7 a	30.7±2.3 b	0.0±0.0 c	2.40±0,00 a	1.34±0,02 b
Fermented	7.0	25.8±1.9 ab	14.3±1.4 a	78.6±9.5 a	41.3±2.3 b	51.5±2.6 a		
Variable					**Significance**			
treatment		0.099	0.015	0.989	0.000	0.087	0.118	0.025
pH		0.000	0.001	0.035	0.296	0.000	/	/
treatment * pH		0.492	0.004	0.491	0.039	0.001	/	/

Table 3 Effect of pH and of Fermentation treatment on tehcnofunctional properties of pumpkin seed proteins

Treatment	pH	Solubility (%)	EAI (m^2/g)	ESI (%)	FC (%)	FS (%)	WAC(g/g)	OAC(g/g)
Control	4.5	20.7±0.9 bc	3.9±0.5 c	143.3±53.8 a	0.0±0.0 b	0.0±0.0	1.74±0.04 a	2.15±0.04 a
Control	7.0	27.9±2.7 a	15.7±0.1 a	23.5±1.2 b	0.0±0.0 b	0.0±0.0		
Fermented	4.5	19.2±1.5bc	5.1±0.7 c	83.7±3.4 ab	0.0±0.0 b	0.0±0.0	1.75±0.03 a	2.02±,0.11 a
Fermented	7.0	28.1±1.1 a	6.9±0.6 b	82.9±11.6 ab	133±2.3 a	0.0±0.0		
Variable					**Significance**			
Treatment		0.523	0.000	0.992	0.000	/	0.773	0.131
pH		0.000	0.000	0.012	0.000	/	/	/
Treatment * pH		0.401	0.000	0.013	0.000	/	/	/

Fermentation negatively affected the foaming capacity of pea proteins, while pumpkin protein samples showed no foaming at all, except for the fermented sample at pH 7, which exhibited a weak but measurable foaming ability. Emulsifying properties were generally more favorable at pH 7 for both protein types. However, fermentation led to a marked reduction in emulsifying activity for pumpkin proteins at pH 7 and for pea proteins at pH 4.5.



Regarding water and oil absorption capacities, pea proteins showed a higher ability to absorb water than oil, while the opposite trend was observed for pumpkin proteins, no effect of fermentation was observed on pumpkin seed protein, but a slight increase was observed in the OAC of fermented pea proteins. The effect of fermentation

can be attributed to hydrolysis, and changes in secondary and tertiary structures (Emkani et al.,2022). Moreover, different gelling abilities in the fermented proteins have been proved by oscillatory rheology (Figure 3), showing less intense hardening phenomena during heating in pumpkin seed protein. This effect can be attributed to acidic irreversible coagulation of proteins during fermentation or the modification of their structure by microorganisms.

4. Effect of different starches on the properties of starch-based gels and plan-based cheese analogues

4.1 State of the art

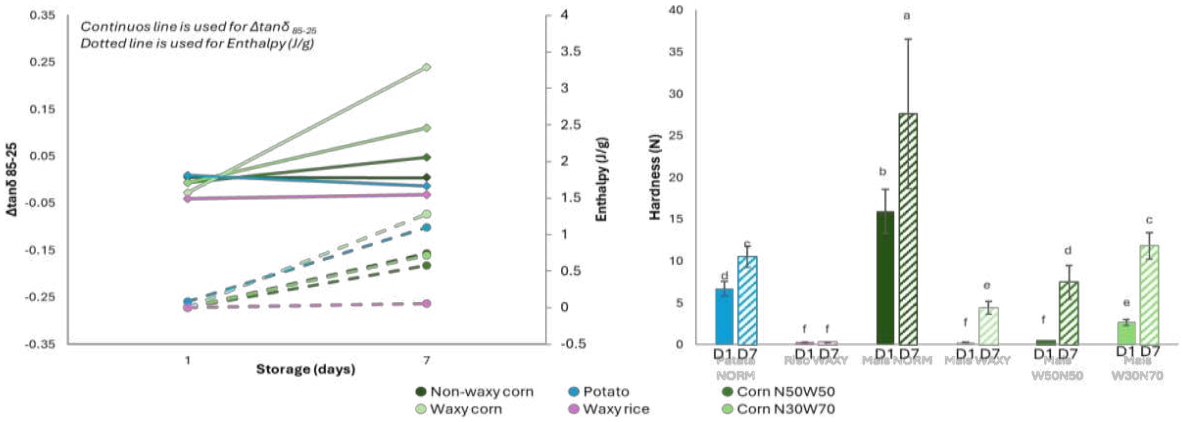
Key characteristics of plant-based cheese analogues often include firmness in the raw state and the ability to melt and stretch when heated. Since plant proteins can't replicate the properties of dairy caseins, starches and hydrocolloids are often added. However, previous work managed to obtain a high percentage of inclusion of protein through its combination with waxy corn starch and coconut oil, containing a cheese with good meltability and stretchability properties (Dobson & Marangoni 2023). Aged amylopectin has previously demonstrated the ability to melt (Yuan & Thompson, 1998). Moreover, some studies have explored the melting properties conferred by different starches in plant-based cheese analogues (Yun et. Al, 2024). However, a direct comparison of waxy corn starch with other starches over time, and their impact on the textural and thermo-rheological properties of both starch gels and cheese analogues, is poorly explored and is investigated in this work.

4.2 Material and Methods

Gels from different starches and their combinations were observed over seven days, and selected combinations were tested in protein-rich plant-based cheese analogue formulations and evaluated through DSC, oscillatory rheology, and textural analysis (TPA). For the starch gels, starch from waxy and non-waxy corn, waxy rice and potato were utilized. Moreover, waxy and non-waxy corn were combined in the two following proportions: 30% non-waxy/70% waxy (Corn N30W70) and 50% non-waxy/50% Waxy (Corn N50W50). The starch gels were produced according to previous author (Lyu et al., 2023) with some modification by mixing starch (20%) with phosphate buffer at pH 5.5 (80%). The two types of corn starches and their combinations were also tested in the plant-based cheese analogue prototype that were produced according to Dobson & Marangoni (2023).

4.3 Results

Significant differences were observed among the different starches, with waxy corn starch showing increasing meltability (represented here by Δtanδ) over time, possibly influenced by the development of amylopectin crystallites during storage. However, waxy rice starch exhibited non-significant retrogradation with lower meltability, underlying the importance of botanical origin on the properties of starch as an ingredient. Non-waxy corn and potato starches formed firmer gels with poor meltability, suggesting a role of amylose in limiting melting



and maintaining structure, even in the presence of amylopectin retrogradation (as observed by DSC).

In cheese analogues, waxy corn starch showed low hardness (data not shown) and elastic modulus, and all the samples showed poor melting. However, aged waxy corn starch after 7 days showed comparable firmness compared to normal corn starch and conferred good melting with an increasing decrease of the elastic modulus with an increasing amount of waxy starch in the formulation. This study provides insights into the role of starch retrogradation in modulating

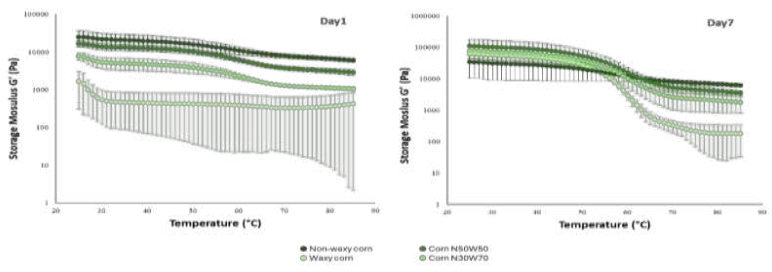


*Figure SEQ Figure * ARABIC 5 Elastic modulus G'as a function of the temperature in Plant Based Cheese analogue with varying proportions of waxy and non-waxy corn starch*

functional properties, addressing the design of plant-based cheese alternatives with improved performance.

5.Conclusions and Future Perspectives

In conclusion, these activities provide a comprehensive overview of the current landscape and future potential of plant-based food analogues. The initial characterization of commercial plant-based cheese analogues highlighted significant challenges, particularly their inability to replicate the desired texture and cooking properties of dairy products and confirmed a lack of consumer acceptance due to limitations in texture, flavor, and especially stretchability, underlying the structural difficulties in mimicking casein functionality with plant-based ingredients. Moreover, it provided insights into the effect of different formulations on the properties of the products. Regarding ingredient modification, the investigation into lactic acid fermentation revealed a protein type-dependent impact on techno-functional properties like foaming, oil absorption capacity, and gelling ability, although it did not produce statistically significant improvements in solubility or water absorption capacity. These observed effects are likely attributed to partial protein hydrolysis and structural modifications induced by the fermentation process. Furthermore, the work demonstrated the influence of starch botanical origin and retrogradation on meltability and texture in both gels and cheese analogues. Notably, waxy corn starch showed promising melting behavior after storage, indicating its potential for improving cheese-like functionality. Future efforts must focus on optimizing formulations by combining modified proteins and starches and further exploring how processes like fermentation can be explored not only for functional modification but, possibly, to enhance the sensory profiles of plant-based ingredients, leading to products with improved performance and consumer acceptance.

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Exploring the physical, structural and technological properties of ball-milled starches from different origins from granules to porous gels

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This PhD thesis investigates how ball milling (BM) treatment time (5, 15 and 30 minutes) modulates the physical, structural, and techno-functional properties of starches from diverse botanical origins. It further examines the utilization of these mechanically modified starches for development of gels. Comprehensive characterization of starch powders was carried out including physicochemical, techno-functional and microstructural characteristics. Additionally, the ball-milled starches were used to develop structured systems such as hydrogels, aerogels and cryogels with enhanced functionalities, and their technological performance was systematically evaluated.

Dai granuli ai gel porosi: esplorare le proprietà fisiche, strutturali e tecnologiche degli amidi macinati a sfere di diverse origini

Questa tesi di dottorato indaga come il tempo di trattamento (5, 15 and 30 minuti) della macinazione a sfere (BM) modula le proprietà fisiche, strutturali e tecno-funzionali degli amidi provenienti da diverse origini botaniche. Esamina inoltre il potenziale di questi amidi modificati meccanicamente per lo svilupo di gel. È stata effettuata una caratterizzazione completa dei campioni, comprese le caratteristiche fisico-chimiche, tecno-funzionali e microstrutturali. Inoltre, gli amidi macinati a sfere sono stati utilizzati per sviluppare sistemi strutturati come idrogel, aerogel e criogel con funzionalità avanzate e le loro prestazioni tecnologiche sono state valutate sistematicamente.

Key words: Starch; ball milling; techno-functional properties; aerogels;

1. Introduction

In accordance with the PhD thesis project previously described (Boateng, 2024), this oral communication reports the main results of the following three major research activities for 3 years directed to:

A1) Evaluate the technological functionality (water and oil holding capacity swelling power, cold water solubility), microstructure, particle size distribution of ball-milled modified starches from different botanical origin (corn, potato, tapioca and wheat).

A2) Develop hydrogel structures from ball-milled starches obtained from various botanical origins (corn, potato, tapioca, and wheat), and evaluate their thermal, rheological and microstructural properties.

A3) Develop porous structures, i.e., aerogels and cryogels using ball-milled starches derived from different botanical origins (corn, high-amylose corn, potato, and wheat) and investigate their key physicochemical properties including densities (bulk and skeletal) water holding capacity, porosity, volumetric shrinkage, specific surface area, pore volume and pore diameter.

2. State-of-the-Art

Starch is a widely available natural biopolymer found in cereals and other plant-based sources. It exhibits a complex multi-scale structure derived from the molecular organization of amylose and amylopectin. Its functional attributes such as contributing to the texture, appearance, and structural integrity of food products have led to their growing significance in the food industry. However, native starch is limited by poor solubility in cold water, slow hydration and swelling rates, thermal instability, and a tendency to retrograde quickly during storage and processing. To overcome these limitations, chemical, enzymatic, and physical modification techniques have been developed to enhance starch functionality for food applications. These methods improve solubility, swelling capacity, thermal resistance, and water and oil binding while reducing crystallinity (Baranowska and Kowalczewski, 2022). Physical modification technologies such as high-pressure processing, cold plasma, pulsed electric fields, and ball milling are increasingly employed to alter starch properties. These environmentally friendly methods, with broader consumer acceptance, offer considerable potential for enhancing ingredient functionality in formulated food systems while also enabling manufacturers to circumvent the “modified starch” label designation. Among these techniques, ball milling (BM) has gained attention as a promising green technology for starch modification. As a solvent-free, mechanical process, BM has demonstrated the ability to improve key structural and functional attributes of starch. Recent studies have highlighted its potential to enhance cold-water solubility, reduce gelatinisation temperature, and increase apparent amylose content in various starches (Bangar *et al*., 2023). Starch, whether in its native or modified form, is widely utilized in the food industry for various applications. Among these, its role in encapsulation and the development of gel-like structures has gained increasing interest. The growing interest in starch-based gels is driven by their affordability, availability, and digestibility, making

them valuable in food formulations. Upon gelatinization and subsequent cooling, starch undergoes retrogradation, reorganizing into a structured three-dimensional network (hydrogel) with desirable functional properties and diverse applications. Starch-based aerogels and cryogels, highly porous matrices obtained via starch dissolution and drying by supercritical CO_2 or freeze-drying respectively, are increasingly used for non-food delivery purposes (Zou and Budtova, 2021). A key research gap remains in the systematic evaluation of ball-milled starches from diverse botanical origins, particularly in their application as innovative gels (hydrogels, aerogels, and cryogels). Despite extensive research on starch-based materials, studies have largely focused on unmodified polysaccharide and protein-derived aerogels, exploring their potential in oil structuring (Plazzotta *et al*., 2019), bioactive delivery systems, and novel material applications. However, the broader functionality of food-grade porous structures, including ball-milled starches, remains largely unexplored (Manzocco *et al*., 2022). Addressing this gap could offer new possibilities for tailored starch-based materials in food and other applications.
This PhD study directly addresses this research gap by systematically evaluating ball-milled starches from diverse botanical origins. For the first time, it explores the use of BM starches in the development of aerogels and cryogels through supercritical CO_2 ($SCCO_2$) and freeze-drying processes, characterize the physicochemical and technological properties of these novel materials and assess their potential as functional delivery systems.

3. Experimental Procedure

This PhD project comprised two main phases. The first phase involved the investigation and characterization of starches from different botanical origins at the structural and techno-functional levels. This included assessing how ball milling treatment time modulated key properties such as granule morphology and particle size distribution water and oil holding capacity, cold water solubility and swelling capacity. The second phase focused on the application of these mechanically modified starches in the development of structured delivery systems such as hydrogels, aerogels and cryogels. The objective was to evaluate how treatment duration influenced the ability of the starches to form gels with desirable structural and functional attributes. Comprehensive analyses were conducted to determine thermal, rheological and microstructural properties of the resulting hydrogels. Also, physicochemical properties of (e.g., volumetric shrinkage, densities (bulk and skeletal), porosity, water holding property, surface area and pore diameter) were analysed on both aerogels and cryogels.

4. Materials and Methods

Native starches of different botanical origins (corn, high amylose corn, potato, tapioca and wheat) were modified using planetary ball miller equipped with zirconium oxide grinding jar (80 ml) and 23 zirconium oxide grinding balls (⌀ 8 mm). Processing conditions tested were the following: weight ratio of balls-to-starch was 23:1, constant rotational speed and milling times of 5, 15, and 30 min. The technological functionalities studied included: swelling power (SP), cold water solubility (CWS), oil and water holding capacity (OHC and WHC). Particle size distribution was measured by starching solutions of 1% (w/w) using particle size analyser. Analysis was carried out at a stirring speed of 2500 rpm using water as the dispersing agent, while the refractive index (1.52) and absorptivity index (0.01) were kept constant. The following parameters were determined: specific surface area (SSA), the mean volumetric diameter (D4;3), and the surface area diameter (D3;2). Microstructure of the starch powders and hydrogels were analysed using a Nikon A1r confocal laser Scanning microscope (CLSM) and scanning electron microscope (JEOL, Tokyo, Japan).
Rheological analyses were conducted on hydrogels prepared from both native and ball-milled (BM) starches using 10% (w/w) starch dispersions. Measurements were performed with a rheometer (MCR 302, Anton Paar, Graz, Austria) equipped with a parallel plate geometry (diameter: 50 mm; gap: 1 mm). A temperature sweep was applied from 30 to 90 °C at a rate of 5 °C/min under a constant strain of 0.1%, during which changes in storage modulus (G′), loss modulus (G″) and Tan δ (G″/G′) were determined.
Thermal properties of the starch dispersions were assessed using differential scanning calorimetry with a temperature scan ranging from 10 to 90 °C at a heating rate of 10 °C/min. Regarding aerogel and cryogel preparation, both native and BM starches were gelatinized at 15% (w/w) at 121 ºC, aged for 48 h, and subjected to 72 hours solvent exchange prior to supercritical CO_2 drying to obtain aerogels. Cryogels were produced by freeze-drying hydrogels that had been frozen for 72 h. The skeletal density (ρ_{skel}) of native and BM aerogels and cryogels samples was measured using a helium pycnometer operating at room temperature (25 °C) and a pressure between 1.01-1.2 bar. The measurements were performed in five replicates. Bulk density (ρ_{bulk}) of the samples was determined by calculating their weight and dimensions. Overall porosity (ε) was subsequently computed using Eq. (2) (Santos-Rosales *et al*., 2020).

$$\varepsilon\ (\%) = \left(1 - \frac{\rho bulk}{\rho skel}\right) * 100 \qquad 1$$

The volumetric shrinkage (VSt) was derived from the volumes of $V_{hydrogel}$ and $V_{aerogel}$, using the formular provided in Eq. (3) (Dhua and Mishra, 2023).

$$VSt = \frac{Vhydrogel - Vaerogel}{Vhydrogel} \times 100 \qquad 2$$

The physical properties of native and BM aerogels and cryogels samples were evaluated using low-temperature N_2 adsorption–desorption analysis. One-way ANOVA (GraphPad prism software) was used for statistical analysis.

Significant differences between individual samples were determined by Tukey's test (p = 0.05).

5. Results and Discussion

5.1 Technological functionality, physical and microstructural properties of starch

Starches from different botanical origins showed significant variations in their technological functionality with BM treatment time (Table 1), highlighting a significant increase in their overall technological functionality compared to the corresponding native. In general, the starches showed a positive correlation between milling time and the improvement of the CWS, SP, OHC, and WHC properties. This increase can be attributed to the disruption of hydrogen bonds within the starch polymer chains. The increased availability of free hydrophilic hydroxyl groups facilitates greater interaction with water molecules. The increase in oil-holding capacity (OHC) with milling time is attributed to the formation of surface cavities and dents, which enhance the starch molecule's ability to trap and retain oil (Chen *et al*., 2024). Interestingly, tapioca starch subjected to 30 minutes of ball milling exhibited a threefold increase in water-holding capacity (WHC) and swelling power (SP) compared to the other BM samples. Moreover, potato starch exhibited a distinct response compared to other botanical sources, reaching maximum SP and WHC after 5 minutes of BM treatment (data not shown). In contrast, other starch samples showed a continuous increase in the technological functionality studied with prolonged milling duration. This suggests that potato starch is more sensitive to ball milling treatment, which is likely due to its larger native granule size and potentially more fragile structural integrity. The inherent crystalline structures of the native starches, likely contributed to the lower baseline techno-functional properties (Liu *et al*., 2020).

Prolonged milling increased the D4,3 particle size and broadened its distribution due to agglomerate formation during the process (Table 1). Conversely, specific surface area (SSA) showed a decreasing trend, attributed to the formation of these agglomerates (data not shown). Furthermore, CLSM and SEM imaging validated the disruption of starch granules, revealing the presence of damaged, fragmented, and agglomerated insoluble particles in both ball-milled starch suspensions and hydrogels (Figure 1).

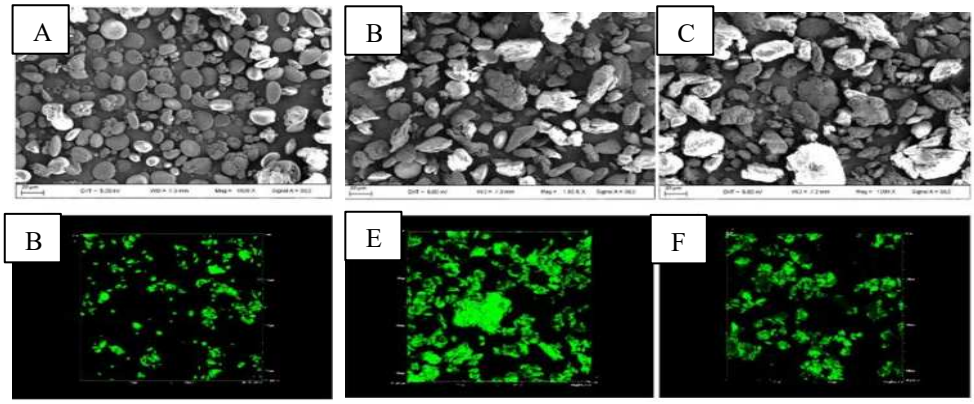


Figure 1 *SEM (top) and CLSM (bottom) of wheat starch, where A and B is 0 min, B and E is 15 min, C and F is 30 min respectively. Scale bar: 20 µm and Magnification of 1000X for SEM and Scale bar: 50 µm and Magnification of 40X for CLSM.*

5.2 Thermal and rheological properties of the starch hydrogels from different botanical origins

BM induced significant structural modifications in starch granules, leading to the reduction of short-range crystalline order, reduced double-helix content, amylose depolymerization, and amylopectin degradation within damaged granules (Han *et al*., 2022). These alterations directly influenced the gelatinization process, resulting in a progressive decrease in gelatinization temperature (T_{gel}) and enthalpy (ΔH) with prolonged milling duration (Table 1). This trend suggests a negative correlation between milling time and ΔH. Furthermore, Tan δ values remained below 1 for all starch samples, except for PS-BM30, which exhibited a slightly higher value, indicating predominantly solid-like (gel) behaviour (data not shown).

Rheological analysis (Figure 2) showed that BM reduced hydrogel strength, as indicated by lower storage modulus (G′) values and the formation of weaker gel structures compared to untreated starch. This weakening behaviour was consistently observed across all BM starch samples.

Table 1 Variation (% in respect to the native) of selected technological functionality and thermal properties of BM starch

Sample& treatment(min)	CWS (%)	SP (%)	WHC (%)	OHC (%)	D [4;3] (%)	T_{gel} (%)	ΔH (%)
CS-BM15	9.07±0.98^{e}	5.54±0.27^{d}	330.61±20.19^{c}	69.42±9.2^{d}	-70.92±22.40^{b}	-21.09±2.10^{b}	-99.71±22.1^{a}
PS-BM15	161.98±10.73^{b}	3.88±0.27^{ed}	140.28±30.19^{e}	107.26±10.03^{c}	-104.54±26.93^{d}	-15.12±2.20^{a}	-98.73±25.2^{a}
TS-BM15	2.05±0.73^{f}	178.82±10.07^{b}	289.54±23.73^{d}	-7.96±0.73^{g}	-122.19±17.23^{e}	-19.19±3.20^{b}	-92.41±20.2^{a}
WS-BM15	14.01±1.85^{d}	20.28±2.33^{c}	243.91±26.15^{e}	37.00±11.02^{e}	-22.73±7.32^{a}	N.d	N.d

CS-BM30	9.90±1.35^{e}	4.91±0.43^{d}	392.52±25.49^{b}	77.96±12.04^{d}	-76.73±20.20^{b}	-27.54±2.54^{d}	-99.78±22.02^{a}
PS-BM30	191.89±0.91^{a}	-16.68±2.53^{e}	78.81±26.0^{g}	114.19±14.04^{b}	-94.19±22.55^{c}	-14.65±3.07^{a}	-99.84±26.62^{a}
TS-BM30	10.83±1.23^{e}	185.31±18.07^{a}	693.64±47.3^{a}	20.73±2.73^{f}	-135.48±15.93^{f}	-24.40±2.80^{c}	-97.30±22.2^{a}
WS-BM30	23.64±0.79^{c}	5.78±0.62^{d}	206.17±34.4^{f}	35.05±7.03^{e}	-24.80±6.00^{a}	N.d	N.d

$^{a\text{-}f}$: Means within the same column followed by different letters are significantly different ($p < 0.05$)
CS, PS, TS and WS; corn, potato, tapioca and wheat starch respectively. BM15, BM30: Ball milled starch for 15 and 30 min. Tgel and ΔH; Temperature and enthalpy of gelatinization respectively. N.d means not detected

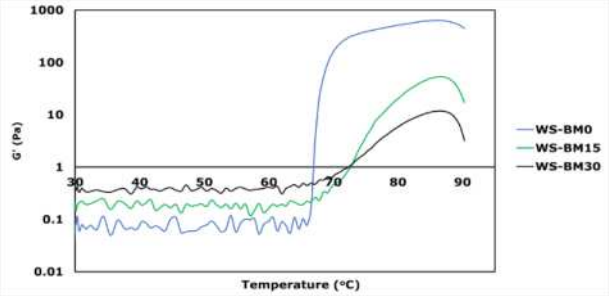


Figure 2 *Change of G' of native and BM wheat starch subjected to temperature sweep analysis*

5.3 Physicochemical properties of starch aerogels and cryogels from different botanical origins

The volumetric shrinkage (VSt %) observed after supercritical CO_2 drying represents the cumulative shrinkage across all processing steps. BM starch aerogels generally exhibited lower shrinkage compared to their native counterparts, except for potato starch (PS) and wheat starch (WS), suggesting that BM treatment reinforced the gel network in most cases (Table 2). The mechanical forces exerted during ball milling disrupted both intramolecular and intermolecular hydrogen bonds within starch chains, facilitating the fragmentation of α-1,6 branching points in amylopectin (Han *et al*., 2022) and leading to an increase in amylose content. The newly generated amylose molecules, characterized by their linear structure, formed tightly packed networks, contributing to a stronger gel matrix (Druel *et al*., 2017). In addition, high amylose corn starch (HACS), with 52% amylose content, was expected to exhibit the lowest shrinkage compared to other studied starches with amylose content below 25%. In contrast, freeze-drying produces materials with little to no shrinkage (data not shown).

Ball milling treatment (BMT) had a significant influence on the bulk and skeletal densities of starch-based aerogels and cryogels, as well as their overall porosity (Table 2). Both skeletal and bulk densities exhibited a statistically significant decreasing trend ($p < 0.05$) with increasing milling duration. However, this trend was inconsistent across BM samples, particularly at extended milling times. The reduction in packing density within these amorphous domains facilitated greater pore formation, thereby enhancing porosity (He *et al*., 2014). Given the inverse relationship between porosity and density, the observed decrease in density corresponded directly with increased porosity. Aerogels exhibited higher density than cryogels, likely due to structural densification from shrinkage during solvent exchange, whereas cryogels, which underwent no solvent exchange, maintained lower density (Baudron *et al*., 2019). Among cryogels, potato starch (PS) showed the highest porosity and the lowest density, aligning with a sharp increase in WHC (data not shown).

Water-holding capacity (WHC) increased with prolonged ball milling, with starch cryogels (data not shown) exhibiting approximately twice the WHC of their corresponding aerogels. This difference is likely due to the larger pore size and more open structure of cryogels, which enhance water retention. Among the starch types, PS-BM aerogels showed the highest WHC (Table 2). BM- HACS demonstrated the lowest WHC in both aerogels and cryogels, with a notable decline observed in its cryogels as milling time increased. This reduction is due to the tightly packed structure of high-amylose starch, which resists BM-induced mechanical disruption, limiting hydrogen bond breakdown and restricting water retention. (Lv *et al*., 2021). Overall, WHC is strongly influenced by starch type, granule characteristics, and structural integrity.

Surface area (SA) and pore diameter showed significant increases with prolonged milling duration in some cases ($p < 0.05$) (Table 2); however, the overall trend remained inconsistent across treatments. The increase was likely due to ball milling (BM) promoting glycosidic bond degradation at amylopectin α-1,6 branch points, resulting in higher amylose content. This rise correlated with increased surface area (SA), particularly in CS, HACS, and WS samples treated for 5 and 10 minutes (data not shown). The pore diameter measurements for all samples ranged from (4–24 nm), confirming their classification within the mesopore range for aerogels. In contrast, both pore diameter and surface area of the starch cryogels fell below the instrument's detectable limit.

Table 2 Variation (% in respect to the native) of selected physicochemical properties of BM starch aerogels

Sample& treatment(min)	Bulk density (%)	Skeletal density (%)	Porosity (%)	WHC (%)	SA (%)	Pore diameter (%)	VSt (%)
CS-BM15	29.81±0.98^{b}	6.58±0.67^{a}	8.80±0.89^{b}	15.57±2.39^{e}	48	21.2	31.17±4.10^{ba}
HACS-BM15	4.47±0.49^{fe}	-16.83±1.19^{f}	4.55±0.10^{c}	9.37±1.19^{g}	-12	-74.4	19.80±2.50^{c}
PS-BM15	-5.42±0.73^{e}	3.97±0.47^{b}	-1.77±0.19^{d}	58.95±9.51^{a}	-7	-15.0	-10.02±2.20^{f}
WS-BM15	-4.28±0.85^{fe}	1.36±0.33^{dc}	-1.69±0.12^{d}	18.54±2.15^{d}	-2	229.4	-1.5±0.08^{e}
CS-BM30	43.36±4.35^{a}	7.71±0.93^{a}	13.67±2.49^{a}	-8.52±0.62^{h}	52	21.2	35.65±2.54^{a}
HACS-BM30	12.37±2.19^{d}	-9.58±0.99^{e}	5.18±0.26^{b}	25.48±3.49^{c}	-7	-22.4	15.92±1.50^{c}
PS-BM30	-5.76±0.91^{e}	4.59±0.43^{b}	-2.02±0.19^{d}	32.18±5.26^{b}	-23	500.0	2.47±0.07^{d}
WS-BM30	21.07±3.79^{c}	2.86±0.12^{c}	4.68±0.19^{c}	11.68±1.04^{f}	12	-21.6	-8.26±0.97^{f}

$^{a\text{-}f}$: Means within the same column followed by different letters are significantly different ($p < 0.05$)
WHC was calculated from the 6th hourly interval. CS, HACS, PS and WS; corn, high amylose corn, potato and wheat starch respectively. BM15, BM30: Ball milled starch for 15 and 30 min.

6. Conclusions and Future Perspectives

This study demonstrated that ball milling (BM) significantly enhanced the technological functionality of starches while reducing the gel strength. The impact of BM treatment duration on the physicochemical properties of aerogels and cryogels was systematically evaluated, revealing notable differences between processing techniques. Supercritical CO_2 drying preserved the hydrogel nanostructure more effectively than freeze-drying, increasing specific surface area and porosity, making it a valuable method for aerogel stabilization. In contrast, freeze-dried cryogels exhibited minimal shrinkage, resulting in enhanced aqueous stability. The high water-holding capacity of the BM-modified samples highlights their potential for applications requiring superior hydration properties, such as food formulations. Ball milling is a viable, eco-friendly, and efficient technique for modifying starch to develop gel-based systems. It enables tunable porosity, increased surface area, and improved mechanical stability. Studies on the crystallinity and microstructure of the gels are ongoing. Additionally, the use of BM starch gels as innovative carrier structures for bioactive compound encapsulation is currently under investigation.

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Oral presentation sessions
PhD students – 3rd year
Abstracts

PARALLEL SESSION 2
Smart Packaging and Green Technologies for Food Preservation

Microbial Biopolymers for Potential Applications in Food Packaging

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The PhD project focused on the recovery of various microbial biopolymers to develop active and sustainable food packaging. Brewer's spent yeast (BSY) was treated using non-thermal technologies (HPH, PEF) and autolysis processes to extract β-glucans and mannoproteins, which were then incorporated into edible and multilayer films. Pullulan was produced by *Aureobasidium pullulans* cultivated on agro-industrial by-products. Nisin, an antimicrobial peptide produced by *Lactococcus lactis* subsp. *lactis,* was incorporated into bio-based films made from poly(trimethylene furanoate) (PTF). An internship at the European Food Safety Authority (EFSA) enabled a systematic review of the safety of biopolymers intended for food contact applications.

Biopolimeri microbici per potenziali applicazioni nel packaging alimentare

Il progetto di dottorato si è concentrato sul recupero di diversi biopolimeri microbici per un loro potenziale utilizzo in imballaggi alimentari attivi e sostenibili. Il lievito esausto da birrificazione (BSY) è stato trattato con tecnologie non termiche (HPH, PEF) e processi di autolisi per estrarre β-glucani e mannoproteine, poi integrati in film edibili e multistrato. Il pullulano è stato prodotto da *Aureobasidium pullulans* coltivato su sottoprodotti agroindustriali. La nisina, peptide antimicrobico prodotto da *Lactococcus lactis* subsp. *lactis*, è stata incorporata in film bio-based a base di poli(trimetilene furanoato) (PTF). Un tirocinio presso l'Autorità Europea per la Sicurezza Alimentare (EFSA) ha permesso una revisione sistematica sulla sicurezza dei biopolimeri impiegati nei materiali a contatto con gli alimenti.

Keywords: agro-industrial by-products, microbial biopolymers, food contact materials, shelf-life, safety assessment

1. Introduction

In recent years, growing environmental concerns and the need to reduce reliance on fossil resources have driven interest in biodegradable alternatives to synthetic petroleum-based films in food packaging. Conventional polymers like polyethylene, polypropylene, and polystyrene offer excellent mechanical and barrier properties but are non-biodegradable and derived from non-renewable sources, posing serious environmental challenges. Biodegradable materials based on natural polymers—such as polysaccharides, proteins, and lipids—are increasingly explored as sustainable alternatives. Among these, yeast biomass shows promise due to its cell wall components, including β-glucans, mannoproteins, and chitin. In particular, β-glucans constitute up to 65% of the yeast cell wall and are effective in forming multicomponent films with proteins. Innovative, non-thermal extraction methods such as high-pressure homogenisation (HPH) and pulsed electric fields (PEF) can efficiently break down yeast cells, facilitating the recovery of valuable biopolymers (González-López et *al.*, 2023). Notably, brewer's spent yeast (BSY), a widely available by-product, has emerged as a sustainable source of β-glucans and mannoproteins for use in active and biodegradable packaging. Other microbial biopolymers, such as pullulan (from *Aureobasidium pullulans*) and bacterial cellulose (e.g., from *Komagataeibacter xylinus*), also hold potential for packaging applications due to their film-forming properties and biodegradability (Saharan *et al.*, 2024). Biopolymers are not only environmentally friendly but can also serve as carriers for antimicrobial and antioxidant agents, enhancing food preservation. Unlike direct addition to food, which may lead to rapid diffusion and loss of activity, incorporating such agents into packaging materials enables controlled release, maintaining efficacy during storage. For instance, nisin, a bacteriocin produced by *Lactococcus lactis* subsp. *lactis*, retains its antimicrobial activity after processing and is being studied for integration into biopolymer matrices (Bukvicki *et al.*, 2023). Furthermore, agro-industrial by-products such as cheese whey, beet molasses, grape and olive pomace are suitable substrates for microbial fermentation, offering a circular approach to biopolymer production (Horue *et al.*, 2021). This valorisation of food industry residues reduces waste, cuts costs, and aligns with circular economy goals. However, the use of biopolymers in food packaging demands rigorous safety evaluations, addressing potential contaminants, microbial hazards, and non-intentionally added substances (NIAS). Ensuring food contact safety throughout the production chain is essential for regulatory compliance and consumer protection (EFSA, 2023). In summary, biopolymers derived from waste and microbial fermentation represent a promising strategy for sustainable packaging. Their development requires optimisation of production processes and comprehensive safety assessments to ensure functionality, biodegradability, and food safety.

This report presents the main results of the PhD project (Ciccone, 2023, 2024):
A1) Systematic Literature Review and EFSA Internship:
A six-month internship was conducted at the European Food Safety Authority (EFSA) as a Guest Scientist within

the Food Contact Materials team (FCM) of the Food Packaging and Ingredients Unit (FIP). During this time, a systematic literature review (SLR) was performed to evaluate the safety of microbial biopolymers intended for food contact applications, starting with a critical analysis of the EFSA Technical Report (EFSA, 2023).

A2) Valorisation of Brewer's Spent Yeast (BSY) for Biopolymer Extraction:
Two different sources of *Saccharomyces cerevisiae* (commercial baker's yeast (BY) and brewer's spent yeast (BSY)) were selected for the extraction of β-glucans and mannoproteins from yeast cell walls. The study aimed to optimise biopolymer recovery using two non-thermal technologies: high-pressure homogenisation (HPH) and pulsed electric fields (PEF). Additionally, a biorefinery approach was implemented by applying varying autolysis durations (24 to 72 hours) to characterise the extracted biopolymer fractions. The BSY biomass was supplied by the ENEA Research Centre (Italian National Agency for New Technologies, Energy and Sustainable Economic Development).

A3) Film and Coating Formulations:
Both solid and freeze-dried BSY biomasses (from ENEA) were incorporated into multilayer composite films based on polybutylene succinate (PBS), polylactic acid (PLA), and polyethylene terephthalate (PET) at 5% and 10% concentrations. Edible films for direct food contact were developed using sodium alginate (3%) and glycerol (2.5%) enriched with 5% or 10% of BSY. These activities were conducted in collaboration with the Department of Civil, Chemical, Environmental and Materials Engineering (DICAM) at the University of Bologna. The edible films were also tested as coatings on cherry tomatoes at the Department of Food Science - University of Aarhus (Denmark).

A4) Pullulan Production:
Pullulan was produced by two *Aureobasidium pullulans* strains cultivated on various agro-industrial by-products, including cheese whey, olive pomace, grape pomace, and beet molasses, to evaluate these substrates as low-cost and sustainable fermentation media.

A5) Incorporation of Nisin into Bio-based Polymers:
Nisin, a natural antimicrobial peptide, was incorporated into fully bio-based poly (trimethylene furanoate) (PTF) blends at 0.05% and 2.5% by weight. The resulting materials retained the thermal and barrier properties of pristine PTF, while mechanical properties and surface wettability were modified. The antimicrobial functionality of the films was demonstrated through tests on ACE juice inoculated with *Listeria monocytogenes*.

2. Materials and Methods

A1) The methodology integrated EFSA principles to assess the safety of naturally derived food contact materials. Specific keywords were chosen to identify potential substances of concern during biopolymer production. Four research questions structured the review around carbon sources, microorganisms, fermentation, and recovery. A rigorous screening of titles, abstracts, and full texts led to a comprehensive systematic literature review focused on two case studies: bacterial cellulose and nisin.

A2) Two different sources of *S. cerevisiae* (commercial baker's yeast (BY) and brewer's spent yeast (BSY)) were investigated to extract β-glucans and mannoproteins from cell walls. Biopolymer recovery was optimised through high-pressure homogenization (HPH) at 125 MPa for 3 passes and pulsed electric fields (PEF) at 1.5 kV/cm with 45 pulses, alone or combined, with and without thermal treatment (TT - 90 °C for 20 min). Dispersions of 10% w/v (dry yeast/volume) were prepared in distilled water, treated, then centrifuged at 19000g for 15 min to separate supernatants. β-glucans and mannoproteins content were quantified using Megazyme kits (K-EBHLG and K-MANGL) (Ciccone *et al.*, 2024). The BSY biomass from the ENEA Research Centre was further processed by autolysis at 50°C under agitation for 24 to 72 hours. Autolysates were thermally inactivated and centrifuged to yield fractions: liquid supernatant (LIS), protein hydrolysate (PH), and yeast cell debris (YCD). Characterisation included pH, dry matter, β-glucans, mannoproteins, fatty acid profiles, antioxidant capacity, and antimicrobial activity.

A3) To explore the potential of brewer's spent yeast (BSY) in sustainable packaging, both solid and freeze-dried BSY biomasses were used to develop three film types: coated, edible, and composite. Coated films were obtained by dispersing BSY in water at various concentrations and applying it onto corona-treated substrates (Polylactic acid (PLA), polyethylene terephthalate (PET)) using a wire bar applicator for uniform coverage. Edible films were formulated with sodium alginate (3%) and glycerol (2.5%), enriched with BSY biomass, heated, cast in Petri dishes, and air-dried to obtain thin, flexible films. Composite films were produced by incorporating BSY powder or pellets into a biodegradable polybutylene succinate (PBS) matrix. The materials were milled, dried, and processed via twin-screw extrusion and compression molding to obtain flat films. In addition, a preliminary application involved coating cherry tomatoes to evaluate postharvest preservation. The coatings, crosslinked with

calcium chloride ($CaCl_2$) (3%), were monitored over 10 days for weight loss, color, texture, pH, titratable acidity, total soluble solids (TSS) and overall migration.

A4) To recover pullulan, two strains (*A. pullulans* var. *melanogenum* DSM 2404 and *A. pullulans* DSM 3042) were tested using four agro-industrial by-products: cheese whey, olive pomace, grape pomace, and beet molasses. Cheese whey, olive pomace, and grape pomace were sterilised in an autoclave (121°C for 15 min), while the beet molasses were diluted 1:20 and filtered with a sterile filter. Sterile falcon tubes were prepared, each containing one of the substrates: 10 g of olive pomace or grape pomace (diluted 1:3 with sterile distilled water), 30 g of cheese whey and beet molasses (without further dilution). A 5% inoculum of microorganisms was added to each tube. The samples were incubated at 30°C with agitation (150 rpm) for 9 days to assess fermentation over different periods. To recover pullulan, isopropyl alcohol (1:3) was added to the supernatants obtained after centrifuging each fermented sample (10000*g* for 20 min). Subsequent centrifugation was performed to collect the precipitate, which was then dried overnight at 80°C in an oven to obtain pullulan powder (Mujdeci *et al.*, 2024).

A5) PTF films incorporating 0.05% and 2.5% nisin were prepared via solvent casting followed by compression molding. The resulting films were characterised by morphology (SEM), thermal stability (TGA/DSC), mechanical properties (tensile test), surface wettability (contact angle), and gas permeability. Antimicrobial activity was assessed in vitro by agar diffusion against *Listeria monocytogenes* Scott A and *Lactiplantibacillus plantarum* B391 4A; inhibition zones were measured after 24 and 72 h. A challenge test in ACE juice inoculated with *L. monocytogenes* (10^2 cfu/mL) was performed at 4°C using 2.5% nisin films, and microbial counts were monitored over 12 days to evaluate antibacterial effectiveness in a food matrix.

3. Results and Discussion

A1) The study integrates systematic literature review methodologies with experimental approaches to valorise agro-industrial by-products and microbial biomass. During a six-month research period as a Guest Scientist at the European Food Safety Authority (EFSA) (Parma, Italy), a systematic literature review (SLR) was conducted in accordance with EFSA guidelines to assess the safety of microbial biopolymers. The review addressed four key research questions related to carbon sources, microbial strains, fermentation processes, and biopolymer recovery methods. From over 9000 initially screened articles, 46 were selected for in-depth analysis, including case studies on bacterial cellulose and nisin. The findings emphasise the need to characterise natural carbon sources for contaminants and pesticides, evaluate the QPS status of microorganisms, and analyse metabolites and reaction products. Information on composition, adverse effects, impurities, and migration potential is crucial for food additives like nisin in food contact materials.

A2) Experimental investigations focused on the extraction of β-glucans and mannoproteins from *S. cerevisiae* biomass, using both bakery yeast (BY) and brewery spent yeast (BSY). High-pressure homogenization (HPH) and pulsed electric fields (PEF), applied alone and in combination, were used to disrupt yeast cell walls. HPH proved effective in achieving complete cell disruption, while PEF induced membrane permeabilisation. The combined treatment (HPH+PEF) yielded the highest extraction levels: carbohydrates (7.02 mg/mL in BY; 2.04 mg/mL in BSY), proteins (2.39 mg/mL in BY; 1.64 mg/mL in BSY), β-glucans (3.90 g/100 g dry matter in BY; 10.44 g/100 g in BSY) (Fig.1), and mannoproteins (3.26 g/100 g in BY; 3.54 g/100 g in BSY). This non-thermal extraction approach offers an eco-friendly alternative to conventional methods and aligns with circular economy principles. In addition, a comprehensive bio-refinery approach was adopted to characterise valuable biopolymer fractions in BSY. The biomass of BSY was provided by ENEA Research Centre. At the beginning, eight sample sets (SC1–SC8/9) were obtained after varying durations of autolysis (up to 72 hours). The longest autolysis period resulted in the highest β-glucan content (22.63 g/100 g), while sample SC4 showed the highest mannose concentration (3.34 g/100 g). Autolysis time influenced fatty acid profiles, favouring saturated fatty acids. Sample SC8/9 (24h of autolysis) displayed the greatest antioxidant activity and notable adhesive properties, highlighting BSY's potential in sustainable packaging applications. Further fractionation of ten BSY samples—including untreated controls and autolysates—into liquid supernatant (LIS), protein hydrolysate (PH), and yeast cell debris (YCD) revealed a significant increase in β-glucan and mannoprotein recovery after 24 and 72 hours of autolysis, particularly in the LIS fraction. Fatty acid analysis identified palmitic acid (C16:0) and oleic acid (C18:1) as the predominant fatty acids, along with a significant presence of medium-chain fatty acids, including lauric acid (C12:0) and myristic acid (C14:0). The YCD fraction, including its lyophilised form, exhibited selective antimicrobial activity against *Listeria monocytogenes* at concentrations of 5% and 10%. This effect can be attributed, at least in part, to the presence of lauric acid, which has been shown to exert strong antimicrobial effects at relatively low concentrations, partly by disrupting virulence regulation via the PrfA system (Thomasen *et al.*, 2022). Similarly, myristic acid has demonstrated significant antimicrobial activity against this type of pathogenic microorganism, suggesting a potential synergistic contribution to the observed inhibitory effect (Chen *et al.*, 2019). All tested fractions also demonstrated measurable antioxidant activity, which is likely associated with the presence of bioactive compounds such as polyphenols and glutathione. LC-MS/QTOF analysis confirmed the retention of

these bioactive molecules in the BSY extract, further supporting its antioxidant and antimicrobial potential.

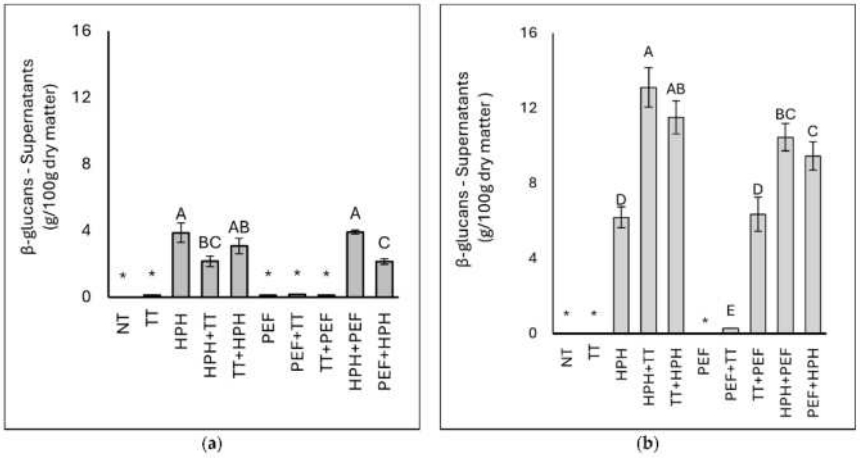


Figure 1 *β-glucan content (g/100 g dry matter) of the supernatants of BY (**a**) and BSY (**b**). NT represents the control sample. Different letters indicate significant differences ($p < 0.05$).*

**: data below the detection limit (1 g/100 g).*

A4) Pullulan production was explored using two *Aureobasidium pullulans* strains (DSM 2404 and DSM 3042) cultured on cheese whey, olive pomace, grape pomace, and beet molasses. Both strains grew on all tested substrates, reaching maximum cell load within 2 days at 30°C. The pullulan production was strained and substrate-dependent. The strain DSM 2404 resulted in higher pullulan production than the strain DSM 3042 regardless of the substrate considered. Considering the substrates, higher pullulan production was observed on whey and molasses, and 2-5 days of fermentation seemed to be enough to reach the highest yield of pullulan (Fig. 3). This study highlights the potential of agri-food by-products as sustainable substrates for pullulan production, though the further quality characterisation of pullulan is needed for food packaging applications.

A3) Biomasses at 5% and 10% concentrations showed good integration with PBS, PLA, and PET matrices. Edible alginate-glycerol films enriched with BSY demonstrated enhanced functional properties. When applied as coatings on cherry tomatoes, they retained moisture, reduced weight loss, and preserved texture and colour for up to 10 days. $CaCl_2$ crosslinking further improved film stability and adhesion. The coatings exhibited strong adherence, effective moisture retention, and surface protection. Visual and structural assessments were supported by Videometer imaging (Fig. 2). Preliminary Challenge tests with *Listeria monocytogenes* on coated cherry tomatoes indicated a moderate delay in bacterial growth. However, further microbiological evaluations are required to confirm and quantify antimicrobial activity over longer storage periods.
These findings highlight BSY as a promising source of bioactive compounds for use as natural antimicrobials and antioxidants, and as a sustainable raw material for food packaging aligned with circular economy principles.

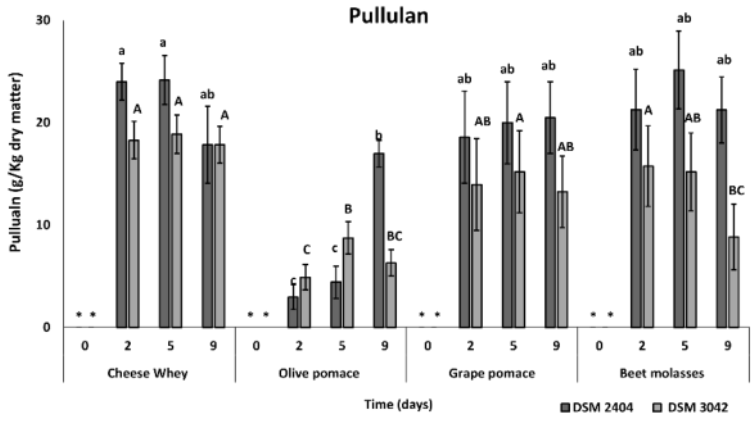


Figure 2 *Alginate-based coating solution enriched with 5% BSY powder applied to cherry tomatoes.*

A5) Moreover, fully bio-based blends were developed by incorporating the natural antimicrobial peptide nisin (0.05 and 2.5 wt%) into poly(trimethylene furanoate) (PTF), a furan-based polyester. The blends maintained the thermal and mechanical stability of pristine PTF and preserved its excellent gas barrier properties. The formulation containing 2.5% nisin showed antibacterial activity against *Lactiplantibacillus plantarum* and *Listeria monocytogenes*, confirmed through disc diffusion assays. When tested in ACE juice (pH 4.5) inoculated with *L. monocytogenes*, the nisin-enriched PTF reduced bacterial counts below detection limits, unlike the untreated control (Fig. 4).

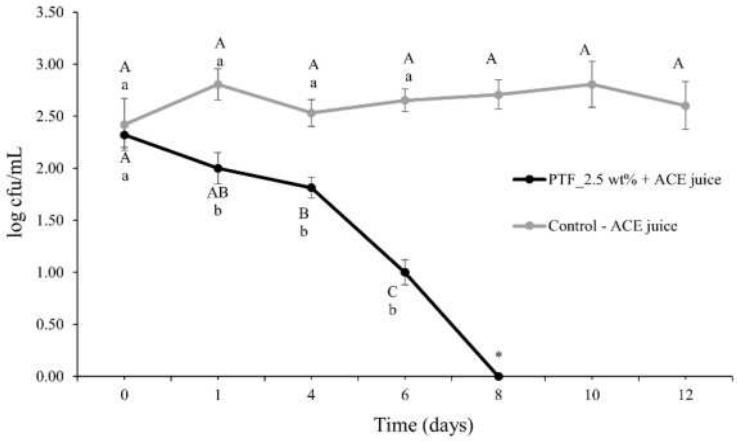


Figure 4 *Microbial cell loads of Listeria monocytogenes Scott A inoculated in ACE juice sample with PTF films containing 2.5% nisin (PTF_2.5 wt%) and control samples (no film). The results are presented as the statistical mean and standard deviation (SD) of three independent replicates. (a-b): Different lowercase letters indicate statistically significant differences between samples at the same time point ($p < 0.05$). (A-B): Different uppercase letters indicate statistically significant differences within the same sample across different time points ($p < 0.05$).*

**: data below the detection limit (< 0.5 log cfu/mL)*

4. Conclusions and Future Perspectives

This doctoral research demonstrated the valorisation of agro-industrial by-products, particularly brewer's spent yeast (BSY), as a sustainable source for microbial biopolymers used in food packaging. Through the integration of non-thermal extraction techniques—high-pressure homogenisation (HPH) and pulsed electric fields (PEF)—with biorefinery strategies, it was possible to efficiently extract β-glucans, mannoproteins, and other bioactive compounds. Controlled autolysis processes enhanced both the yield and functional properties of these fractions, improving their antioxidant capacity and selective antimicrobial activity when applied in edible films and multilayer packaging materials. Additionally, pullulan production using *Aureobasidium pullulans* grown on agro-industrial residues confirmed the feasibility of converting waste streams into microbial polysaccharides, though further optimisation is required to improve polymer purity and performance. Bio-based poly(trimethylene furanoate) (PTF) blends incorporating nisin, a natural antimicrobial peptide, retained mechanical and thermal stability while providing effective antimicrobial action against *Listeria monocytogenes*, indicating strong potential for active food packaging applications. A six-month internship at EFSA focused on the safety and regulatory aspects of microbial biopolymers, underlining the need for comprehensive risk assessments addressing contaminants and food contact compliance. Overall, this research advances the circular bioeconomy by transforming low-value waste into safe, functional, and environmentally friendly packaging solutions.

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Biodegradable Innovative Packaging: A Sustainable Solutions for Food Shelf Life Extension

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This PhD project developed and evaluated biodegradable packaging and edible coatings for food preservation as sustainable alternatives to conventional plastic packaging, with the aim of reducing environmental impact and preserving product quality. Innovative formulations were applied to food products, quality parameters were monitored over time, and their evolution was analyzed using kinetic modelling approaches. The integration of these packages with kinetic models capable of describing quality changes over time as a function of temperature represents a promising basis for optimizing sustainable packaging systems, improving production and distribution technologies to prevent food spoilage.

Packaging Innovativo Biodegradabile: Una Soluzione Sostenibile per Prolungare la Shelf Life degli Alimenti

Questo progetto di dottorato ha sviluppato e valutato imballaggi biodegradabili e rivestimenti commestibili per la conservazione degli alimenti come alternative sostenibili agli imballaggi in plastica convenzionali, con l'obiettivo di ridurre l'impatto ambientale e preservare la qualità dei prodotti. Le formulazioni innovative sono state applicate ai prodotti alimentari, i parametri di qualità sono stati monitorati nel tempo e la loro evoluzione è stata analizzata utilizzando approcci di modellazione cinetica. L'integrazione di questi imballaggi con modelli cinetici in grado di descrivere i cambiamenti di qualità nel tempo in funzione della temperatura rappresenta una base promettente per ottimizzare i sistemi di imballaggio sostenibili, migliorare le tecnologie di produzione e distribuzione per prevenire il deterioramento degli alimenti.

Keywords: edible coating; biodegradable packaging; sustainability; kinetics modelling; quality monitoring.

1. Introduction

In accordance with the PhD thesis project, this oral communication reports the main results of the following activities:

(A1) Formulation development and application of biodegradable packaging and edible coating to foods;
(A2) Monitoring of the product's shelf life and study of the degradation kinetics of quality indexes.

2. Materials and Methods

2.1 Formulation and application of innovative packaging on PGI Sila potatoes

Sila potatoes (*Solanum tuberosum L. cv. Agria*) (Protected Geographical Indication-PGI) were washed and sliced to a uniform thickness of 6 mm. Then, they were coated with arabic gum and an enriched arabic gum formulation obtained with the addition of an antioxidant lemon (*Citrus limon* (L.) Osbeck) pomace extract (E). E was obtained following the method reported by Giacondino *et al.* (2024). Specifically, 10% w/v Arabic gum solution (GA) was prepared in distilled water, then it was heated at 40°C for 60 min, and glycerol (1% w/v) was incorporated. The pH of GA solution was adjusted to 5.6 with 1 N NaOH (Ali *et al.,* 2021). Another coating (GAE) was formulated by adding 10% of E to the GA solution. Potato slices were immersed for 2 min in GA (sample PGA) and GAE (sample PGAE) coatings, while uncoated one (sample PB) was considered as a control. Subsequently, all samples were dried at 70 °C for 6 hours until semi-dry, packed in biodegradable cellulose bags, and then stored at 15 °C, 20 °C, and 30 °C. Physico-chemical and sensory parameters (colour, texture, aw, moisture, total polyphenols, visual descriptors) were monitored every 20 days.

2.2 Formulation and application of innovative packaging on fennel ('Finocchio di Isola Capo Rizzuto' PGI)

Fennels (*Foeniculum vulgare* Mill.) 'Finocchio di Isola Capo Rizzuto' PGI were washed, sliced to a thickness of 1 cm, and sanitized with sodium hypochlorite solution (200 ppm) for 2 min. Then, fennel slices were packaged in polypropylene (PP) trays sealed with polyethylene terephtalate (PET)/PP film (sample T) and in PP bags (sample B) as conventional plastic materials. Sample stored in cellulose bags (C) was considered as a biodegradable alternative packaging. All fennel samples were stored at 4 °C, 10 °C, and 15 °C. The effectiveness of the packaging system in maintaining quality was evaluated by monitoring texture, moisture, colour, and total bacterial count after 0, 3, 6, 10, 14, and 17 days.

2.4 Evaluation of degradation kinetics of quality parameters during storage

Food quality degradation during storage was described by kinetic models, as shown in the following equation (1):

$$\frac{dC}{dt} = -kC^n \qquad (1)$$

where C is the quality index value at time t, t the storage time (day), k the rate costant (d^{-1}), and *n* the reaction order. The temperature effect was modeled via the Arrhenius equation (2):

$$k = k_0 \exp - \frac{E_a}{RT} \qquad (2)$$

where k is the reaction rate constant, k_0 the temperature-independent constant, E_a the activation energy (J mol^{-1}), R the gas constant (8.314 J mol^{-1} K^{-1}), and T the storage temperature (K) (Liao *et al.,* 2024). Zero- and first-order kinetics were evaluated using regression analyses of experimental data to describe the qualitative degradation, estimate $R^{2,}$ and thermodynamic parameters, forming the basis for predicting product quality evolution.

2.5 Statistical Analysis

The obtained results are presented in tables as mean ± Standard deviations. One-way analysis of variance (ANOVA) with Tukey's *post-hoc* test ($p < 0.05$) was performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1 Analysis of quality changes of potato slices during storage and degradation kinetics

Producing semi-dry foods with fresh-like qualities and longer shelf life is a promising option for industrial needs. Among the strategies, edible coatings are gaining interest not only for their role in reducing the use of plastics, but also as a pre-treatment before drying, helping to preserve texture and appearance, and conveying bioactive substances (Ignaczak *et al.,* 2025). Table 1 shows the evolution of quality parameters of potato slices stored at 20 °C. The L* values of the potato slices decreased over time, consistent with browning phenomena. The coated samples, and particularly PGAE, show higher values of the a* and b* components, indicating that the enriched coating may have inhibited the initial browning to some extent, stabilising the colour component, as confirmed by literature (Mehyar *et al.,* 2025). However, the differences of blu-yellow tone (b*) among coated samples decreased in the second half of storage, indicating a common tendency for pigment loss and colour variation towards less warm tones. All samples exhibit a marked reduction ($p < 0.01$) in moisture content and water activity over time. PGAE sample showed higher initial moisture retention, and no significant difference was observed between treatments during storage. In general, edible coatings can act as a barrier to moisture transfer by maintaining a higher initial level; the higher moisture found in PGAE can be attributed to the barrier action exerted by the solid materials present in the extract, which deposited on the surface and slowed down the diffusion of water vapour. Crispness decreased significantly over time in all samples. Initially, it was lower in coated samples, particularly in PGAE, and it could be attributed to the higher moisture retention induced by the coating (Ignaczak *et al.,* 2025). However, values tended to converge towards similar levels, consistent with the expected soft and flexible texture typical of semi-dry products. In other cases, edible coatings had no significant effect on the colour and texture of fresh-cut products like diced pineapple (Meerasri e Sothornvit 2023). Coating composition and bioactive compounds can improve or worsen performance. In this regard, an increase in total polyphenols was significantly observed on potato coated with the antioxidant-enriched coating, which was also appreciated from the visual descriptors of the sensorial analysis (data not shown).

Table 1 *Changes in the main quality parameters of potato slices during storage at 20 °C*

	Time/ Samples	0	40	80	Sign.		Time/ Samples	0	40	80	Sign.
	PB	55.78±1.7^{a}	56.34±1.83^{aA}	52.29±2.77^{b}	**		PB	130.74±2.94^{aC}	90.15±1.28^{b}	58.17±6.52^{cB}	**
*L**	PGA	56.06±1.12^{a}	54.09±2.01^{bB}	50.44±1.49^{c}	**	*TPC (mg 100 g^{-1} dw)*	PGA	153.42±4.74^{aB}	92.46±8.7^{b}	68.11±7.36^{bAB}	**
	PGAE	56.91±1.12^{a}	54.58±1.44^{bB}	52.34±2.15^{c}	**		PGAE	169.89±0.47^{aA}	100.38±1.33^{b}	90.38±0.87^{cA}	**
	Sign.	*ns*	*	*ns*			*Sign.*	**	*ns*	**	
	PB	2.21±0.67^{cB}	3.08±1.2^{bB}	4.33±0.77^{aB}	**		PB	17.97±0.29^{aB}	11.84±0.21^{b}	4.52±0.14^{c}	**
*a**	PGA	2.65±0.54^{bB}	3.97±0.71^{aB}	3.92±1.08^{aB}	**	*Moisture content (%)*	PGA	19.11±0.39^{aAB}	13.3 6±0.5^{b}	3.88±1.13^{c}	**
	PGAE	4.85±0.62^{A}	5.59±0.92^{A}	5.43±0.85^{A}	*ns*		PGAE	21.41±0.84^{aA}	12.73±1.45^{b}	4.94±0.4^{c}	**
	Sign.	**	**	**			*Sign.*	*	*ns*	*ns*	
	PB	16.29±1.32^{B}	16.8±2.17^{B}	17.94±1.31	*ns*		PB	20.76±2.52^{Aa}	12.37±4.62^{b}	10.41±1.44^{abAB}	*
*b**	PGA	22.05±1.69^{aA}	21.23±2.08^{aA}	16.72±2.75^{b}	**	*Crispness (kg sec^{-1})*	PGA	18.8±3.02^{aAB}	14.45±3.96^{ab}	12.88±3.99^{bA}	*
	PGAE	22.54±2.36^{aA}	21.82±2.87^{aA}	17.74±1.66^{b}	**		PGAE	15.56±2.6^{aB}	13.98±2.42^{a}	8.03±2.55^{bB}	**
	Sign.	**	**	*ns*			*Sign.*	*	*ns*	*	
	PB	0.76±0^{aB}	0.75±0.02^{a}	0.7±0.01^{b}	**						
aw	PGA	0.76±0^{aB}	0.75±0^{a}	0.68±0^{b}	**						
	PGAE	0.84±0^{aA}	0.76±0.02^{b}	0.69±0.01^{c}	**						
	Sign.	**	*ns*	*ns*							

*The results are reported as mean ± d.s.. Small letters within a row and capital letters within a column show significant differences as assessed by Tukey's post hoc test. Abbreviations: **, significance at p < 0.01; *, significance at p < 0.05*

Kinetic modeling quantifies biochemical and physical changes, facilitating reaction control and product quality preservation (Choosuk *et al.,* 2022). The changes in crispness and water activity (*aw*) of potato slices were modelled based on the zero- and first-order models (Table 2). The observed quality indices showed a progressive and temperature-dependent trend, with a more rapid decrease at higher temperatures, as shown by the increase of the reaction rate constants with temperature in all samples. For crispness deterioration, this indicates accelerated loss at higher temperatures. PGAE demonstrated the lowest degradation rates, indicating enhanced structural protection. Higher R^2 values for the first-order model indicate that it may better describe the loss of crispness. The activation energies (Ea) indicate higher resistance to thermal degradation of crispness in the coated samples, particularly with the addition of the extract. The higher Ea values in PGA and PGAE evidence that more energy is required to lose crispness, probably due to the barrier effect of the coating or its interactions with the product. Water activity data show a linear decrease in time and negative kinetic gradients. This behavior suggests that increasing temperature accelerates the process. The activation energies results suggest that PGAE sample, characterized by an improved textural stability, showed higher sensitivity to aw changes induced by temperature. This can be attributed to differences in water mobility resulting from the addition of the extract, due to changes in matrix porosity or interactions induced by polyphenols or other constituents of the extract, as confirmed by the literature (Saidi *et al.,* 2025). The estimated activation energies are consistent with mechanisms driven by water diffusion and matrix modifications due to the thermal effect (Jassin *et al.,* 2022). Higher R^2 values in the zero-order model indicate that it better describes water activity kinetic behavior under these experimental conditions.

Table 2 *Kinetic parameters of crispness and water activity (aw) changes in potato slices stored at different temperatures.*

		Zero order		Arrhenius Model		First order		Arrhenius Model	
	T (°C)	**Equation**	**R^2**	**R^2**	**Ea (kJ mol^{-1})**	**Equation**	**R^2**	**R^2**	**Ea (kJ mol^{-1})**
					Crispness (kg sec^{-1})				
	15	-0.1201x±20.29	0.643			-0.0076x±3.00	0.609		
PB	20	-0.1238x±19.82	0.888	0.906	39.81	-0.0083x±3.00	0.911	0.913	88.20
	30	-0.2607x±19.89	0.867			-0.0425x±3.25	0.910		
	15	-0.0633x±18.26	0.893			-0.0040x±2.91	0.906		
PGA	20	-0.0706x±18.25	0.924	0.932	61.82	-0.0045x±2.90	0.943	0.912	122.81
	30	-0.2132x±16.00	0.888			-0.0437x±3.09	0.993		
	15	-0.0338x±14.66	0.501			-0.0024x±2.68	0.464		
PGAE	20	-0.0837x±15.70	0.890	0.947	77.04	-0.0073x±2.78	0.871	0.995	130.55
	30	-0.1773x±13.70	0.888			-0.0367x±2.81	0.954		
					Water activity (aw)				
	15	-0.0008x±0.75	0.721			-0.0011x±-0.29	0.723		
PB	20	-0.0007x±0.77	0.875	0.812	81.59	-0.0009x±-0.26	0.867	0.811	89.87
	30	-0.0037x±0.79	0.868			-0.0061x±-0.22	0.837		
	15	-0.0007x±0.74	0.633			-0.0010x±-0.30	0.635		
PGA	20	-0.0008x±0.76	0.730	0.918	80.78	-0.0011x±-0.27	0.734	0.915	89.50
	30	-0.0036x±0.77	0.863			-0.0059x±-0.24	0.826		
	15	-0.0017x±0.79	0.695			-0.0023x±-0.23	0.712		
PGAE	20	-0.0017x±0.84	0.966	0.888	48.15	-0.0022x±-0.18	0.964	0.875	57.15
	30	-0.0043x±0.82	0.895			-0.0068x±-0.19	0.872		

3.2 Analysis of quality changes of fennel during storage and degradation kinetics

Fresh-cut fennels undergo enzymatic browning mediated by polyphenol oxidase (PPO) after tissue damage (Giacondino *et al.,* 2025). The quality of fresh-cut products depends on both post-harvest treatments and packaging technologies. Plastic packaging is widely used, but it contributes to environmental pollution. So, biodegradable materials represent a promising alternative (Agustin-Salazar *et al.,* 2024). Table 3 shows the main quality parameters of fennels during storage at 4 °C. Samples C and T showed a significant increase in brightness (L*) over time, while B maintained more stable values. All samples showed a slight decrease in H°, which may indicate early stages of enzymatic browning or pigment degradation, due to the enzymatic activities (PPO). However, no significant differences were observed among samples at the end of storage. The saturation, expressed by Chroma (C*), tended to reduce in B and T samples stored in plastic packaging, while it was constant in C samples. Our previous study confirmed the observed results that demonstrated a limited effect of the packaging materials on brightness and hue stability of fresh-cut fennel (Giacondino *et al.,* 2025). Furthermore, the colour change (Chroma*) tended to decrease only in samples stored in PP, and it was stable in samples stored in biodegradable packaging. Cellulose-based and PP packaging showed comparable effectiveness in preserving color stability in fresh-cut vegetables (Paulsen *et al.,* 2024; Agustin-Salazar *et al.,* 2024). This suggests that color changes are dependent on different storage factors than on the type of packaging material alone. A general decrease in hardness

was observed in all samples, with a significant reduction over time and no significant differences among treatments. Both conventional plastic and biodegradable packaging did not significantly influence the consistency of fresh-cut vegetables, as confirmed by González-Buesa *et al.* (2014) who tested celery stick firmness, similar to fennel slices. The moisture content of fennel slices remained almost constant over time, with a lower value in C compared with the other samples at the end of storage, in line with what was observed by Agustin-Salazar *et al.* (2024). This is probably due to the higher moisture sensitivity and permeability of biobased films compared to polyolefin films (Mahajan e Lee, 2023). Total bacterial count increased in all samples, as expected for fresh-cut vegetables, with a lower growth observed in T during storage. Packaging materials, with different gas permeability had so influenced this observed result. Cellulose-based film has then shown potential applications in the preservation of fresh-cut fennel, showing comparable performances to conventional plastic packaging.

Table 3 *Changes in the main quality parameters of fennels during storage at 4°C*

	Time/ Samples	0	6	17	Sign.		Time/ Samples	0	6	17	Sign.
	B	70.89±5.18^{bB}	73.83±1.03	72.93±3.79	*ns*		B	33.51±6.81^{a}	28.36±4.65^{ab}	24.55±5.6^{b}	*
*L**	C	70.89±5.18^{bB}	74.09±1.02^{a}	75.51±2.56^{a}	**	*Hardness (N)*	C	33.51±6.81^{a}	26.89±2.72^{b}	21.18±2.34^{b}	**
	T	70.89±5.18^{bB}	74.5±1.29^{a}	75.26±3.26^{a}	**		T	33.51±6.81^{a}	26.4±4.03^{b}	24.1±4.48^{b}	**
	Sign.	*ns*	*ns*	*ns*			*Sign.*	*ns*	*ns*	*ns*	
	B	10.23±1.66^{a}	9.22±0.77^{b}	8.95±1.75^{bAB}	**		B	93.8±0	94.21±0.58	94.75±0.68^{A}	*ns*
*C**	C	10.6±1.34	9.93±0.57	10.08±2.19^{A}	*ns*	*Moisture content (%)*	C	93.8±0	93.61±0.3	92.33±1.03^{B}	*ns*
	T	10.6±1.34^{a}	9.1±0.84^{ab}	8.3±1.05^{bB}	*		T	93.8±0	94.32±0.15	94.95±0.17^{A}	*ns*
	Sign.	*ns*	*ns*	*			*Sign.*	*ns*	*ns*	**	
	B	94.62±2.42^{a}	92.38±0.83^{bB}	92.12±2.67^{b}	**	*Total bacterial*	B	3.44±0.01^{c}	6.32±0.05^{bA}	10.52±0.03^{aA}	**
H°	C	94.62±2.42^{a}	93.29±1.32^{abAB}	92.63±1.99^{b}	*	*count (Log*	C	3.44±0.01^{c}	6.56±0.04^{bA}	10.58±0.06^{aA}	**
	T	94.62±2.4	93.61±1.95^{A}	93.13±2.37	*ns*	*CFU g^{-1})*	T	3.44±0.01^{c}	5.15±0.21^{bB}	9.11±0.14^{aB}	**
	Sign.	*ns*	*	*ns*			*Sign.*	*ns*	**	**	

*The results are reported as mean ± d.s.. Small letters within a row and capital letters within a column show significant differences as assessed by Tukey's post hoc test. Abbreviations: **, significance at p < 0.01; *, significance at p < 0.05.*

Food quality parameters evolve over time, and storage temperature acts as one of the key factors in accelerating or decelerating degradation mechanisms. Table 4 presents the results of a regression study of zero- and first-order reaction kinetics of quality parameters of fennel samples under various storage conditions. Both quality parameters exhibited a clear trend influenced by storage temperature, with faster hardness loss and microbial growth observed at higher temperatures (10 °C and 15 °C compared to 4 °C) as evidenced by the absolute increase in the slopes of the kinetic curves with increasing temperature. The results of hardness (N) showed R^2 values higher for the first-order model, indicating that it can better describe the degradation process for this parameter. The activation energies (Ea) showed a lower value in C, which implies that their consistency is more thermally susceptible, while B seems more stable, requiring more energy for structural change. Reported activation energies for quality parameters, like consistency, range from 30 to 67 kJ mol^{-1}, using zero- and first-order models (Zhang *et al.,* 2021). The microbial growth accelerates with increasing temperature in all samples, as expected. The linearity of the kinetic curves with positive slopes confirms an exponential growth pattern, which is coherent with microbial proliferation (Tsironi *et al.,* 2017). For all samples, the microbial growth rate increased with temperature due to the general enhancement of metabolic and enzymatic activity at higher thermal conditions. R^2 values for zero order (> 0.99), indicating a good fit for all samples. T showed the highest temperature sensitivity, with Ea values reaching 49.42 kJ mol^{-1}. Tsironi *et al.* (2017) reported a great dependence of total microbial count on temperature, with activation energies of the same order of magnitude for minimally processed salads. This confirms the validity of the adopted kinetic model and highlights the dependency of the parameter on different storage temperatures.

Table 4 *Kinetic parameters of hardness and total bacterial count changes in fennel samples stored at different temperatures*

		Zero order		Arrhenius Model		First order		Arrhenius Model	
	T (°C)	Equation	R^2	R^2	Ea (kJ mol^{-1})	Equation	R^2	R^2	Ea (kJ mol^{-1})
					Hardness (N)				
	4	-0.4212x±31.23	0.745	0.999	48.42	-0.0148x±3.44	0.769	0.997	54.64
B	10	-0.6649x±31.61	0.896			-0.0255x±3.46	0.921		
	15	-0.9386x±31.60	0.905			-0.0364x±3.46	0.934		
	4	-0.6466x±31.32	0.883			-0.0247x±3.45	0.915		
C	10	-1.0147x±31.24	0.785	0.703	24.79	-0.0370x±3.44	0.820	0.847	26.77
	15	-0.9587x±33.96	0.956			-0.0380x±3.53	0.990		
	4	-0.4297x±30.81	0.720			-0.0152x±3.43	0.754		
T	10	-0.6149x±30.92	0.751	0.999	37.23	-0.0228x±3.43	0.769	0.997	40.60
	15	-0.7953x±31.78	0.691			-0.0297x±3.46	0.669		
					Total bacterial count (Log CFU g^{-1})				

	4	0.3772x±4.33	0.928			0.0564x±1.48	0.836		
B	10	0.4989x±3.59	0.976	0.999	31.27	0.0797x±1.31	0.946	0.988	32.19
	15	0.6335x±4.40	0.922			0.0958x±1.49	0.823		
	4	0.3732x±4.47	0.892			0.0560x±1.50	0.807		
C	10	0.5156x±3.80	0.948	0.991	40.97	0.0750x±1.42	0.888	0.975	41.74
	15	0.7389x±3.71	0.973			0.1125x±1.37	0.924		
	4	0.4511x±4.71	0.888			0.0625x±1.54	0.797		
T	10	0.5204x±4.05	0.949	0.994	49.42	0.0732x±1.48	0.846	0.992	46.34
	15	0.7088x±3.60	0.966			0.1097x±1.35	0.930		

4. Conclusions and Future Perspectives

We observed the impact of edible coating and different packaging types on the quality parameters of potato and fennel slices during storage at different temperatures. Results showed that the tested treatments affected the quality parameters of the potatoes and fennel slices differently, depending on the specific considered parameter. Zero- and first-order kinetic models were able to describe these changes, as the different degradation processes follow distinct reaction kinetics. The biodegradable packaging and edible coatings helped to maintain quality or did not adversely affect it, indicating that they can be considered valuable, even as an efficient and sustainable alternative to more conventional preservation methods. With a promising potential for commercial applications, these findings emphasize the significance of kinetic modeling in understanding the mechanisms of quality degradation at various temperatures. This will lead to the optimization of production and distribution technology processes to prevent or reduce both quantitative and qualitative food losses.

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Sustainability and innovation of spray packaging applied to food and nutraceutical products: development of innovative sprayable solutions and study of their application in bakery and confectionery

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This PhD thesis research project is aimed at setting up an experimental procedure for the identification and the development of different formulations (such as mousses, dressings, and nutraceuticals) that can be packaged in aerosol spray cans used in confectionery and bakery. They will be first produced through pilot plants, then rheologically and technologically characterized; the process will be finally validated in order to be applied on an industrial scale.

Sostenibilità e innovazione del confezionamento spray applicato ai prodotti alimentari e nutraceutici: sviluppo di soluzioni spray innovative e studio della loro applicazione per la produzione di prodotti da forno e prodotti dolciari.

Questo progetto di tesi di dottorato mira a mettere a punto un procedimento sperimentale per l'individuazione e lo sviluppo di diverse formulazioni (quali mousse, decorazioni, preparazioni e prodotti nutraceutici) che possano essere confezionate in bombolette spray successivamente impiegate nelle filiere dei prodotti dolciari e da forno. Queste verranno prodotte attraverso impianti pilota, caratterizzate dal punto di vista reologico e tecnologico, e il processo verrà poi validato in modo da poter essere applicato anche su scala industriale.

Key words: Aerosol and spray packaging; foam; rheology; innovation; sustainability; emulsion.

1. Introduction

Aerosol spray packaging can represent a good alternative to traditional packaging due to its high recyclability, ease of use, and adaptability. This PhD research project aimed to investigate and evaluate the application of this packaging to different products. This oral communication reports the main obtained results of the following activities and objectives investigated throughout the three years of PhD:

A1) constant deepened research focused on scientific articles and commercial products during the entire PhD project;
A2) application of the aerosol spray packaging to already existing products intended for bakery applications and subsequent characterization and evaluation of the developed samples to investigate their technological and microbiological stability;
A3) development of a new sprayable product that can be packaged in an aerosol spray bottle, definition of the processing parameters, technological and rheological evaluation of the samples (plant-based foams);
A4) investigation of the effect of thermal treatment, mild technologies, and preservatives on the technological and microbiological stability of the samples;
A5) recipe implementation of some of the most performant foams with the addition of extracts and study of their bioaccessibility through in vitro digestion.

2. Aerosol Spray Applications

Aerosol spray packaging represents a good solution for food products: it is versatile, easy to use, clean and efficient; moreover, aerosol cans are made of completely recyclable material, they can guarantee longer storage times without the use of cold chain, they can reduce the chances of contaminations, and they contribute to waste reduction (for no unused product to be disposed of) (Stahl et al., 2017; Marsh & Bugusu, 2007; Associazione Italiana Aerosol, AIA). These reasons made it possible to hypothesize its application even to foods and products that currently have another type of container, thus implementing their shelf life, durability and practicality of use. By acting in this way, it would be able to develop innovative and more sustainable products. Since bakery and confectionery are investing a lot in these, the application of this packaging technique was investigated on some already existing preparations (such as glazers) and on completely new formulations (a plant-based foam). Rheological measurements were fundamental during both formulation and characterization of the samples to completely understand any possible change in viscosity and visco-elastic properties.

3. Experimental Procedure

During the first year of PhD, the application of aerosol spray packaging was investigated applied on a plant-based

glaze mixture intended for bakery productions. The glaze was characterized before and after spray packaging, the effect of ethanol as a preservative was also examined to improve the product shelf-life. After obtaining positive results, the development of a completely new product to be packed into a spray can was studied. Deepened bibliographic research (i) was fundamental to understanding the ingredients that could be used for the new formula: more than 60 ingredients were considered, found in both scientific papers and commercial products. Among the analysed commercial products, three plant-based UHT-treated preparations were technologically characterized (ii) to understand their physical properties and the methodologies that could better explain the behaviour of this kind of matrix. This was useful to determine the first formula (iii) and to define the production process parameters (iv). Different recipes were developed (v) and tested before and after whipping through siphon charged with N_2O (vi). Formulations were consequently adjusted and improved, the most performant samples were technologically characterized and packaged inside a spray can by Volcke Aerosol Italy S.r.l. (specialized in aerosol packaging) to verify their adaptability and erogation yield (vii). The effect of preservatives addition and the application of some hurdle technologies (viii) were investigated to improve the stability of the samples. Finally, samples were subjected to in vitro digestibility test (ix); a recipe implementation, through the addition of a vegetable extract, was also considered and bioaccessibility of the contributed antioxidant compounds was assessed too (x).

4. Materials and Methods

Samples were produced through the all-in-one system machine RoboQbo Qb8-4 (Bologna, Italy) that could easily mix the different ingredients grinding up the largest particles and capable of maintaining a specific treatment temperature for the desired minutes. Water was heated to the set temperature of 60°C and powder ingredients were then mixed in at 750 rpm for 1 minute, depending on the formulation. The interested vegetable oil was first melted, added, and left under continuous agitation at 750 rpm for 10 minutes; and lastly powdered sugar was included by mixing for 15 minutes at 150 rpm. Samples were produced and analyzed after cooling, on the same day of production.

The liquid mixtures were characterized by their color, pH, particle size distribution, and viscosity. Samples (300g) were then whipped through a siphon charged with N_2O (8g); physical and chemical properties (color, and pH) were measured together with viscosity, viscoelasticity, and textural properties through a test profile analysis (TPA, double compression test through a 40 mm stainless-steel compression plate). Rheology was investigated through a MCR 302 RheoCompass (Anton Paar GmbH) using a parallel plate geometry (PP50) at 20.00±1.00°C; viscosity was measured applying a shear stress from 0.1 to 100 s^{-1} in 1 mm gap for liquid samples and 6 mm gap for whipped foams; viscoelasticity was investigated carrying out an amplitude sweep test at 1 Hz with 0.01% to 100% strain, followed by a frequency sweep test from 0.1 to 1000 ω within the linear range at the same strain (0.01%).When required, the homogenization treatment was carried out through a high-pressure homogenizer (GEA PandaPLUS Lab Homogenizer 2000, GEA Group AG) applying a single cycle homogenisation at 500 bar. In vitro digestibility was measured through INFOGEST® methodology (Brodkorb et al., 2019) in collaboration with Universitat Politécnica de Valéncia (UPV). Some samples were then packaged by Volcke Aerosol Italy S.r.l. into aluminium cans where 90g of product were inserted in a 45x125 mm bottle and then pressurized with 2g of N_2O. The same analytical procedures were applied in the trials conducted on the UHT-treated plant-based glaze.

5. Results and Discussion

The study and the evaluation of the effect of ethanol addition on a UHT-treated plant-based glaze for bakery applications were fundamental to understanding the potential of this technology, its weaknesses and strengths. Starting from this trial it was possible to define the recipes, the methodologies and the parameters to reach in terms of an innovative plant-based foam formulation. The results obtained can provide a comprehensive view of the world of foams and of the necessary technological stability to be achieved.

5.1 Sprayable plant-based glaze

A UHT-treated plant-based glaze made with water, pea protein, dextrose, starch, and acidity regulator (disodium phosphate) was physically characterized before applying the aerosol-spray packaging technology. To guarantee its microbiological stability, ethanol as a preservative (Sauerbrei, 2020) was added at different percentages (5%, 7.5%, 10%, 15%, 20%) and its ability was tested under vacuum and non-vacuum at two storage temperatures (4°C, 25°C). A microbial challenge test was carried out to determine its effectiveness in inhibiting bacteria (*Escherichia coli, Latilactobacillus sakei, Staphylococcus xylosus, Bacillus subtilis*), yeasts (*Saccharomyces cerevisiae, Zygosaccharomyces bailii*), and molds (*Penicillium chrysogenum*). Physical and rheological tests (such as pH, water activity, moisture, color, viscosity, viscoelasticity, flocculation, particle size distribution) were fundamental in defining the new glazes characteristics. Ethanol showed to be effective in preserving glaze quality and stability, but its addition had an impact on glaze viscosity and particle size distribution: at increasing percentages, there was a higher microbial growth reduction, but also a decrease in viscosity making the glazes denser making consequently harder the erogation through the nozzle. All glazes containing maximum 10% ethanol showed a viscosity and particle size distribution suitable with aerosol packaging: 10% addition stored at 25°C resulted in the

best sample in both microbiological (Figure 1) and technological terms.
Samples were than analysed applying three glazing techniques such as pastry brush, aerosol-spray can (mixture of N_2 propellant and glaze), and bag-on-valve system (compressed air surrounding a welded bag containing the glaze) with the help of Volcke Aerosol Italy. Glazes were then poured on frozen croissants using the different techniques, baked, and evaluated for color and texture (cutting test). From this trial, it was possible to define that ethanol addition did not affect the crispiness of glazed croissants; comparing the glazing techniques, 33% less glaze was used when glaze was sprayed together with a reduction in the erogation time obtaining equivalent color and texture of brushed goods. Among spraying techniques, bag-on-valve system was the most efficient providing continuous spraying. In conclusion, this first part of the study demonstrated that ethanol can be easily added to a plant-based glaze to improve stability and microbiological safety; also, aerosol-spray packaging technology results in a sustainable, anti-waste, easy- and ready-to-use, and stable product without the need for a cold chain.

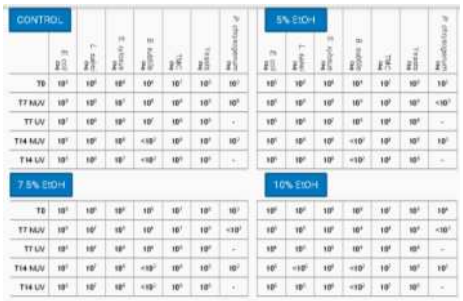


Figure SEQ Figure * ARABIC 1: *Growth trend of the microorganisms inoculated in the samples (CTR, 5% EtOH, 7.5% EtOH, 10% EtOH). Results are expressed as logarithm concentration (CFU/mL).*

5.2 Foams formulations and production

The research conducted during the first year of PhD (section 5.1) allowed to understand more information about the spray packaging technique and the physical characteristics that a product requires to be inserted and erogated. From this, the formulation of a new sprayable plant-based preparation that can be packaged into a spray bottle ready to be whipped was investigated. In particular, the new product should be able to maintain its structure after whipping, have a neutral taste and be stable at room temperature for almost 12-18 months. More than 30 already existing products and more than 20 papers were analyzed to understand the main ingredients which were used to prepare similar products; from the bibliographic research conducted, sucrose, vegetable fats, milk proteins, isolates from pulses, and ester and mono-diglycerides of fatty acids were the most common for creams, mousses and fresh doughs preparations. Three commercial products (a spray plant-based cream, a ready to be whipped UHT-treated plant-based cream, and a preparation for a neutral plant-based mousse) were then selected and analyzed to be physically characterized and to evaluate their whipping capacity. Samples were whipped through different techniques: whisk in planetary, IKA stirrer, whisk in RoboQbo all-in-one system, and siphon charged with N_2O. At this phase, it was possible to affirm that samples showed a neutral pH, any color difference was due to the different ingredients used, and that the whipping technique can affect the final textural properties. Samples particles dimension was affordable for the nozzle of the spray bottle (remaining below 150 μm). Investigating rheology, samples exhibited a shear thinning behavior with a prevalence of the storage modulus (G') on the loss one (G'').
Considering this information, the production process was developed at UPV using a Thermomix 31-1 (Vorwerk & Co. KG, Wuppertal, Germany): heat water up to 60°C, add emulsifiers under strong agitation, melt fats at 65°C and then add to the water phase under a strong agitation for 10 min, continue agitating for 10 min, finally add sugars and salt and mix 15 min, collect and cool at 25°C. Samples compositions are reported in Table 1, they were analyzed before and after whipping through a siphon charged with N_2O. The same procedure was followed at Università Cattolica del Sacro Cuore (UCSC) by using RoboQbo Qb8-4: setting 750 and 150 rpm velocity to reply respectively strong and continuous agitation (samples reported in Table 2).

Table 1 *Samples composition produced at UPV, specific quantities and names of ingredients cannot be detailed for copyright reasons (a double X represents a higher amount of the same ingredient).*

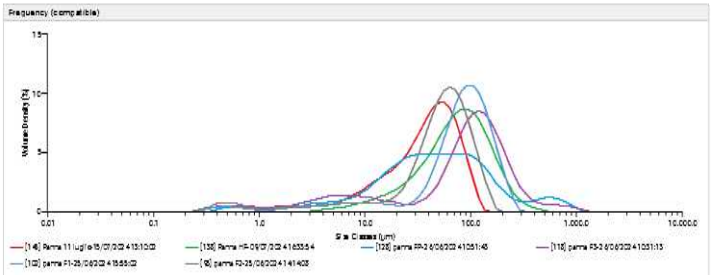
Ingredients	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Vegetable fat	X	X	X	X	X	X	X	-	X	X
Sugar	XX	XX	XX	XX	X	X	X	XX	XX	XX
Thickener	X	XX	X	X	X	X	X	X	X	-
Emulsifier	-	-	X	XX	XX	XX	XX	XX	XX	-
Salt	X	X	X	X	X	X	X	X	X	X
Fiber 1	-	-	-	-	X	-	-	XX	-	-
Sweetener 1	-	-	-	-	-	X	-	-	-	-
Sweetener 2	-	-	-	-	-	-	X	-	-	-
Fiber 2	-	-	-	-	-	-	-	-	-	X

Table 2 *Samples composition produced at UCSC, specific quantities and names of ingredients cannot be detailed for copyright reasons (a double X represents a higher amount of the same ingredient).*

Ingredients	F1	F2	F3	F4	F5	F6	F7

Vegetable fat 1	X	X	X	-	-	-	-
Vegetable fat 2	-	-	-	X	XX	XX	XX
Sugar	X	-	XX	-	XX	XX	XX
Sweetener	X	XX	-	XX	-	-	-
Thickener	X	X	X	X	-	-	-
Emulsifier	X	X	X	X	-	-	-
Salt	X	X	X	X	XX	XX	XX
Fiber 1	-	X	-	X	-	-	-
Fiber 2	-	-	-	-	X	X	-
High protein flour 1	-	-	-	-	X	-	X
High protein flour 2	-	-	-	-	-	X	-
Fiber 3	-	-	-	-	-	-	X

All samples exhibited a neutral pH (near or equal to 7) that didn't change after whipping. The TPA showed some differences among the samples: both firmness and adhesiveness changed depending on the different ingredients used (the highest variability was recorder with the addition of fibers and proteins). All samples exhibited a bright light color, with some shades of green or yellow depending on their composition. Through the rheology measurements it was possible to evaluate samples viscosity at 20°C before and after whipping: a shear thinning behavior was demonstrated in both cases. Considering the viscoelasticity, the formulas showed a greater G' compared to G'': foams were quite stable under the stress, but they all exhibited really low values of LVER (0.01%). Particle size distribution was also monitored before and after whipping: the majority of the particles are below 150µm, meaning that they could pass through the nozzle of the spray bottle (Figure 2); additionally, the particle dimension seemed not to be affected by whipping. At this stage, it was possible to obtain a sprayable plant-based foam that can be whipped through a siphon, formulations should be refined to obtain a product that could be placed easily inside a spray bottle and maintain a good structure once sprayed. The recipe has to be adjusted also to obtain a more neutral taste.



5.3 Investigation of preservatives and treatments on foams stability

The formulations previously developed were implemented and modified to reach a lower viscosity and allow better filling and spraying. To do that water content was increased, one vegetable oil was selected as the most performant, and the kind of sugars added helped in increasing the product flow. New recipes were developed (as reported in Table 3) investigating the role of different protein sources (flour 1, 2, and 3), the combination of various preservatives, and the effect of sweeteners addition on the final taste.

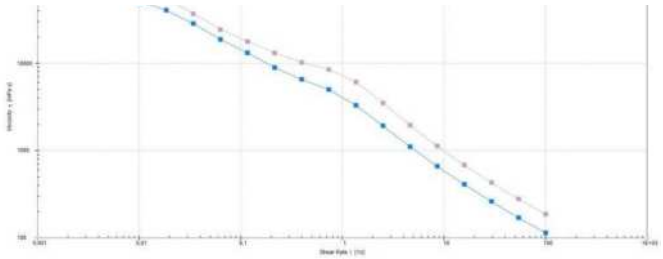
Table 3 *Composition of the sprayable foams, specific quantities and names of ingredients cannot be detailed for copyright reasons(a double X represents a higher amount of the same ingredient).*

Ingredients	CPS	CPSC	CPSB	C	CPSB1	CPSBC	CPSBC1	CE	CEP	CPSBCE	CPSBCM	CPSBCME
Sugar 1	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	X	X
Vegetable oil	X	X	X	X	X	X	X	X	X	X	X	X
Sugar 2	X	X	X	X	X	X	X	X	X	X	X	X
Emulsifier	X	X	X	X	X	X	X	X	X	X	X	X
Flour 1	-	X	-	-	-	-	-	-	-	-	-	-
Flour 2	X	-	-	X	-	-	-	X	X	-	-	-
Flour 3	-	-	X	-	X	X	X	-	-	X	X	X
Preservative 1	XX	XX	XX	-	X	X	X	-	X	X	X	X
Preservative 2	XX	XX	XX	-	X	X	X	-	-	X	X	X
Acidifier	-	-	-	-	-	XX	X	-	-	XX	XX	XX
Preservative 3	-	-	-	-	-	-	-	XX	X	X	-	X
Sweetener	-	-	-	-	-	-	-	-	-	-	X	X

Samples were technologically and rheologically analysed to verify their stability, their erogation yield was also evaluated after packaging in aluminium spray cans. Foam made using legume flour resulted in being more stable in visco-elastic terms when compared to other flours with high protein content. Samples were thermally treated at 60°C (as reported in section 5.2), for 5 min at 80°C, and for 30 min at 110°C: foams containing legume flour showed a good adaptability to the treatment and a lower protein denaturation, thus reaching a higher stability a

lower viscosity variability (Figure 3). To contain microbial contamination, a pH reduction (below 4.5) was carried out adding an acidifying solution.

Figure SEQ Figure * ARABIC 3*: Viscosity vs Shear Rate of CPS B treated at 60°C (blue) and 80°C (violet).*



CPS BC sample was selected as "base recipe" to which some variations were applied: a preservative was added to improve the sample stability (CPS BC E), a sweetener was tested to reduce sweetness (CPS BC M) (Helstad., 2019), a combination of both techniques was also considered (CPS BC M E). These samples were analysed from a rheological point of view exhibiting a shear thinning behaviour and a higher G' compared to G''; furthermore, the reached viscosity allowed the aerosol spray packaging. The application of thermal treatment (10 min at 80°C) and of homogenization (single cycle at 500 bar) was also considered to understand how they could impact on the different recipes. Samples treated at 80°C showed a lower viscosity thus suggesting a more liquid consistency preferred for the spray can; homogenization seemed not to have an effect.

5.4 Foams in vitro digestibility and recipe implementation

The most performant samples in terms of technological stability and erogation yield were then subjected to the in vitro digestion analysis (IVD). CPS BC, CPS BC E, CPS BC M, and CPS BC M E treated at both 60°C and 80°C were digested through the INFOGEST® methodology to understand the impact of the thermal treatment on the foam digestibility. All samples treated 10 min at 80°C showed a higher percentage of IVD compared to samples treated at 60°C due to protein denaturation and a higher fat dispersion related to the temperature reached. The differences in the formulations' composition seemed not to affect the digestibility of the final product.

The same four samples treated at 80°C were then implemented adding a vegetable extract made with pumpkin and carrot with colouring function. IVD was carried out together with a bioaccessibility evaluation of some compounds such as phenolic compounds, antioxidants, and carotenoids (Igual et al., 2022). The extract addition changed the color of the foam to pale yellow, it didn't impact on the taste or on technological characteristics. Considering IVD, it was lower for all samples added with Y also if always maintaining an IVD higher than 96%. The Y enrichment showed positive results in terms of carotenoids bioaccessibility meaning that CPS BC formula can be implemented from a nutritional point of view with extracts, vitamins, or nutraceutical compounds.

6. Conclusions and Future Perspectives

This research demonstrated that aerosol spray packaging represents a viable method for a range of food preparations: following multiple trials, final samples exhibited high compatibility with the packaging system and maintained satisfactory performance upon erogation. Recipe implementation and corresponding process modifications could be considered to develop specialty products and nutraceuticals; indeed, the results suggest that the formulation exhibits good adaptability for enrichment with extracts and dietary supplements.

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Free and microencapsulated olive pomace extracts to improve the phenolic content and shelf-life of fresh pasta

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This PhD thesis dealt with the scale-up of innovative food solutions proposed by research, to a level closer to industrial application. Food prototypes developed at laboratory level (Technology Readiness Level, TRL 2-3), were validated and designed at the industrial level (TRL 5-6). The project issues are in line with the mission 4 component 2 "M4C2" purpose of the National Recovery Plan and Resilience document, that provide support for investments in research and development and promote innovations and their transfer to food companies.

Impiego di estratto di sansa in forma libera o microincapsulata per migliorare il contenuto fenolico e prolungare la shelf-life della pasta fresca

Questa tesi di dottorato ha riguardato lo scale-up di soluzioni innovative alimentari proposte dalla ricerca, su una scala quanto più vicina possibile all'applicazione industriale. I prototipi alimentari sviluppati a livello di laboratorio (Technology Readiness Level, TRL 2-3) sono stati validati e progettati a livello industriale (TRL 5-6). Le tematiche affrontate sono in linea con l'obiettivo della Missione 4 Componente 2 "M4C2" del Piano Nazionale di Ripresa e Resilienza Italia, che prevede il supporto agli investimenti in ricerca e sviluppo, promuovendo l'innovazione e il suo trasferimento alle imprese del settore alimentare.

Key words: scale-up; food by-products valorisation; fresh pasta; shelf-life extension.

1. Introduction

In accordance with the PhD thesis project previously described, this oral communication reports the main results of the following two activities directed to:

A1) obtain a functional fresh pasta added with free or microencapsulated olive pomace extracts (OPE);
A2) investigate the effects of OPE (in both forms), on the physic-chemical and microbiological quality of fresh pasta samples during storage, in order to assess its potential for shelf-life extension.

2. Functional fresh pasta and shelf-life extension

Pasta is a cereal-based food of the traditional Mediterranean diet, appreciated worldwide due to its convenience, palatability, and nutritional quality. According to Italian Law (Presidential Decree n. 187 of 9 February 2001) fresh pasta should contain at least 24% of moisture and a_w ranging from 0.92 to 0.97. It is considered a rich source of carbohydrates, with low content of proteins, fibers, and bioactive compounds. Being pasta a highly consumed product, easy to prepare, and cheap, the Food and Drug Administration (FDA) recognized it as an excellent vehicle to be fortified with functional compounds with demonstrated biological activity. In addition, the increasing awareness of diet–disease consequences, together with consumers' preference for functional foods, encouraged food scientists to research new formulations for functional pasta manufacturing. These products are usually obtained by the addition of ingredients (as is or extracts) containing bioactive compounds (i.e., dietary fibers or phenolic compounds such as flavonoids, phenolic acids and others). Moreover, due to its high-water content, fresh pasta is particularly prone to spoilage and alteration. It requires refrigerated storage temperatures and a thermal treatment at least equivalent to pasteurization, to ensure shelf-life periods ranging from 30 to 60 days (Marzano et al., 2022). Fresh pasta spoilage is mainly due to the microbial activity of yeasts, bacteria and molds which can rapidly proliferate in this product (Del Nobile et al., 2009). In order to improve the microbial stability and extend the shelf-life of fresh pasta, different methods have been applied in literature: i) the modified atmosphere packaging (MAP) technique; ii) chemical preservatives, such as calcium propionate, potassium sorbate, sodium benzoate and sodium dehydroacetate (Marzano et al., 2022); iii) gamma irradiation and bio-preservation (Marzano et al., 2022). On the other hand, the use of natural ingredients with antimicrobial properties has gained considerable attention from scientific researchers (Maddaloni et al., 2025), driven by the increasing consumer demand for more natural and high-quality food. From a sustainability perspective, fruit and vegetable by-products could be used as a valuable source of phytochemicals, which can be exploited as functional ingredients and as natural preservatives, as well (Maddaloni et al., 2025), reducing their environmental and economic impact. Olive pomace (OP), the major by-product from the olive oil industry (Difonzo et al., 2021), is rich in functional compounds, such as polyphenols, tocopherols and sterols, with well-known antioxidants and anti-inflammatory properties (Difonzo et al., 2021). In addition, authors stated that OP has antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi (Nunes et al., 2021). Thus, the aim of these activities were to i) evaluate the effects of olive pomace extract

(OPE) as such and in microencapsulated form on the physicochemical, nutritional and sensory characteristics of fresh pasta; ii) assess the potential of OPE to extend the shelf-life of fresh pasta samples.

3. Experimental Procedure

During this PhD thesis, in order to achieve the two aforementioned objectives, seven types of fresh pasta samples were prepared: one control (CP, control pasta made with durum wheat semolina), three experimental samples added with Microencapsulated-OPE (MP-1, MP-2, and MP-3), and three samples added with Free-OPE (FP-1, FP-2, and FP-3). The experimental products were manufactured with different concentrations of OPE, obtaining pasta samples with increasing total phenolic concentrations: 0.17 mg GAE g^{-1} of pasta (MP-FP-1), 0.35 mg GAE g^{-1} of pasta (MP-FP-2) and 0.53 mg GAE g^{-1} of pasta (MP-FP-3). These fresh pasta samples were produced at industrial level in a factory located in Southern Italy (Pastificio Maffei, Cutrofiano, Lecce, Italy). The general manufacturing process was performed following the recipe used by Maffei company: 1000 g of durum wheat semolina (Casillo, Corato, Italy) were kneaded with 330 g of tap water for 15 min with a mixer (Kenwood Cooking Chef XL KCL95.424SI, United Kingdom). Doughs were extruded in order to obtain the typical "cavatelli" form, which were pasteurized at 100°C for a minute and a half. For MAP a combination of N2/CO2 of 70/30 was selected. Samples were stored at 4 °C and then analysed immediately after packaging and then after 15, 30, 45, 60, 70, 90, 110 and 120 days of storage.

4. Materials and Methods

4.1 Production of olive pomace extract and microencapsulation

Phenolic extract from olive pomace was obtained following the method proposed by Natrella et al. (2025). As first, inulin used as a wall material for the encapsulation process was recovered from artichoke roots, as described by Difonzo et al. (2022). Then, the microencapsulation of OPE was carried out as reported by Natrella et al. (2025).

4.2 Sensory analysis

Sensory analysis was carried out in order to have information about the effect of OPE on the sensory properties of cooked fresh pasta just after production, as reported by Natrella et al. (2025).

4.3 Chemical physical analysis

Fresh pasta samples were analysed for moisture, water activity, pH and colour as described by Natrella et al. (2025).

4.4 Total phenolic compounds and antioxidant evaluation

Total phenolic compounds (TPC) and ABTS and DPPH assays for antioxidant activity were measured on raw fresh pasta samples during storage time and on cooked samples just after production. Each analysis was performed in triplicate, as described by Caponio et al. (2022) and Difonzo et al. (2022).

4.5 Microbiological analysis

Microbiological analyses were carried out on pasta samples immediately after packaging (D0) and after 15, 30, 45, 60, 70, 90, 110 and 120 days of refrigerated storage at 4 °C. Twenty five grams of samples were aseptically removed from each package and homogenized with 225 mL of sterile buffered peptone water (0.1% w/v) in a stomacher (Bag Mixer, Interscience International, Roubaix, France) for 2 min, as described by Marzano et al. (2022). Each sample was tested in triplicate.

5. Results and Discussion

5.1 Sensory analysis

Figure 1 shows the GPA of the sensory analysis performed on cooked fresh pasta samples. The analysis explained 89.02% of variance, 76.77% of which was along the first component (PC1) and 12.25% along the second component (PC2). The first component clearly showed the effect of the addition of different OPE concentrations. In fact, it is possible to observe a clear separation among samples within three different clusters. The one on the positive side of PC1 represents the samples with the mildest and most typical sensory characteristics, in fact, this area comprises CP and the experimental samples with the lowest OPE concentration, indicating that these samples resulted in very similar results for judges. Then, on the negative side of the PC1 clustered the other two groups, representing the samples at higher OPE concentration used. These two groups differed by considering PC2, meaning that they were perceived differently by judges. Concerning the descriptors, the group that lay on the IV quadrant was characterized by a characteristic odor and taste. On the other hand, as shown in Figure 1, the other two groups were characterized by color, astringency, and bitterness, surely provided by the increasing OPE concentration.

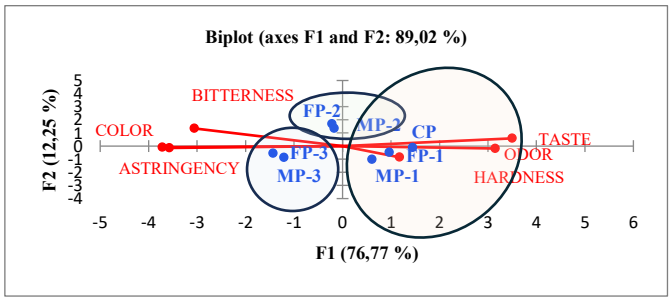


Figure 1 *Generalized Procrustes Analysis (GPA) of the sensory analysis results.*
Abbreviations: CP, Control fresh pasta; MP, fresh pasta sample added of microencapsulated OPE at three different concentrations (MP-1, MP-2, MP-3); FP, fresh pasta added of free OPE at three different concentrations (FP-1, FP-2, FP-3).

5.2 Loss of TPC during cooking

Figure 2 shows the TPC values in raw and cooked fresh pasta samples immediately after production. Cooked pasta samples showed lower TPC, compared to raw samples. The reduction observed could be attributed to the molecules' heat degradation during boiling treatment or their solubilization into the cooking water. Despite the reduction, MP-3, MP-2, and FP-3 samples still had the highest polyphenol content. In general, MP samples showed a higher reduction percentage after the cooking process than FP samples. These outcomes could be accounted to the imperfect formation and low stability of the gluten matrix (especially for MP-3 sample, data not shown), which could promote losses during cooking. Nonetheless, MP-cooked samples had a final polyphenol concentration, on average, similar to or higher than that of FP samples. In addition, despite the recorded losses, the experimental samples showed higher antioxidant activity than CP, according to the DPPH and ABTS assays (data not shown).

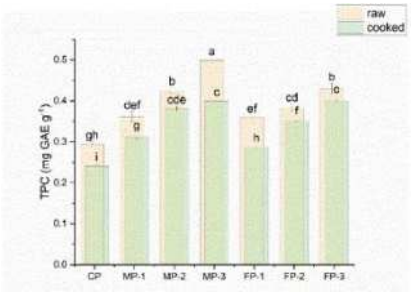


Figure 2 *Total polyphenol content of raw and cooked fresh pasta samples just after production. The values represent means of triplicates ± standard deviation; different letters mean significant statistical differences ($p < 0.05$) to one-way ANOVA followed by Tukey's HSD test.*
Abbreviations: CP, Control fresh pasta; MP, fresh pasta sample added of microencapsulated OPE at three different concentrations (MP-1, MP-2, MP-3); FP, fresh pasta added of free OPE at three different concentrations (FP-1, FP-2, FP-3).

5.3 Moisture content, water activity and pH

Moisture and a_w of CP and experimental samples immediately after production and after 120 days of storage are reported in Figure 3A. Moisture showed a constant decreasing trend from 0 to 120 days of storage, because of water release into the surrounding atmosphere, resulting in the condensation within the packaging (Sanguinetti et al., 2011). Moisture content of CP decreased from 31.10% to 28.18%. On the other hand, moisture in MP and FP samples declined on average from 29.10% to 25.87% and from 29.78% to 27.07%, respectively. It could be hypothesized that the overall increase in dry matter could account for the moisture reduction in samples with OPE. Notably, MP samples showed significantly lower moisture content compared to CP and to the corresponding samples with F-OPE, throughout storage time. Water activity showed a significant decrease for all the samples during storage. This phenomenon may result from the water migration from samples to atmosphere inside the packaging, as confirmed by the results on moisture. In fact, after 120 days of storage, CP had the highest a_w (0.945), followed by FP samples (0.943) and MP samples (0.939). It could be hypothesized that the wall material, used to encapsulate the vegetable extract, had the ability to bind water molecules. However, throughout storage time, all pasta samples still complied with Italian law (Presidential Decree n. 187 of 9 February 2001), according to which fresh pasta must have at least 24% of moisture and a_w ranging from 0.92 to 0.97.

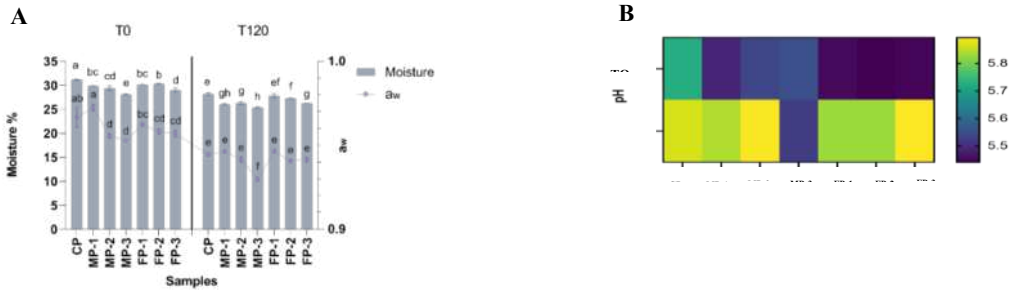


Figure 3 *Evolution of moisture (%) and a_w (A) and pH (B) of fresh pasta samples at the time of production (T0) and after 120 days of storage (T120). The values represent means of triplicates ± standard deviation; different letters mean significant statistical differences ($p < 0.05$) to two-way ANOVA followed by Tukey's HSD test.*
Abbreviations: CP, Control fresh pasta; MP, fresh pasta samples with M-OPE (microencapsulated OP extract); FP, fresh pasta samples with F-OPE (free lyophilised OP extract); the numbers 1,2,3 indicate the 3 different concentrations of OPE used.

Changes of pH can occur in food products as a result of chemical reactions and activities of microorganisms. pH showed a slight but significant increase, except for MP-3, from day 0 up to 120 days after production (Figure 3B). Several authors reported that proteins in fresh pasta could be decomposed by some proteolytic bacteria and molds, releasing some basic compounds, such as amine and ammonia, which can increase pH values. Immediately after production, the addition of OPE had a significant effect on pH ($p < 0.05$). Acid compounds, such as phenolic acids in OPE extracts could account for pH reduction (Difonzo et al., 2021).

5.4 Results of TPC and antioxidant activity

Total polyphenols content (TPC) and antioxidant activity of samples at T0 and T120 are presented in Figure 4A and 4B, respectively. It's worth noting that, increasing OPE concentration, TPC and antioxidant parameters increased ($p < 0.05$). Particularly, samples with M-OPE showed, on average, a lower TPC loss (84.46 %) from the time of production to 120 days of storage, compared to FP samples (86.33%). The OPE addition resulted in a significative increment of the antioxidant activity in the pasta samples, and the assessed antioxidant activity resulted to be strictly correlated to the amount of OPE added to the semolina, according to both DPPH and ABTS assays (Figure 4B). In line with TPC results, FP-1 at the end of storage, showed antioxidant activity, according to DPPH assay similar to CP. On the contrary, MP samples recorded higher antioxidant activity (for both DPPH and ABTS assays) (Fig. 4B). Comparing data at T0 to data at T120 for both TPC and antioxidant activity assays, a general decrease has been observed. The phenomena occurring during storage, such as hydrolytic and oxidative reactions, resulting from O_2 increase in the packaging, could have promoted the recorded losses and consequently compromise the availability of phytochemicals (Maddaloni et al., 2025).

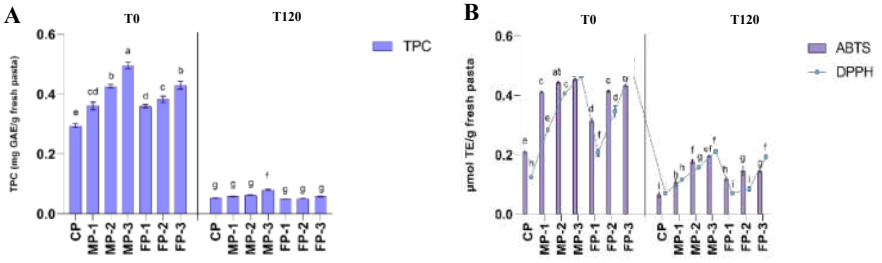


Figure 4 *Evolution of TPC (A) and antioxidant activity according to ABTS and DPPH assays (B) of fresh pasta samples at the time of production (T0) and after 120 days of storage (T120). The values represent means of triplicates ± standard deviation; different letters mean significant statistical differences ($p < 0.05$) to two-way ANOVA followed by Tukey's HSD test.*

5.5 Changes of color

Color is an important parameter of food products' acceptability for consumers, as it refers to their freshness and quality. L* values declined significantly during storage, until 70 days after production (for CP and FP-3), 90 (for all MP samples) and 60 days of storage (for FP-1 and FP-2) (data not shown). Subsequently, L* showed an increasing trend. Wang et al. (2023) stated that the decreasing lightness could be attributed to the oxidation of phenols and browning reaction. On the other hand, increase of L* values could be due to the wet surfaces of samples, which consequently caused an increase in the light scatter, as confirmed by water releases in the packaging atmosphere. Progressive decrease in lightness value was also observed, increasing level of M-OPE and F-OPE. a* values of all samples decreased during storage (data not shown). Anthocyanins degradation or their polymerization with other phenolic compounds may be correlated to a* reduction of processed foods. As expected, the addition of OPE extracts to the dough, significantly increased a* values ($p < 0.05$). This is probably due to the anthocyanins, responsible for red color, present in OPE. On average, cavatelli samples with M-OPE had higher a* values, compared to FP samples ($p < 0.05$). It could be assumed that inulin used as wall material for microencapsulation could increase redness index, as confirmed by Difonzo et al. (2022) on fresh tagliatelle with inulin. Concerning b* parameter, consumers greatly appreciated durum wheat pasta with high yellowish color. On the whole, all pasta samples were characterized by a decreasing trend of b* values throughout the storage time (data not shown). On average, for every sampling time, especially for samples with F-OPE, the greater the addition of OPE, the lower the yellow index. Furthermore, samples with microencapsulated extracts were characterized by higher b* value, compared to the corresponding samples with extract as such.

5.6 Microbiota of fresh pasta

Microorganisms play a priority role by contributing to the quality and desirable properties of the products (Marzano et al., 2022). Culture dependent analyses were performed on microorganisms that naturally occur and that produce alterations in fresh pasta during refrigerated storage. All pasta types were appropriate under the hygienic profile, as highlighted by the absence of pathogenic microorganisms (*Salmonella* spp., *L. monocytogenes*) and presumptive *Bacillus cereus* and the detection of beta glucuronidase-positive *E. coli*, *Clostridium spp*, coagulase-positive *Staphylococcus* and coliforms cell densities below the detection limit (<1 log CFU g^{-1}) in all samples up to 120 days (data not shown). The addition of OPE reduced the growth of spoilage microbes. Mesophilic aerobic, molds and yeast microbial groups (Figure 5A,B,C) investigated registered a significant reduction ($p < 0.05$) of ca. 0.5–1 logarithmic cycles in samples added with extract in free or encapsulated form compared to CP. Total mesophilic aerobic cell densities maintain values near the accepted value by the reference

standard (< 6 log CFU g^{-1}) for up to 120 days in all pasta samples, although CP pasta exhibits ca 0.5 - 1 Log CFU g-1 over at days 110 and 120 compared to experimental ones. Significantly ($p < 0.05$) lower counts were recorded for mould (Fig. 5B) and yeast (Fig. 5C) counts in fresh pasta added of free and microencapsulated olive pomace extract compared to CP, especially at 110 days of refrigerated storage. The significant reduction observed can be due to the presence of the extract, both in free and encapsulated form. In comparison to free, microencapsulated OPE delayed microbial growth during 120 days of storage at 4 °C and this outcome represents a key factor for the feasibility of the use of OPE for improved stability of fresh pasta during storage. Lactic acid bacteria (LAB) not reached a value greater than 1 Log CFU g^{-1} at the final collection time (D120) in samples, without significantly differences for addition of OPE (data not shown), as LAB are generally more resistant to phenolic products than other bacteria, for their abilities to develop structural or metabolic resistance towards this compound. Enterobacteriaceae (ENB) cell density (data not shown) does not change among samples, reaching values < 2 Log CFU g^{-1} at D120.

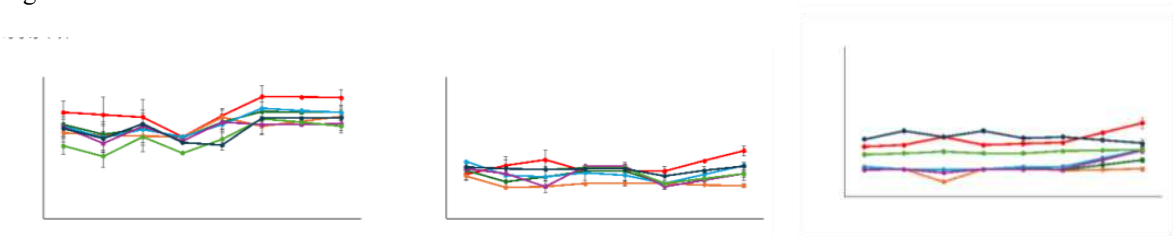


Figure 5 *Line chart concerning the growth of microorganisms (expressed as Log CFU/g) in fresh pasta samples analysed after 15, 30, 45, 60, 70, 90, 110 and 120 days (D) of refrigerated storage. Panel A: total aerobic mesophilic bacteria (TAMB); Panel B: Molds (MO); Panel C: Yeast (YE). Abbreviations: CP, Control fresh pasta; MP, fresh pasta samples with M-OPE (microencapsulated OP extract); FP, fresh pasta samples with F-OPE (free lyophilised OP extract); the numbers 1,2,3 indicate the 3 different concentrations of OPE used.*

6. Conclusions and Future Perspectives

Olive pomace (OP) is the major by-product of olive-oil chain, rich in sterols and phenolic compounds. As fresh pasta is a perishable food product, in this study, olive pomace extracts (OPE) in free (F-OPE) and microencapsulated (M-OPE) form were applied in "cavatelli" fresh pasta samples to assess their efficacy on the shelf-life extension. Thus, microbial quality, pH and moisture content, a_w and total phenolic compounds (TPC), were monitored during a refrigerated storage period of 120 days. Results showed that both M-OPE and F-OPE reduced moisture, a_w and pH, compared to control pasta sample (CP). Particularly, lower values of these parameters were observed for fresh pasta samples with M-OPE, compared to the corresponding samples with F-OPE. Microencapsulation process was useful to protect bioactive compounds during storage, as samples with M-OPE showed greater values of TPC and antioxidant activity than samples added of F-OPE. As expected, TPC, antioxidant activity, moisture and a_w decreased during storage. Notably MP samples at the end of storage had higher b* values compared to CP and to samples with F-OPE. Moreover, the addition of OPE, and particularly M-OPE reduced the growth of spoilage microbes; experimental samples had significantly lower counts for total aerobic mesophilic bacteria, mold and yeast, compared to CP. In the future it may be worth to quantitatively determine the effectiveness of OPE in inhibiting the growth of spoilage microorganisms under study and assess the time at which the viable cell concentration reached its acceptability limit.

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Oral presentation sessions
PhD students – 3rd year
Abstracts

PARALLEL SESSION 3
Innovative and Green Approaches for Food Safety and Sustainability

Use of Non-Thermal Treatments to Improve the Quality, Safety, and Shelf-Life of Raw Pork Sausages

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This PhD thesis research project is aimed at improving the safety, shelf-life and quality of raw pork sausages using non-thermal treatments including High Hydrostatic Pressure and bioprotective cultures.

Utilizzo di trattamenti non termici per il miglioramento della sicurezza, della qualità e della shelf-life di salsicce fresche di carne suina

Questa di tesi di dottorato ha come obiettivo il miglioramento della sicurezza, della shelf-life e della qualità di salsicce fresche di carne suina utilizzando trattamenti non termici tra cui le alte pressioni idrostatiche (HHP) e le colture di biocontrollo.

Key words: High Hydrostatic Pressure; raw pork sausages; food safety; shelf-life; color, texture.

1. Introduction

In accordance with the PhD thesis project previously described (Angelucci, 2023), this oral communication reports the main results of the following three activities directed at:

A1) Investigate the effects of thermal abuse on raw sausages quality both before High Hydrostatic Pressure (HHP) treatment and/or during the shelf-life of the product;
A2) Evaluate the effect of HHP treatment on the survival and proliferation of three pathogenic bacteria: *Salmonella* Enteritidis, *Staphylococcus aureus* and *Listeria innocua* (used as a surrogate of *Listeria monocytogenes*);
A3) Evaluate the effect of HHP on the color and texture of sausages stored at 4°C during the shelf-life.

2. Application of HHP treatments on raw pork sausages

Raw pork sausages are highly perishable due to their relatively high pH (not below 5.5) and water activity (a_w of about 0.97). Since this product is not subjected to fermentation during production, the sanitary quality of raw meat is crucial in ensuring quality and safety. Various factors can affect the quality and, therefore, the shelf-life of raw pork sausages. Spoilage involves complex mechanisms that may not only cause financial losses but also pose safety risks. This deterioration often leads to noticeable changes in the product's appearance, smell, flavor, and texture, making it unappealing and unacceptable to consumers (Zhou *et al*., 2010).
To increase the shelf-life limiting this spoilage phenomena, non-thermal technologies can be proposed. Among non-thermal technologies, HHP is one of the most promising. The latter is a non-thermal food processing technology that applies pressures between 200 and 700 MPa using a non-compressible fluid, typically water at temperatures below 100°C. This rapid method has a minimal impact on the sensory and nutritional qualities of food, enhances food safety and shelf-life by effectively inactivating a wide range of spoilage and pathogenic microorganisms, including bacteria like *E. coli* and *Staphylococcus aureus*. Microbial inactivation results from accumulated damages to essential cellular components, including proteins, membranes, ribosomes, cell walls, and DNA. Key factors influencing this process are treatment pressure, duration, intrinsic properties (such as pH, a_w, and chemical composition), and the physiological state of the cells (Simonin *et al*., 2012; Aganovich *et al.*, 2021). Under commercially applied conditions, HHP cannot be classified as a sterilization method. Consequently, products treated with HHP must be maintained within the cold chain (0-4°C) throughout distribution and storage to preserve both their sensory attributes and microbiological stability. When these conditions are upheld, a notable extension of the product's shelf-life can be achieved (Rendueles *et al*., 2011). However, exposure to thermal abuse at any stage, whether during production, storage, transport, or retail, can significantly compromise the shelf-life and safety of HHP-treated foods.

3. Experimental Procedure

In this PhD thesis, a three-step experimental procedure was set up by performing in sequence the following independent tests, in order to: i) evaluate the effect of HHP treatment (600 MPa for 5 min) on raw pork sausages thermally abused both prior and/or during the storage time; ii) ascertain the influence of the HHP treatment (600 MPa for 5 min) on the survival and proliferation of *Salmonella* Enteritidis, *Staphylococcus aureus* and *Listeria innocua* using challenge tests; and iii) investigate the effect of that treatment on two of the most important quality

parameter such as color and texture in samples stored under appropriate storage conditions (0-4°C).

4. Materials and Methods

Fresh pork meat and subcutaneous fat were obtained from hot-deboned shoulder cuts (53 kg) and utilized for sausages preparation 24 hours post-mortem. The lean meat to fat ratio was maintained at approximately 80:20. The raw material was coarsely ground using a 4-5 mm plate at 0°C and mixed with 2.1% sodium chloride (NaCl) (w/w), 0.1% ground black pepper (w/w), 0.15% ascorbate (w/w), and 0.01% sodium nitrite ($NaNO_2$) (w/w). The seasoned meat batter was then stuffed into natural casings, forming sausages weighing approximately 100 g each, and vacuum packaged in sets of four per package. HHP was applied 24 hours after sausage production. Prior to HHP application, half of the packages were stored at 15°C to simulate initial thermal abuse, while the remaining packages were maintained at 4°C. Following overnight storage at these two temperatures, a subset of samples was subjected to HHP treatment, whereas the remaining samples remained untreated. All samples, regardless of treatment, were subsequently stored under two different temperature conditions to simulate potential cold chain deviations: one group at 4°C (groups: NT-15-4, NT-4-4, 600-15-4, 600-4-4) and another group at 8°C (groups: NT-15-8, NT-4-8, 600-15-8, 600-4-8), simulating a second thermal abuse during storage and distribution. HHP-treated sausages were sampled over 90 d, while controls were analyzed for 20 d. Total mesophilic counts (TMC) were determined using Plate Count Agar (PCA), while lactic acid bacteria (LAB) were enumerated on de Man-Rogosa-Sharpe (MRS) agar supplemented with 200 mg/L of cycloheximide to inhibit fungal growth. Coagulase-negative cocci (CNC) were counted on Baird-Parker (BP). All media were incubated at 30°C, and colony enumeration was performed after 48 h. Additionally, Enterobacteriaceae were detected using Violet Red Bile Glucose Agar (VRBGA), following incubation at 37°C for 24 h. Each microbiological analysis was conducted in three independent repetitions considering different sausage packaging. For the challenge test, sausages were divided into three groups, and each was inoculated with a specific pathogen to a level of approximately 5 log CFU/g using *Salmonella* Enteritidis 155 (belonging to the collection of the Department of Agricultural and Food Sciences, University of Bologna)*, Listeria innocua* DSM 20649 and *Staphylococcus aureus* DSM 20231T. After vacuum packaging and 24 hours of storage at 0-4°C, samples were split into control (untreated) and HHP-treated (600 MPa for 5 min) groups. All samples were stored at 0-4 °C; controls were analyzed for up to 20 d, while HHP-treated samples were monitored for up to 90 d. The quantitative detection of inoculated pathogens during the challenge test was performed by plate counting, using selective media for each microorganism. Listeria Selective Agar Base (LSO) added with Selective Listeria Supplement was used to enumerate *L. innocua*, after an incubation at 30°C for 48 h. For *S.* Enteritidis XLD medium plates were inoculated and incubated at 37°C for 24 h, while *Staph. aureus* was detected on Baird Parker (BP) plates, incubated at 37°C for 48 h; the colonies were recognized based on the presence of a halo. Each microbiological analysis was conducted in three independent repetitions considering different sausage packaging. When pathogen levels were below detection limits, enrichment methods were applied. All analyses were performed in triplicate using different packages. Texture and color analyses were performed only on sausages prepared from batter stored at 4°C, both untreated and HHP-treated. Texture Profile Analysis (TPA) was conducted at 22°C using a TA-Hdi® analyzer, evaluating hardness, springiness, cohesiveness, chewiness, and gumminess on cylindrical samples (1 cm in height and 1.5 cm in diameter). Color parameters (L*, a*, b*) were measured with a Chroma Meter CR-400. For both tests, 10 measurements were taken per sausage across three independent replicates.

5. Results and Discussion

5.1 Microbial shelf-life of sausages

The data related to total mesophilic bacterial counts are presented in Figure 1. In non-HHP-treated samples (Figure 1a), initial mesophilic counts were approximately 4 log CFU/g. A preliminary thermal abuse (15°C for 24 h) induced a modest increase (approx. 0.3 log CFU/g), whereas subsequent storage temperature exerted a more pronounced effect. At 8°C, microbial proliferation was significantly accelerated, especially in samples previously subjected to thermal stress (NT-15-8), which surpassed the shelf-life threshold (6.5 log CFU/g) within 10 d. Samples stored at 4°C exhibited delayed growth, with NT-4-4 exceeding the threshold only after 20 d. High-pressure processing (HHP, 600 MPa for 5 min) substantially reduced mesophilic counts (approx. 2 log CFU/g). Post-treatment samples stored at 4°C maintained microbial stability, with no counts exceeding initial levels. However, storage at 8°C led to a gradual resurgence of bacterial growth, reaching the spoilage threshold at 43 d (600-4-8) and 53 d (600-15-8), with final concentrations approaching 8 log CFU/g after 90 d. LAB dynamics closely mirrored mesophilic trends (Figure 2). Non-treated samples showed initial LAB levels of 3.6-3.8 log CFU/g, with thermal abuse contributing to a modest increase (Figure 2a). The NT-15-8 sample reached the critical LAB concentration first (10 d), followed by NT-4-8 and NT-15-4. In contrast, NT-4-4 remained below 6.5 log CFU/g after 20 d. HHP treatment significantly suppressed LAB counts to below detection or marginally above it, particularly under cold storage (Figure 2b). However, at 8°C, LAB levels began rising after 20 d and reached the spoilage threshold between 45 and 55 d.

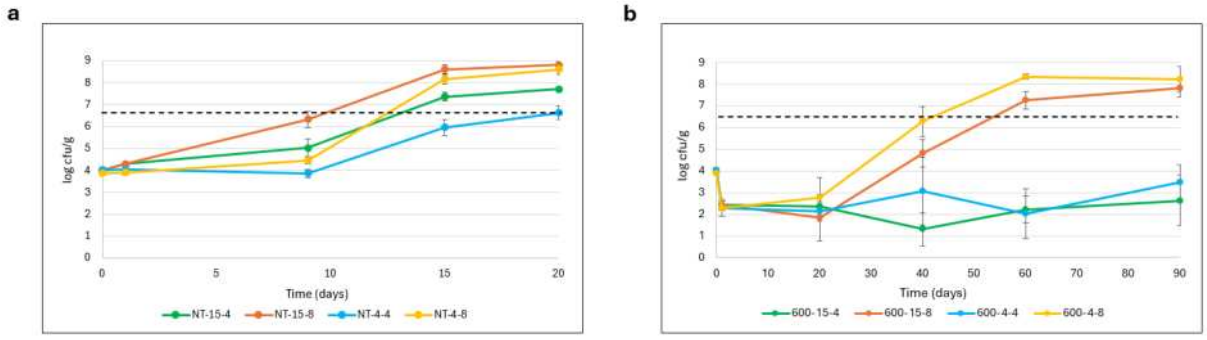


Figure 1 *Microbial counts of not-treated and treated raw pork sausages: Total Mesofilic Counts (a: not-treated; b: samples treated with HHP at 600 MPa for 5 min). 15-4: abused temperature prior to the HHP treatment; 15-8: abused temperature both prior to HHP treatment and during the shelf-life; 4-4: proper conditions; 4-8: abused temperature during the shelf-life.*

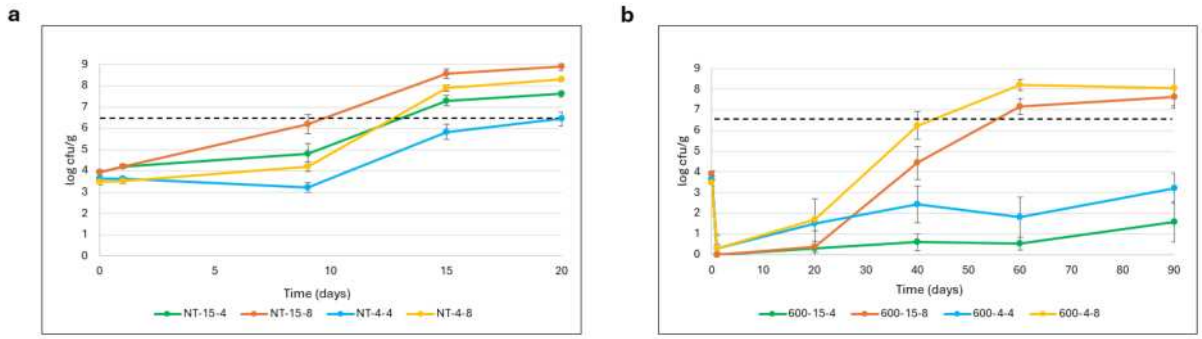


Figure 2 *Microbial counts of not-treated and treated raw pork sausages: Lactic acid bacteria (a: not-treated; b: samples treated with HHP at 600 MPa for 5 min). 15-4: abused temperature prior to the HHP treatment; 15-8: abused temperature both prior to HHP treatment and during the shelf-life; 4-4: proper conditions; 4-8: abused temperature during the shelf-life.*

Regarding Staphylococci, the counts remained stable in untreated samples across all conditions and were notably reduced (approx. 3 log CFU/g) following HHP. Further decline was observed during cold storage, while only minor regrowth occurred at 8°C, never exceeding 2.5 log CFU/g after 90 d. Enterobacteria were initially present at low levels (approx. 1.5 log CFU/g) and remained below detection throughout, particularly after HHP. Overall, the initial microbial burden of the sausages was low and aligned with previously reported data (Cocolin *et al*., 2004; Raimondi *et al*., 2018). LAB appeared to be the predominant group, likely to originate from cross-contamination with fermented products in the processing environment. While short-term thermal abuse had limited impact on initial microbial load, it significantly accelerated spoilage kinetics in untreated sausages. Conversely, elevated storage temperatures markedly reduced shelf-life by promoting recovery and proliferation of bacteria otherwise suppressed at 4°C. Despite the immediate efficacy of HHP in reducing microbial populations, the treatment did not provide definitive shelf-life extension without strict cold chain maintenance. The emergence of viable but non-culturable (VBNC) cells post-HHP (Schottroff *et al*., 2018) and their potential for recovery under favorable conditions (e.g., elevated temperatures) poses additional concerns. HHP can also induce sublethal injuries that delay or mask growth on culture media, further complicating microbial risk assessments (Koseki et al., 2006). LAB exhibited psychrotrophic traits enabling growth at low temperatures, in contrast to staphylococci, which were poorly competitive at 8°C and below. The differential adaptability of microbial groups to refrigeration underscores the importance of precise temperature control in extending the microbiological shelf-life of raw pork sausages.

5.2 Challenge Test

The study examined the survival and growth of *S.* Enteritidis 155, *L. innocua* DSM 20649, and *Staph. aureus* DSM 20231T in raw sausages stored at 4°C, with and without high-pressure processing (HHP) at 600 MPa. All pathogens were initially inoculated at 4.5 log CFU/g. In untreated samples, *S.* Enteritidis declined slightly, *Staph. aureus* remained stable, and *L. innocua* showed limited growth (~0.7 log CFU/g) over 20 d. HHP treatment led to a ~3 log CFU/g reduction in *Staph. aureus* and undetectable levels of *S.* Enteritidis and *L. innocua* by plate count; however, *L. innocua* was qualitatively detected in 60% of samples after 90 d, suggesting potential survival in a viable but non-culturable (VBNC) state. The effectiveness of HHP depends on microbial species, morphology,

food matrix, and cell condition (Yamamoto *et al*., 2021). Gram-negative bacteria like Salmonella are more susceptible to pressure than Gram-positive species such as *Staph. aureus*. Different previous studies highlight variability in pathogen inactivation across different foods and conditions. Temperature control at 4°C is crucial to limit recovery of injured cells, particularly for *Listeria*, which can remain metabolically active and regain culturability even at refrigeration temperatures (Myers *et al.,* 2013; Bover-Cid *et al*., 2015).

5.3 Texture Profile Analysis

Textural changes were evaluated in sausages stored at 4°C, comparing non-treated (NT-4-4) and high-pressure processed (600-4-4) samples. In NT sausages (Table 1), hardness and gumminess increased significantly from T0 (initial sampling time) to T9 (9 d of storage), followed by a decline after 15 d (T15), while cohesiveness steadily decreased. These changes are attributed to protein–water interactions and gel formation during mixing, along with oxidative and structural modifications occurring during storage. Oxidation and water entrapment likely contributed to firmer textures at T9, while further protein oxidation and water redistribution weakened the gel structure by the end of storage. In HHP-treated samples (Table 2), the most notable differences occurred immediately after treatment (T1), with significant increases in all texture parameters. This is attributed to protein denaturation, conformational changes, and the formation of new molecular bonds induced by high pressure (Torres & Velasquez, 2005). However, hardness, gumminess, and chewiness decreased significantly over time post-treatment, likely due to protein and lipid oxidation and water migration within the gel matrix. Additionally, storage led to increased cohesiveness, possibly due to enzyme inactivation and further molecular rearrangements within the meat matrix.

5.4 Color measurements

Regarding color analysis, for NT samples (Table 1), lightness (L*) significantly decreased by day 20, indicating darker sausages likely due to moisture loss, surface dehydration, and protein/lipid oxidation affecting light scattering. Redness (a*) initially increased by day 9, possibly from surface dehydration enhancing myoglobin's light interaction, then declined by day 20, likely due to myoglobin oxidation causing discoloration. In HHP-treated samples (Table 2), the most notable color changes occurred immediately after treatment (T1), with a sharp increase in L* attributed to protein denaturation, aggregation, and myoglobin denaturation. Correspondingly, redness (a*) and yellowness (b*) decreased, reflecting myoglobin denaturation and oxidative effects on lipids and proteins. During refrigerated storage, color trends in treated sausages resembled NT samples, showing gradual declines in L*, a*, and b*, likely due to continued protein oxidation, moisture loss, and myoglobin oxidation, which collectively affect the stability of the HHP-induced gel network and cause discoloration (Møller *et al.,* 2011).

Table 1 *Evolution in color and textural parameters (assessed by Texture Profile Analyses) in non-treated raw pork sausage samples (NT-4-4) over the storage time. Each value is the means of 30 measurements on three independent samples (10 measurements for sample).*

	T0	T9	T15	T20	sem	P-value
Color						
Lightness – L*	50.7 a	49.1 ab	49.8 ab	46.2 b	0.43	***
Redness – a*	21.9 b	24.6 a	23.8 ab	18.5 c	0.40	***
Yellowness – b*	13.7 ab	15.1 ab	17.5 a	11.0 b	0.77	*
Texture Profile Analyses						
Hardness (kg)	1.19 b	1.46 a	1.24 b	1.19 b	0.02	***
Cohesiveness	0.49 a	0.44 c	0.45 bc	0.46 b	0.01	***
Gumminess (kg*cm)	0.58 b	0.64 a	0.56 b	0.55 b	0.01	***
Springiness (cm)	0.60 a	0.58 ab	0.54 b	0.55 ab	0.01	*
Chewiness (kg*mm)	0.35 ab	0.37 a	0.30 b	0.30 b	0.01	***

*sem=standard error of mean; *=p<0.05; ***=p<0.001; a-c=mean values followed by different letters significantly differ over the storage time (p<0.05)*

Table 2 *Evolution in color and textural parameters (assessed by Texture Profile Analyses) in HHP-treated raw pork sausage samples (NT-4-4) over storage time. Each value is the means of 30 measurements on three independent samples (10 measurements for sample).*

	T0	T1	T20	T40	T60	T90	sem	P-value
Color								
Lightness – L*	50.7 de	56.7 a	53.3 bc	55.1 ab	51.8 cd	48.6 e	0.32	***
Redness – a*	21.9 a	18.8 b	19.1 b	19.3 b	16.0 c	16.5 c	0.22	***
Yellowness – b*	13.7 a	8.7 c	9.0 c	10.4 b	9.0 c	8.3 c	0.17	***
Texture Profile Analyses								
Hardness (kg)	1.19 a	2.81 ab	2.82 ab	2.44 bc	2.18 c	2.17 c	0.06	***
Cohesiveness	0.49 d	0.67 c	0.67 c	0.72 b	0.73 ab	0.75 a	0.01	***
Gumminess (kg*cm)	0.58 d	1.89 a	1.89 a	1.83 ab	1.55 c	1.59 bc	0.04	***

Springiness (cm)	0.59 b	0.88 a	0.93 a	0.89 a	0.88 a	0.85 a	0.02	***
Chewiness (kg*mm)	0.35 c	1.66 ab	1.76 a	1.65 ab	1.35 b	1.35 b	0.05	***

*sem=standard error of mean; *=p<0.05; ***=p<0.001; a-c=mean values followed by different letters significantly differ over the storage time (p<0.05)*

6. Conclusions and Future Perspectives

The present study showed that the use of HHP treatments at 600 MPa for five minutes could represent a procedure that can extend the shelf-life of raw pork sausages up to 90 d (compared with 15-20 d for conventional products). If products are not thermally abused (*i.e.* with storage at 8°C), the microbial load remains well below the concentration identified as the limit for spoilage throughout the period considered.
In addition, according to the challenge test conducted, this treatment is effective in eliminating high concentrations of *Salmonella* Enteritidis. While, in the case of *Listeria innocua*, although after 90 d of storage at 4°C this pathogen was not detectable quantitatively, some samples were positive at qualitative assessments by sampling 25 g of product. However, the qualitative presence found is also not risky for raw sausages that are cooked prior to consumption. Among the pathogenic bacteria tested, *Staphylococcus aureus* proved to be the most resistant, being able to partially survive the conducted treatment, but moreover, it was not able to multiply at the storage temperature of 4°C.
Regarding the effects on textural characteristics induced by HHP, an increase in all the parameters considered (hardness, cohesiveness, gumminess, chewiness and springiness) is observed, which, moreover, reduces the release of exudates during storage by improving the performance of the product during storage.
Color parameters were also affected by HHP treatment with an immediate decrease in a* and especially b*, while the effects on L* were opposite. At the end of storage, the observed trends were similar to those of raw sausages that had not undergone HHP treatment. The application of HHP has proved to be an effective strategy for increasing the shelf-life of raw pork sausages up to 90 d, however, the effects of HHP on color and texture limit the application of the treatment due to consumer acceptability. For these reasons, other strategies, such as bioprotective cultures, are being evaluated to increase the shelf-life of raw sausages without compromising their quality.

7. Nomenclature

HHP, High Hydrostatic Pressure; a_w, water activity; TMC, Total mesophilic counts; PCA, Plate Count Agar; LAB, Lactic acid bacteria; MRS, Man-Rogosa-Sharpe; CNC, Coagulase-negative cocci; BP, Baird Parker; VRBGA, Violet Red Bile Glucose Agar; LSO, Listeria Selective Agar Base; XLD, Xylose Lysine Desoxycholate; VBNC, viable but non-culturable.

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Nature-inspired compounds: synthesis and interaction with molecular targets for the application in plant protection

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This PhD thesis dealt with the design and synthesis of natural and nature-inspired compounds to find out bioactive molecules with antimicrobial potential, especially antifungal. In this context, based on microbiological screenings, the interaction of both synthesized and natural compounds with molecular targets was investigated through calorimetric techniques to elucidate their possible mechanisms of action.

Composti ispirati alla natura: sintesi e interazione con target molecolari per l'applicazione nella protezione delle piante

Questa tesi di dottorato ha riguardato la sintesi di nuove molecole ispirate a composti naturali con attività antimicrobica, specialmente antifungina. In questo contesto, in seguito a screening microbiologici, l'interazione dei composti sintetizzati e quelli naturali con i potenziali targets è stata studiata grazie all'utilizzo di tecniche calorimetriche in modo tale da poter confermare il loro meccanismo d'azione.

Key words: Natural compounds, Organic synthesis, Phospholipid membranes, Differential Scanning Calorimetry

1. Introduction

In accordance with the PhD thesis project previously described (Sacchi, 2023), this oral communication reports the main results of the following three main activities directed to:

A1) Synthesis of natural and nature-inspired compounds as potential antifungal agents
  A1.1) difucol and diphlorethol as well as their methylated/acetylated derivatives
  A1.2) multitarget molecules: stilbenoid-carboxamide hybrids
A2) Biological evaluation of antifungal activity
A3) Study the interaction of natural and synthesized compounds with model membranes in order to elucidate their possible mechanism of action
  A3.1) Interaction of diphlorethol and its derivatives, using MLBs
  A3.2) Interaction of terpenoids, using MLBs and SUVs

Besides, during my PhD I also worked on:

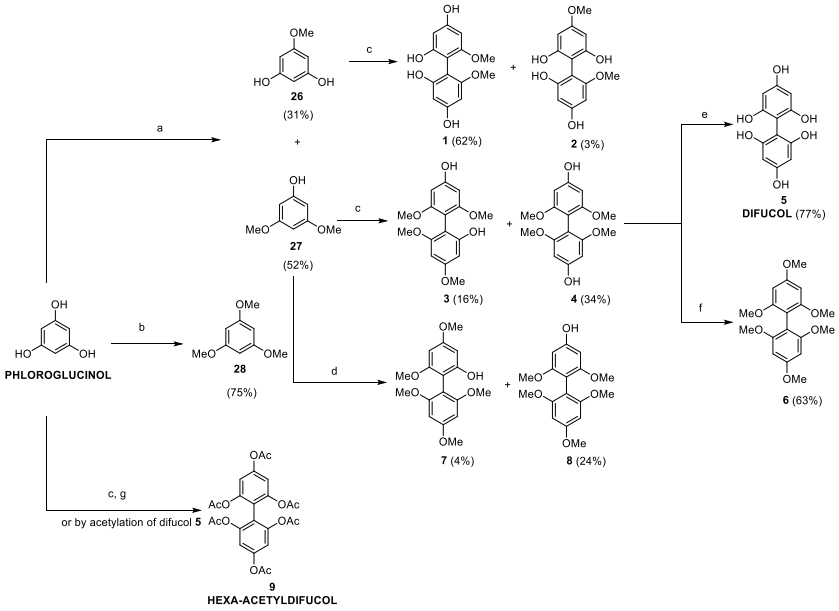
A4) Interaction of natural and nature-inspired chalcones with enolase 1, a protein involved in the glycolysis process, in order to establish if this protein could be a target for the tested compounds.

2. Nature-inspired biphenyls and diphenyl ethers

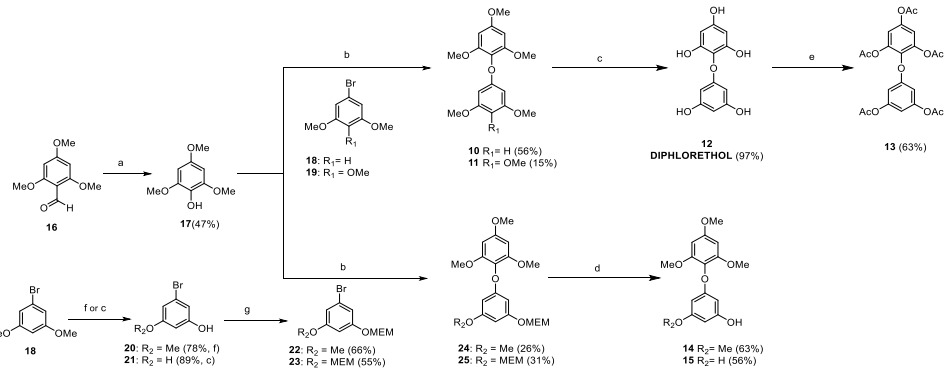
Phlorotannins are polyphenolic compounds made of phloroglucinol units mainly found in brown algae, exhibiting diverse structural features and bioactive properties. For instance, De Corato and colleagues tested the antifungal activity of brown seaweed extracts against phytopathogens, and they showed that phloroethols and fucophloroethols contained in *Laminaria digitata* extracts are effective against *B. cinerea* and *M. laxa* (100% mycelial growth inhibition), at 30 g/L concentration (De Corato et al., 2017). However, the biological evaluation of these compounds is typically carried out on enriched fractions rather than on pure isolated compounds. Nonetheless, direct isolation of phlorotannins from their natural sources is quite impractical because of the low amount and the presence of multiple structural and conformational isomers (Shrestha et al., 2021). In this context, to investigate the potential application of natural biphenyl and diphenyl ether derivatives as antimicrobial agents, a series of natural and derived phloroethols and fucols with diverse methylation/acetylation patterns were designed and synthesized.

2.1 Synthesis

The designed compounds were synthesized following two different synthetic approaches. In particular, biphenyls were obtained by dimerization reactions (Scheme 1), whereas diphenyl ethers through Ullman reactions (Scheme 2).



Scheme 1. *a) Me_2SO_4 (0.3 equiv), K_2CO_3, Acetone, 55 °C, 24 h; b) Me_2SO_4 (3 equiv), K_2CO_3, Acetone, 55 °C, 3 h; c) $FeCl_3{\cdot}6H_2O$ (2 equiv), MeOH/H_2O, r.t., 24−48 h; d) $FeCl_3{\cdot}6H_2O$ (0.15 equiv), $(t\text{-BuO})_2O$, HFIP, r.t., 6 h; e) BBr_3, DCM, N_2, −78 °C to r.t., 18 h; f) Me_2SO_4, K_2CO_3, Acetone, r.t., 21 h; and g) Ac_2O, Pyridine, r.t., 24 h*



Scheme 2. *a) 1. mCPBA, DCM, N_2, 0 °C to r.t, 4 h; 2. KOH, MeOH, 0 °C, 45 min; b) CuI, Picolinic Acid, K_2CO_3, DMSO, 130 °C, 5 d; c) BBr_3, DCM, N_2, −78 °C to r.t., 16 h; d) HCl, MeOH, r.t., 16 h; e) Ac_2O, DMAP, TEA, THF, r.t., 7 h; f) 1-Dodecanethiol, NaOH, NMP, 130 °C, 1 h; and g) MEM-Cl, DIPEA, THF, N_2, 0 °C to r.t., 16 h*

2.2 Biological evaluation

The compounds were tested to evaluate their antifungal activity against four different strains of phytopathogenic fungi, *P. oryzae* (PO21_07 QoI-resistant strain and PO_A252 QoI-sensitive strain), *B. cinerea* (BC-2A10), and *F. culmorum* (FC-UK). The fucol-type dimers showed only low or no inhibition toward fungal mycelium. On the other hand, phloroethols showed more promising activity, in particular toward the two strains of *P. oryzae* and *B. cinerea*. In fact, compounds **10**, **11**, and **14** showed 35-40% inhibition of mycelium growth at 500 µM. To better investigate the potential application of these compounds as antifungals against *P. oryzae* and *B. cinerea*, they were further assessed in terms of the inhibition of spore germination. However, only compound **15** was able to inhibit the appressorium formation of *P. oryzae* by 48% without affecting the germination (Sacchi et al., 2025).

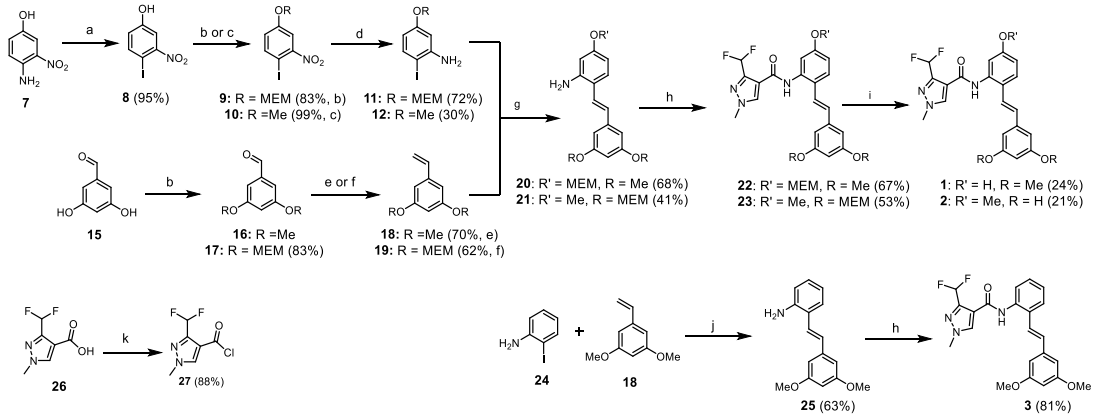
3. Stilbenoid-carboxamide hybrids

The widespread use of a limited number of synthetic fungicides has led to the emergence and spread of resistance and high environmental impact (Kunova et al., 2016). To address the growing urgency in managing fungal diseases, there is the need to find novel compounds with lower human and environmental impact, able to effectively inhibit pathogens and overcome resistance. In this context, a promising strategy to counteract the resistance emergence is to use multitarget compounds, together with the development of natural and nature-inspired agrochemicals. Among the most used synthetic fungicides, succinate dehydrogenase inhibitors (SDHIs) play a pivotal role since they are able to inhibit the pathogen mitochondrial respiratory chain. Their chemical structure is characterized by the presence of a carboxamide group linking a polar core to a hydrophobic tail. (Sierotzki and Scalliet, 2013) Among natural products, stilbenoids are known to inhibit the mycelial growth of

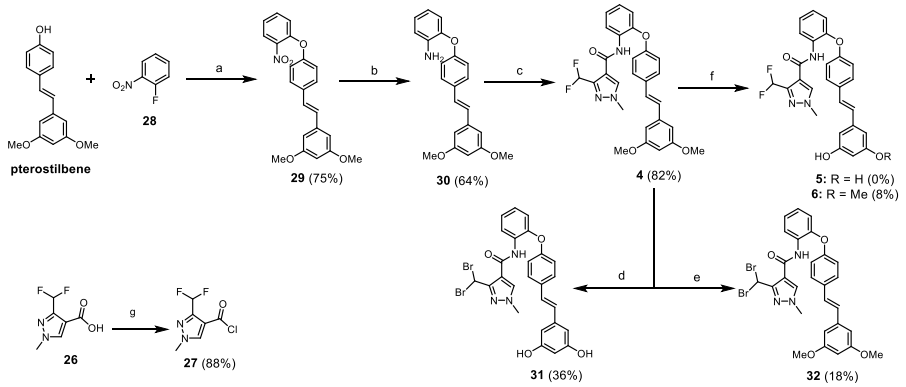
some phytopathogenic fungi with a nonspecific mechanism of action (Kunova et al., 2024).
In this scenario, we have designed a small set of novel multitarget compounds bearing the carboxamide moiety (present in SDHI Fluxapyroxad) connected to the stilbene scaffold. The antifungal potential of the synthetised derivatives was evaluated against *P. oryzae*, *F. culmorum*, and *B. cinerea* in terms of mycelium growth inhibition, showing promising results.

3.1 Synthesis

Two different classes of hybrid were synthesized: in the first one, the carboxamide moiety was installed directly on the stilbene core (scheme 3); in the second one, the carboxamide and stilbene core were connected by an aryl linker (scheme 4).



Scheme 3. *a) 1. HCl, $NaNO_2$ aq, 0 °C, 30 min, 2. KI aq, r.t., 16 h, dark; b) MEMCl, DIPEA, dry DCM, N_2, r.t., 4 h; c) CH_3I, K_2CO_3, dry acetone, N_2, reflux, 4 h; d) $SnCl_2 2H_2O$, AcOEt, MS 4Å, r.t., 2 h; e) $P^+Ph_3CH_3Br^-$, t-BuOK, Et_2O, r.t., 5 h; f) $P^+Ph_3CH_3Br^-$, t-BuOK, THF, r.t., 5 h; g) $Pd(OAc)_2$, P(o-Tol)3, TEA, dry ACN, reflux, 16 h; h) 27, TEA, dry DCM, N_2, r.t., 16 h; i) p-TsOH·H_2O, MeOH, r.t., 16 h; j) $Pd(OAc)_2$, TEA, dry ACN, N_2, reflux, 3 h; k) $SOCl_2$, dry toluene, N_2, 90 °C, 5 h.*



Scheme 4. *a) K_2CO_3, dry DMF, N_2, 70 °C, 6 h; b) Fe (powder), NH_4Cl, MeOH/H_2O 1:1, reflux, 8h; c) 27, TEA, dry DCM, N_2, r.t., 16 h; d) BBr_3 (4eq.), dry DCM, N_2, -78 °C to r.t., 16h; e) BBr_3 (2eq.), dry DCM, N_2, -78 °C to r.t., 5h; f) 1-dodecanethiol, NaOH, dry NMP, 90 °C, 150 min; g) $SOCl_2$, dry toluene, N_2, 90 °C, 5 h.*

4. Cell membranes: the calorimetric approach

Upon heating, phospholipid bilayers undergo a gel-to-liquid crystalline phase transition that affects the van der Waals interactions between hydrocarbon chains, increasing their mobility. Variations in the overall transition temperature and enthalpy can reveal important information related to the membrane physicochemical properties. For this reason, a key technique for the investigation of cell membrane thermodynamic stability is represented by the Differential Scanning Calorimetry (DSC).
A model membrane (MM) constituted as a 30:20:35:15 mixture of DPPC:DOPC:DPPG:DPPS was designed to represent the phospholipid membrane of phytopathogenic fungi, like *B. cinerea*.

4.1 Interaction of diphenyl ethers with the model membrane: Multilamellar Lipid Bilayers (MLBs)

Among the synthesized phloroglucinol dimers, the diphenyl ether derivatives showed the highest biological activity. As a consequence, this class of compounds was taken into consideration to evaluate their possible mechanism of action (Saitta et al., 2021). The calorimetric experiments were performed on the model membrane, represented by multilamellar bilayers (MLBs), incorporating the selected compounds at physiological pH (pH 7.4)

in a 1:10 compound:phospholipid molar ratio. All the tested compounds showed interaction with model membrane with similar effects, with the exception of compound **13**. Indeed, all the thermograms (except for MM+13) showed both entropic (lower transition temperature) and enthalpic (a lower transition enthalpy) membrane destabilization. According to the correlation between the biological assay and the calorimetric data, we may argue that the phospholipid membrane might be a target of diphenyl ethers. The only discrepancy appeared for compound **12**, which was inactive, however showing the best interaction with the model membrane. This might be due to the presence of all free hydroxy groups that make the molecule too hydrophilic to completely enter the membrane hydrophobic core *in vivo*.

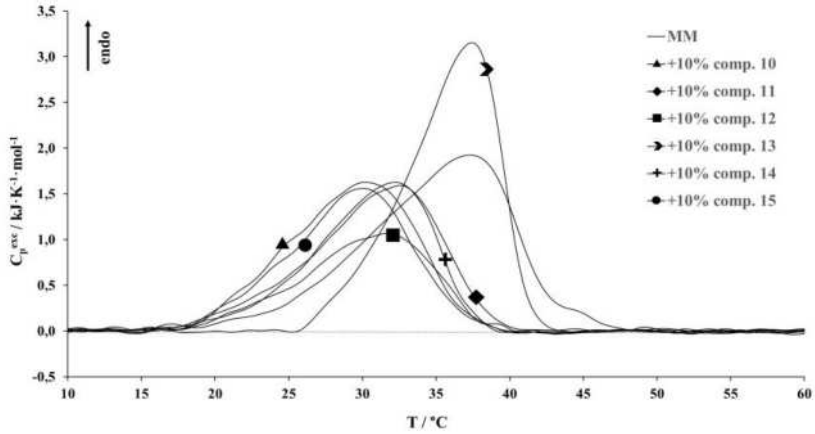


Figure 1. *DSC thermograms for model membrane MLBs alone and with incorporation of compounds 10 to 15 (from scheme 2) at physiological pH (10 mM phosphate buffer pH 7.4).*

4.2 Interaction of terpenoids with the model membrane

Terpenoids, like thymol and carvacrol, together with cuminaldehyde, are well known for their antifungal activity. Although literature illustrates that these compounds can interact with phospholipid membranes, the mechanism of action is not clear yet. Hence, the interaction of these compounds with the designed MM was studied aiming at dissecting the effects related to the MM phospholipid constituents.

As preliminary studies, the selected terpenoids were tested with MLB systems as shown above for diphenyl ethers; basing on the promising results, a High Sensitivity DSC (HS-DSC) analysis was also carried out on Small Unilamellar Vesicles (SUVs), which are single-bilayer vesicles with a diameter around 100 nm that can simulate cell membranes more realistically. The HS-DSC investigation was performed on a MM with the same composition reported above, as well as on binary systems for the "dissection" study. All the three compounds showed the same behaviour. For this reason, only the thymol case study is here reported.

4.2.1 Dissecting the effect of MM composition: the case study of thymol

This study was carried out to elucidate the role of the main phospholipid polar headgroups in the interaction. The presence of thymol was reflected mainly in an increment of the ACI value, which represents a decrease of the cooperativity of the transition (Saitta et al., 2019). In particular, the ACI value increases from 2.5 ± 0.2 °C (for the all the considered binary model membranes) to 3.4 ± 0.2 °C (DPPC:DPPS 3:2) and 4.2 ± 0.2 °C (DPPC:DPPE 3:2), this suggesting that the interaction was mainly driven by the presence of phospholipid with headgroups of small size (namely, serine in DPPS and ethanolamine in DPPE).

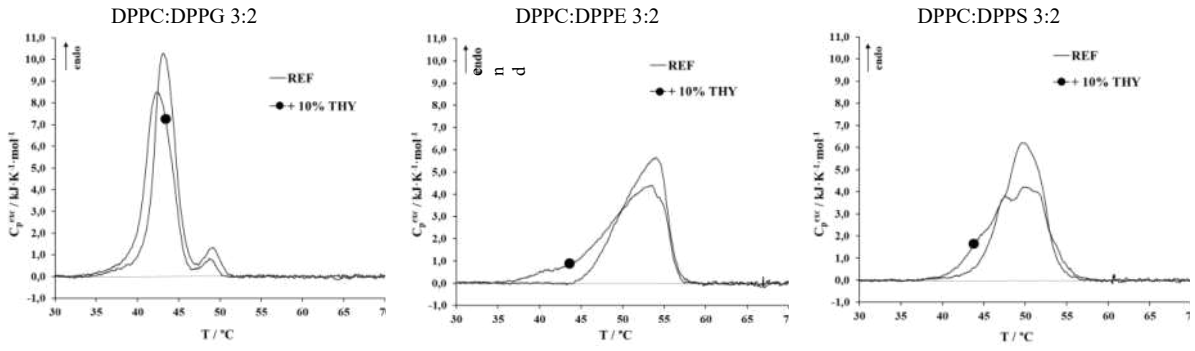


Figure 2. *Micro-DSC thermograms for binary SUVs with and without thymol (+10% mol) at physiological pH (10 mM phosphate buffer pH 7.4).*

4.2.2 Interaction with model membrane: the case study of thymol

Surprisingly, the addition of thymol to SUV MM at a compound:phospholipid 1:10 molar ratio seems not to lead to any compound-MM interaction. Instead, by increasing the molar ratio up to 1:1, the interaction was visible as a drastic decrease of the average transition temperature. This peculiar behaviour is due to the fact that thymol (and the other two terpenoids) mainly acts entropically by reducing the MM transition cooperativity. However, the already reduced transition cooperativity characterizing SUVs (compared to MLBs) together with analogous effects deriving from the presence of a high amount of unsaturated phospholipid (DOPC) (Saitta et al., 2019), were much higher than those arising from the MM interaction with thymol itself. For this reason, higher concentrations of thymol are needed to appreciate the effects of the interaction.

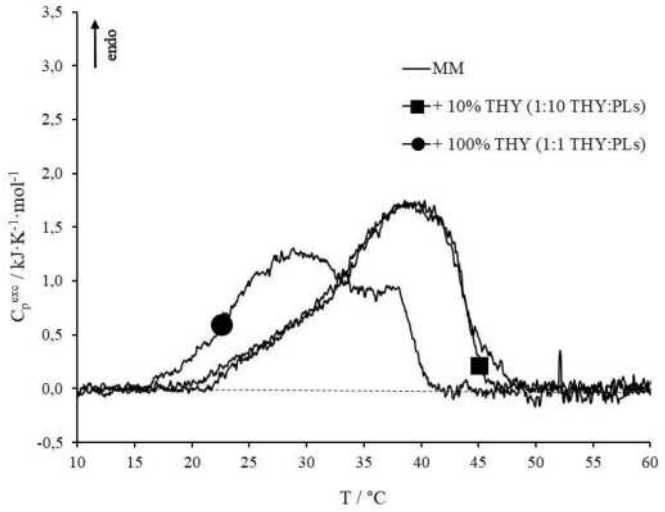


Figure 3. *Micro-DSC thermograms for SUVs of model membrane and model membrane with thymol incorporation at two different concentrations.*

5. Conclusion and Future Prospectives

In conclusion, during this period two sets of compounds with promising antifungal activity, and in the near future, structural modifications on the best compounds will be carried out in order to increase their potency. The modification will be guided both by biological and calorimetric results. Moreover, we were able to associate the biological activity of a new class of compounds to a molecular target, i.e. the four-component model phospholipid lmembrane. For future studies, more complex vesicles could be prepared to enhance the correspondence with real membranes, for instance by incorporating ergosterol into the model membrane

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Biomimetic synthesis of phytoalexins as environmentally friendly antimicrobials

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The PhD research project aims at the development of novel nature-inspired multitarget antimicrobial agents for crop protection, overcoming the emergency of resistant strains and limiting the use of harmful chemicals. A particular emphasis was placed on i) valorizing value-added bioactives present in agrifood residues; ii) exploiting chemo-enzymatic approaches to access desired scaffolds under mild conditions; iii) designing scaffold optimization strategies, to increase both the activity and the bioavailability of the natural compounds.

Sintesi biomimetica di fitoalessine come agenti antimicrobici sostenibili

Il progetto di ricerca ha l'obiettivo di sviluppare nuove molecole ad attività antimicrobica multi-bersaglio da utilizzare per la protezione delle colture, traendo ispirazione da composti di origine naturale. Questa strategia consente di limitare l'uso di sostanze chimiche nocive e mitigare al contempo l'insorgenza di fenomeni di resistenza. Particolare enfasi è stata posta su: i) valorizzazione di bioattivi ad alto valore aggiunto presenti nei residui agroalimentari; ii) impiego di approcci chemo-enzimatici sostenibili per la sintesi dei composti desiderati; iii) messa a punto di strategie di ottimizzazione molecolare, al fine di aumentarne sia l'attività che la biodisponibilità.

Key words: crop protection; chemo-enzymatic synthesis; nature-inspired; phytoalexins, antimicrobial activity

1. Introduction

Agriculture plays a pivotal role in food security as well as in food waste; indeed, plant diseases, and particularly fungi infections, are responsible for massive food losses. For these reasons, new and efficient antimicrobial agents for crop protection need to be promptly available as weapon against this emergency. Plants secondary metabolites are a limitless source of bioactive compounds. Their high structural diversity, novel mode of action, and low environment impact, make them suitable as drug candidates. This oral communication reports the main results of the activities directed to the design, synthesis, and biological evaluation of nature-inspired antimicrobial agents for crop protection. The project has been divided into five chapters.

2. Synthesis of potential multitarget fungicides: methoxyacrylate stilbene hybrids (MOAS)

2.1. Aim of the work

Pyricularia oryzae is the phytopathogenic agent of rice blast, a devastating infection that is causing crop losses in the world rice production. The natural compounds strobilurins are among the most effective agents against *P. oryzae*, however their single-site mode of action is causing insurgence of resistance strains. In this work, the pharmacophoric portion of strobilurins was installed on different substituted stilbenoids, a family of bioactive nonflavonoid phenylpropanoids recovered from Vitacease, Leguminaceae, Gnetaceae and Dipterocarpaceae, aiming at obtaining potential multitarget methoxyacrylate stilbene (MOAS) hybrids to overcome or reduce the insurgence of resistant strains.

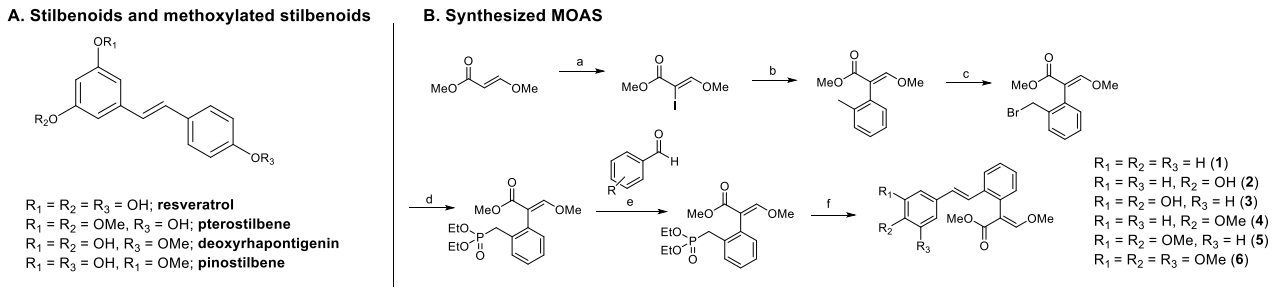


Figure 1 *(**A**) Structure of natural stilbenoids investigated in this study; (**B**) Chemical structure of synthesized methoxyacrylate-stilbene hybrids (MOAS). Reagents and conditions: (**a**) i) NIS, AcOH, DCM, rt, 24 h, ii) TEA, DCM, rt, 16 h, 62%; (**b**) 2-tolylboronic acid, Pd(PPh3)4, K3PO4, dioxane:H2O 5:1, 90 °C, 6 h, 76%; (**c**) NBS, AIBN, CCl4, reflux, 6 h, 69%; (**d**) P(OEt)3, 130 °C, overnight, N2, 54%. (**e**) NaH, THF, N2, 0 °C → rt, 20%; (**f**) From 12 to 3: TBAF, THF 0 °C → rt, 1 h, 25%.*

2.2. Results and discussion

A set of different substituted stilbenoids (Fig. 1A) was first evaluated for their mycelium growth inhibitory activity against wild type (WT) and resistant strains of *P. oryzae*. Methoxylated stilbenoids, such as deoxyrhapontigenin and pinostilbene, resulted to be drastically more active compared to the corresponding fully hydroxylated parent compound resveratrol with 60-80% inhibition at 200 µM even on strobilurin-resistant strains. However, this activity was lost when methoxylated stilbenoids were merged with the strobilurins' pharmacophore. The synthesized MOAS (Fig. 1B) showed low inhibition on *P. oryzae* resistant strains maintaining strobilurin-like behavior.

2.3. Conclusion and future perspectives

This study allowed to identify methoxylated stilbenoids, deoxyrhapontigenin and pinostilbene, as potential antifungals for further investigation as inhibitors of both WT and resistant *P. oryzae*. Unfortunately, merging the stilbenoids with the methoxy acrylate fragment was not sufficient, at the tested concentration, to overcome the strobilurin-induced resistance on the selected strains. However, these results open the possibility to study and apply this strategy on different pharmacophore and chemotypes.

3. Chemical and enzymatic synthesis of cinnamic acid amides

3.1. Aim of the work

Plants phytoalexins are a heterogeneous class of secondary metabolites involved in plant self-defense mechanisms against biotic and abiotic factors. They are chemically divided into the classes of phenylpropanoids, diterpenes, flavonoids, and phenylamides, with the latter constituting the main focus of this project. Phenylamides are obtained from the coupling of different carboxylic acid (cinnamic, *p*-coumaric, caffeic, ferulic, and benzoic) with biogenic amines (tryptamine, tyramine, agmatine, and putrescine). In literature, they are reported to accumulate in plants after infection by pathogens and exerting antifungal and antimicrobial activities. The aim of this work was to investigate the effects of some phenylamides (Fig. 2) on plant fungal (WT and resistant *P. oryzae*, *B. cinerea*, and *F. culmorum*) and food-borne bacterial pathogens to address their possible application in crop protection; moreover, a more sustainable synthetic approach was designed using the commercially available enzyme lipase B from *Candida antarctica* (CaL-B) in parallel with a traditional amidation protocol.

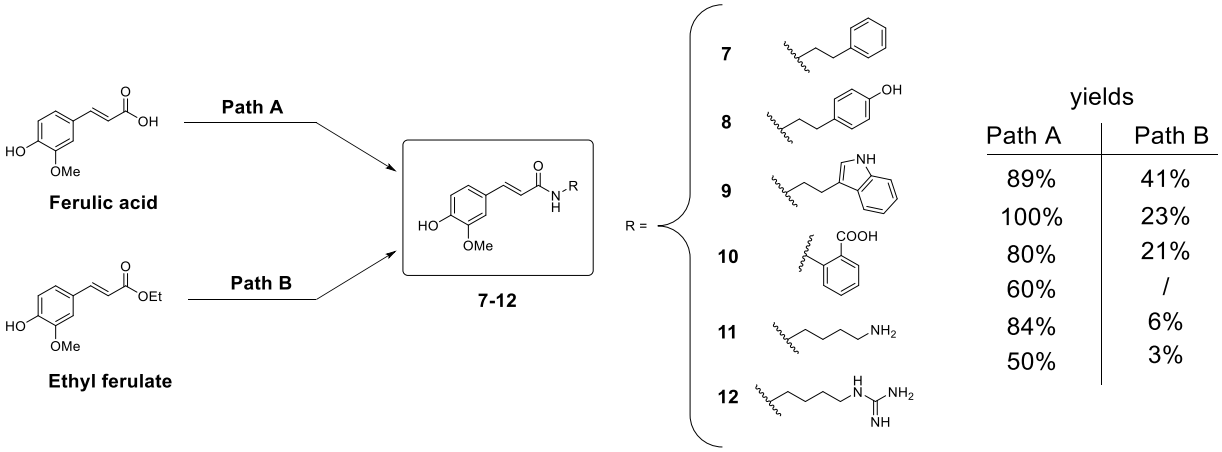


Figure 2 *Chemical structure of synthesized phenolamides considered in this study. Reagents and conditions: (**Path A**) EDC HCl, HOBt, DIPEA, R-NH$_2$, DMF; (**Path B**) imm-CaL-B, R-NH$_2$, tert-amyl alcohol.*

3.2. Results and discussion

Most phenolamides are not commercially available and their recovery from natural matrices is low yielding. Using a traditional amidation protocol, ferulic acid phenolamides **7**-**12** were obtained in excellent yield after 24 h stirring at room temperature in dry DMF and DIPEA using HOBt and EDC HCl as coupling agents. In the case of the chemo-enzymatic approach, ethyl ferulate and CaL-B were stirred in *tert*-amyl alcohol at 80 °C for 24 h. Phenolamides recovered in modest yield when compared to Path A as the enzyme CaL-B showed high substrate specificity.

For the antifungal activity, no evidence of inhibition of the mycelium growth was observed at 500 µM on the selected strains. Additional tests on *P. oryzae* spores highlighted that three phenolamides **8**, **11**, and **12**, were able to inhibit the appressoria (responsible for the fungal infectivity) formation at 500 µM by 76%, 59%, and 94%, respectively. The evaluation of the antibacterial activity revealed that all the compounds affected the normal growth kinetics of the considered strains, with **10** being the most effective against *E. faecalis*, *L. monocytogenes*, and *S. aureus*.

3.3. Conclusion and future perspectives

This study confirmed the important role of phenolamides in plant self-defence and as antimicrobial agents. Compounds **11**, **12** and **10** were selected for further investigation in food preservation and crop protection.

4. Synthesis and derivatization of cinnamic acid dimers

4.1. Aim of the work

The previous project helped to identify FerAgm (**12**) and FerPut (**11**) as promising antimicrobial molecules. In plants hydroxycinnamoylagmatine (HCAgm) are biosynthesized by the enzyme agmatine *N*-coumaroyltransferase (ACT). When biotic or abiotic stress occurs, these molecules can be involved in oxidative coupling reactions and dehydrodiferulates dimers are formed. In literature, several dimers isolated from natural matrices have been already described as antibacterial agents. In particular, hordatines, which are produced exclusively in barley, and poacic acid, recovered mainly from grasses. The aim of this project was i) to develop a chemo-enzymatic synthetic approach to access natural dehydrodiferulates in high yield to study their biological activity; ii) to synthesize a collection of their derivatives bearing agmatine and putrescine chains; iii) to synthesize a collection of new chemically modified analogues to deepen our knowledge on structure-activity relationships.

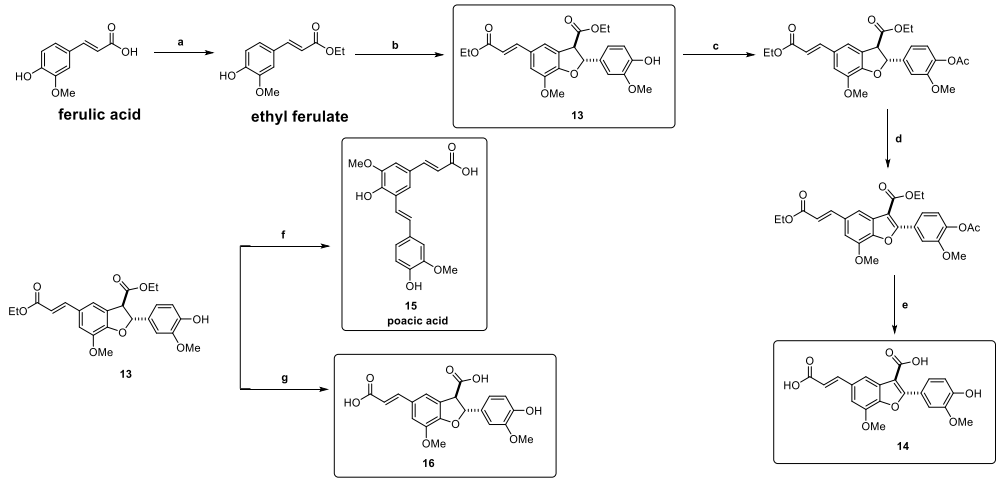


Figure 3 *Chemical structures of dehydrodiferulates **14**, **15**, and **16** considered in this study. Reagents and conditions: **(a)** HCl 37%, ethanol, 78 °C, 72h, 98%; **(b)** HRP/H_2O_2, acetone, water, rt, 20 min, 22%; **(c)** Ac_2O, TEA, DCM, rt, o.n., 88%; **(d)** DDQ, toluene, 100 °C, 30 h, 78%; **(e)** NaOH 2 M, dioxane, rt, 20 h, quantitative; **(f)** NaOH 0.35 M, 90 °C, o.n., 63%; **(g)** NaOH 2 M, dioxane, rt, o.n., 38%.*

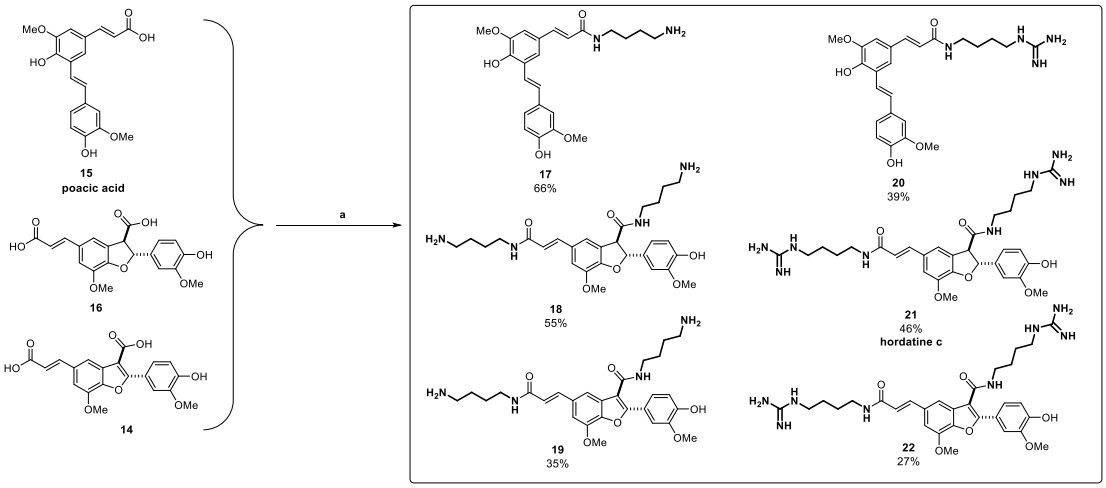


Figure 4 *Chemical structures of agmatine and putrescine functionalized dehydrodiferulates considered in this study. Reagents and conditions: **(a)** i) Boc-protected putrescine or agmatine, EDC HCl, HOBt, DMF, rt, 24 h, ii) TFA 10% in DCM, rt, 2h.*

4.2. Results and Discussion

Dehydrodiferulates were obtained from ferulic acid and ethyl ferulate exploiting the commercially available enzyme horseradish peroxidase (HRP) in presence of H_2O_2. Different hydrolytic conditions were employed (Fig. 3, **f** and **g**) on the obtained key intermediate **13** to give diacid dimers **15** (natural compound poacic acid) and **16**. A chemical modification on scaffold **13** led to isolation of benzofuran analogue **14** for structure-activity relationships investigations. Amidation protocols were applied on **14**, **15**, and **16** and their agmatine and putrescine functionalized analogues were obtained, including the natural compound hordatines C (**21**), and the two newly

designed poacic acid analogues (**17** and **18**) and benzofurans (**21** and **22)** (Fig. 4).

All the compounds described were screened on fungal and bacterial strains to evaluate the antimicrobial activity. Free acid dehydrodiferulates **15**-**17** were not active as mycelium growth (at 500 µM on *B. cinerea*, *F. culmorum*, and *P. oryzae*) or germination (at 500 µM on *P. oryzae* spores) inhibitors. Poacic acid **15** showed a modest ability to inhibit the appressorium formation in *P. oryzae* strains (44%), while benzofuran **14** exhibited relevant activity both at 500 and 200 µM (98% and 74% respectively). Notably, the parent dehydro **16** was inactive at the tested concentrations. Improved activity was observed on *P. oryzae* appressoria formation inhibition assay for all the agmatine and putrescine functionalized dimers **17**-**22** (100% inhibition at 500 and 200 µM). **17**-**22** were also screened on Gram-positive (*S. aureus*) and Gram-negative (*E. coli*, *S. enterica* and *P. aeruginosa*) bacteria, and agmatine functionalized compounds **20** and **22** showed excellent results on *S. aureus* with a MIC of 16 and 8 µg/mL, respectively.

4.3. Conclusion and future perspectives

This study succeeded in developing a chemo-enzymatic strategy to obtain high quantities of dehydrodiferulates. The structure modification on compound **13** and the agmatine and putrescine functionalization led to the isolation of hordatine related compounds **18** and **21**, as well as the first-time synthesized compounds **17**, **19**, **20**, and **22**. Biological evaluations clarified that the functionalization with agmatine and putrescine is essential and responsible for antimicrobial activity. The comparison between compounds **16** and **14** and their respective analogues, showed that the introduction of the benzofuran scaffold is beneficial for antimicrobial activity.

The future perspectives of this project will be: i) the design of other chemically modified derivatives with different functionalization to apply structure-optimization strategies; ii) further investigations on the antifungal and antibacterial activities, considering both lower concentrations and new targets, iii) the possibility of conducting studies aiming at elucidating the molecular target of this panel of molecules. Regarding point ii) collaborations with two different European

universities are ongoing to test the prepared compounds as anti-yeast for low alcohol beer preservation, and as glucosidase inhibitors.

5. Synthesis of cinnamic mono-, bis-, and tris-amides of putrescine and spermidine

5.1. Aim of the work

Starting once again from compound FerPut (**11**), the conjugation with aliphatic di- and polyamines, such as putrescine and spermidine, was investigated. This class of phenolamides is widely distributed in nature, and accumulated in flowers, pollen and leaves where they are involved in plants response to stressors. With kukoamine A and B being the most studied, polyamines and related phenolamides have been described in literature as endowed with anti-inflammatory, antidiabetic, neuroprotective, and anticancer activities. However, considering the previous antimicrobial evaluation conducted in our group on phenolamides, the scope of this project (still ongoing) was to design a synthetic strategy to obtain high amount of a panel of mono-, bis-, and tris-amides of putrescine and spermidine and to evaluate their antimicrobial activity.

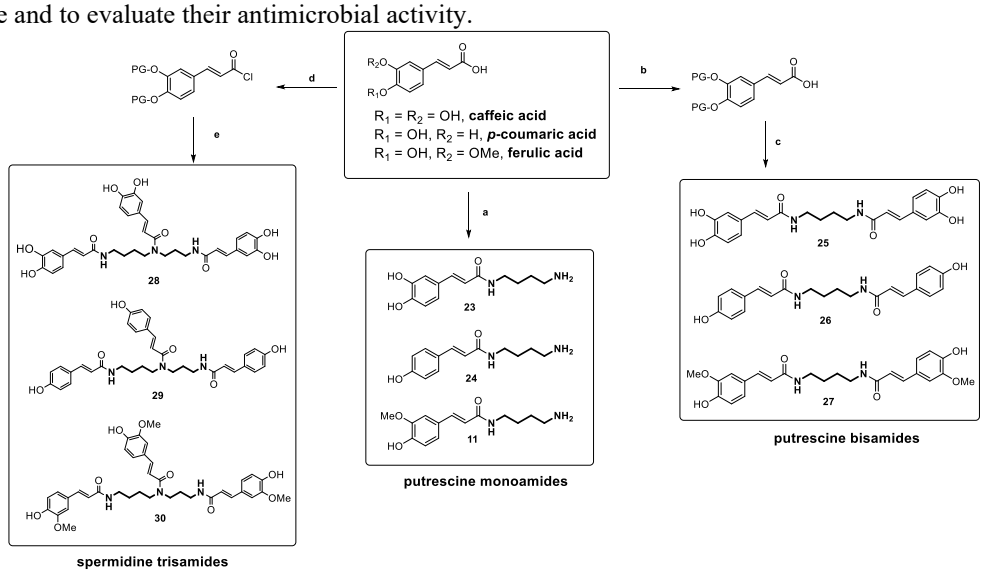


Figure 5 *Chemical structure of cinnamic mono-, bis-, and tris-amides considered in this study. Reagents and conditions:* ***(a)*** *i) Boc-putrescine, EDC HCl, HOBt, DIPEA, DMF, rt, 24 h, ii) TFA 10% in DCM, rt, 2h;* ***(b)*** *free hydroxy groups were protected in form of acetals;* ***(c)*** *i) EDC HCl, HOBt, DIPEA, DMF, rt, 24 h; ii) protecting group removal;* ***(d)*** *i) free hydroxy groups were protected in form of acetals, ii) $SOCl_2$, 80 °C, 16 h,* ***(e)*** *i) spermidine, pyridine, THF, rt, 24 h, ii) protecting group removal.*

5.2. Results and discussion

Using traditional amidation protocol, putrescine mono-amides (**11**, **23**, and **24**), putrescine bis-amides (**25**-**27**) and spermidine tris-amides (**28**-**30**) were synthesized in high yields. Antimicrobial evaluation on mycelium growth inhibition, *P. oryzae* spore and appressoria formation inhibition, as well as antibacterial activity assays are ongoing.

6. Total synthesis of thioholgamide

6.1. Aim of the work

During my stint abroad for the Erasmus traineeship, I was hosted by Prof. Philipp Heretsch at the Leibniz University of Hannover. This project was focused on proposing the first total synthesis of the natural compound thioholgamide A, a ribosomally synthesized and post-translationally modified peptide isolated from *Streptomyces* sp. *MUSC 136T*. This molecule showed potent anti-proliferative activity in cancer cell lines at nanomolar concentrations. Its chemical features are a thiopeptide lateral chain and a β-hydroxy-N^1,N^3 -dimethylhistidinium and *C*-terminal 2-aminovinyl-3-methyl-cysteine containing macrolactone.

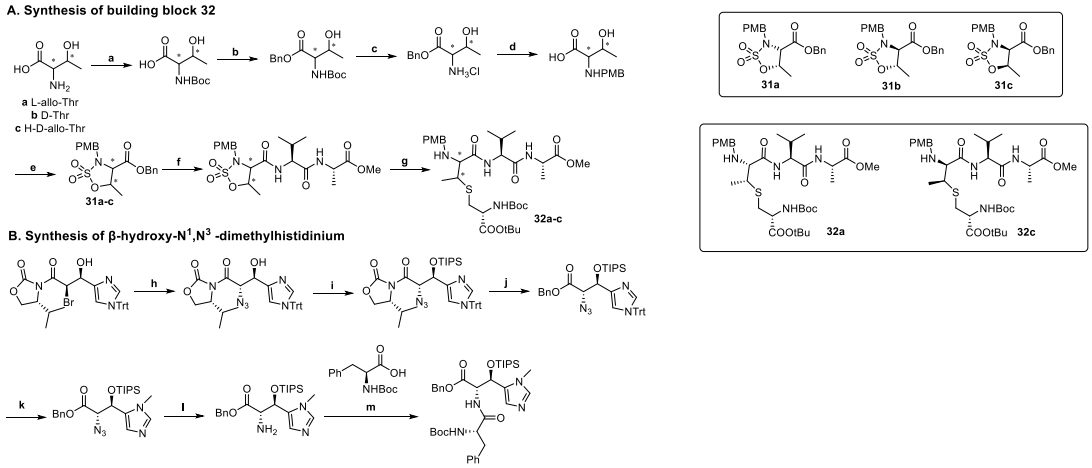


Figure 6 *Reagents and conditions: (**a**) $(Boc)_2O$, $NaHCO_3$, MeOH:H_2O 1:1, rt, 24 h, quantitative; (**b**) BnBr, $NaHCO_3$, DMF dry, 0 °C to rt, o.n., 77%; (**c**) HCl/dioxane 4 M, rt, 30 min, 95%; (**d**) i) $NaCNBH_3$, p-anisaldehyde, AcOH, MeOH, 0 °C to rt, 16 h, ii) $NaHCO_3$, 80%; (**e**) i) $SOCl_2$, Py, DCM dry, –78 °C to rt, 1 h, ii) $RuCl_3$ H_2O, $NaIO_4$, H_2O, MeCN, 0 °C to rt, 20 min, 90%; (**f**) i) Pd/C, H_2, EtOAc, rt, 5 h, quantitative; ii) HCl Val-Ala-OMe, T3P, TEA, DCM dry, 0 °C to rt, 14 h, 80%; (**g**) i) tert-butyl (tert-butoxycarbonyl)-L-cysteinate, Cs_2CO_3, DMF dry, 35 °C, 18 h, ii) BF_3Et_2O, EtSH, DCM, 23 °C, 18 h, 67%; (**h**) NaN_3, DMF dry, 45 °C, 2h, 70%; (i) TIPS triflate, 2,6-lutidine, DCM dry, 0 °C to rt, o.n., 68%; (**j**) i) LiOH H_2O, H_2O_2 (35%), THF:H_2O 4:1, 0 °C, 5 h, ii) BnOH, EDC HCl, DMAP, DCM dry, 0 °C to rt, o.n., 60%; (**k**) i) MeOTf, DCM dry, rt, 15 h, ii) acetone:H_2O 2:1, rt, 20 h, quantitative; (**l**) PPh_3, THF:H_2O 20:1, 50 °C, 18h, quantitative; (**m**) HATU, DIPEA, THF, 0 °C to rt, 48 h, 84%.*

6.2. Results and discussion

During my stint abroad I was able to take part to this ongoing project on designing the first total synthesis of the natural compound thioholgamide A. Following the synthetic route reported in Fig. 6, I was able to prepare and isolate two important building blocks of the macrocycle portion of the natural compound.

7. References

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Dietary Exposure to Chemicals and Implications for Consumers Health

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This PhD thesis aims to strengthen consumer safety by advancing the scientific basis for dietary evaluations and risk assessments, involving the systematic monitoring of potentially toxic chemical contaminants and essential nutrients, in various food products, providing the basis for exposure modeling and characterization of the health effects.

Key words: Food safety, multi-elemental analysis, dietary exposure, risk assessment, montecarlo simulations.

1. Introduction

Dietary exposure assessments to chemicals in foods are a critical component of public health protection, as they can quantify levels of potentially hazardous contaminants, such as pesticide residues, heavy metals, mycotoxins, food additives, persistent Organic Pollutants (POPs) and process contaminants, which may be ingested by consumers through the diet. These environmental contaminants are unintentionally present in food. Factors like global industrialization, climate change, and insufficient waste management contribute to the infiltration of these contaminants into the food chain, particularly in water and soil, thus, ending up accumulating in staple dietary commodities including cereals, leafy vegetables, fruits, milk, dairy products, fish, and meat. The occurrence of environmental contaminants in food has raised significant public health concerns due to their persistence, bioaccumulation, and chronic toxicity. Chronic exposure to low levels of these substances over time can lead to serious health effects, including cancer, hormonal imbalances, immune suppression, and developmental disorders (Eskola et al, 2020). Monitoring and quantifying the inorganic elements in food samples is highly valuable for detecting both essential nutrients and harmful contaminants with precision (Ferreira et al, 2023). Exposure assessments are essential for identifying populations and sub-populations at risk, accounting for safe intake thresholds. Guidance for health risk evaluations are provided by regulatory bodies such as the European Food Safety Authority (EFSA), the U.S. Food and Drug Administration (FDA), and the World Health Organization (WHO). Over the past two decades, the methodologies, scope, and complexity of exposure assessments have evolved significantly, reflecting advancements in analytical chemistry, statistical modeling, and the increasing concern over cumulative exposure to multiple substances with similar toxicological endpoints or mode of action, as well as balancing the possible adverse health effect with the beneficial properties of the studied food or food components within risk/benefit evaluations. The two dominant approaches in dietary exposure assessment are deterministic and probabilistic models, each with distinct advantages and limitations. Deterministic models apply fixed point estimates for both food consumption and contaminant concentrations. This approach is conservative and suitable for screening-level assessments or regulatory compliance purposes. However, it lacks the capacity to capture variability and uncertainty within the population or between individual exposures. Probabilistic models, in contrast, capture the inherent variability and uncertainty in consumer behavior, food contamination levels, and toxicological thresholds. Monte Carlo simulation is a computational method that uses random sampling and statistical modeling to estimate the distribution of possible outcomes, rather than relying on single-point estimates. By generating thousands of exposure scenarios, it allows risk assessors to evaluate the probability of exceeding safety limits across different population groups (EFSA, 2017). Probabilistic assessments are now increasingly favored by regulatory agencies when sufficient data are available. Uncertainty and Variability Management Managing uncertainty is another major evolution in dietary exposure assessment. Probabilistic models inherently incorporate variability (natural heterogeneity) and can also address uncertainty (data gaps, model assumptions) using sensitivity analysis (EFSA, 2018).

Despite methodological advancements, several challenges persist, such as limited or inconsistent occurrence data for some chemicals, lack of harmonized dose/response models for many inorganic elements, which affects the possibility of using standardized approaches for risk and risk/benefit assessments and the need for more refined individual-level data, especially in vulnerable groups. Future directions should include greater reliance on machine learning and toxicokinetic modeling, the incorporation as much as possible of real-world scenarios, and a continued shift toward integrated multi-exposure frameworks.

Given that, the presented work focuses on three core objectives:

I. Monitor and describe the occurrence, concentration levels, and distribution patterns of potentially toxic inorganic contaminants and essential nutrients, in various food commodities, primarily through mass-spectrometry technologies (ICP-MS).
II. Development and refinement of methodologies to estimate dietary exposure to different food components. This will be achieved through the integration of probabilistic modeling approaches, particularly Monte Carlo simulations, to capture the variability and uncertainty inherent in real-world consumption patterns and contaminant levels across different population groups. Designing the models to support both single-substance and cumulative exposure assessments.
III. Characterize risk by comparing exposure estimates to toxicological reference values and assessing potential health risks associated with various dietary scenarios. This includes also risk/benefit evaluations, where the potential health benefits of food consumption are weighed against the associated detrimental effects.

2. Methodologies

2.1 Sample collection

The studies presented in this communication regard two different sets of food samples:

1- Included n = 29 botanical extracts sourced from various origins and different plant species, all of which were available in the EU market and sold in homogeneous powder form.
2- Included n=292 bovine samples, collected from three different Italian regions, Piemonte, Veneto and Toscana, where bovines were slaughtered all within the year 2024. From each region, ten different farms, located within their geographical area, were selected to contribute to this study with five different animals each. From each animal, the diaphragm muscle (n=146) and the liver (n=146), were excised and dried at -80°C.

2.2 Multi Elemental analysis by ICP-MS

For both sample sets, multi-elemental analysis was performed through Agilent 7900 ICP-MS quadrupole technology and an atomic absorption spectrometer, the mercury analyzer AMA-254. These techniques enable the quantification of up to 54 inorganic elements, including macro, micro, trace, and ultra-trace elements, namely: Li, Be, B, Na, Mg, Al, P, K, Ca, Ti, V, Cr, Fe, Mn, Ni, Co, Cu, Zn, As, Se, Rb, Sr, Y, Zr, Mo, Pd, Ag, Cd, Sn, Sb, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Yb, Lu, Hf, W, Pt, Tl, Pb, Bi, Th, U, Hg.

2.3 Dietary exposure assessment

For exposure assessment purposes, chronic food consumption data were retrieved from the "EFSA Comprehensive European Food Consumption Database", by selecting the studied food category and the target population groups. Data were obtained as mean ± Sd grams per kilogram of body weight per day (g kg bw^{-1} day^{-1}). Exposure estimates were obtained through the employment of the following formulas:

$$Daily\ exposure(mg/day) = \frac{Element\ concentration\ (mg\ kg^{-1})\ \times\ Food\ consumption\ rate\ (g\ day^{-1})}{1{,}000}$$

EDI= element concentration (µg/kg^{-1}) / 1000 × food consumption rate (µg/kg bw^{-1} day^{-1})

EWI = element concentration (µg kg^{-1}) / 1000 × food consumption rate (µg/kg bw^{-1} day^{-1}) × 7

*EDI = Estimated daily intake µg/kg bw^{-1} day^{-1}; EWI = Estimated Weekly intake µg/kg bw^{-1} $week^{-1}$

2.4 Risk and benefit characterization

For the assessment of potentially toxic metals and metalloids, such as Al, As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Sn, U, and Zn, Tolerable daily intake (TDI), Tolerable weekly intake (TWI) and benchmark dose lower confidence limits (BMDLs) were retrieved because of their potential health risks based on the thresholds of concern established by "EFSA and the Joint FAO/WHO Expert Committee on food additives" from the "Chemical Hazards Database, OpenFoodTox". To characterize the risks from chronic intake of the potentially toxic elements, either the percentage contribution to the TDI/ TWI or the Margin of Exposure (MOE). These values were calculated employing the following formulas:

%TDI or %TWI = EDI or EWI / HGBV × 100 *and* MOE = BMDL / EDI

For the assessment of essential nutrients, such as Ca, K, P, Mg, Fe, Zn, Dietary reference values, such as Average Intake (AI), Average Requirement (AR), Population Reference Intake (PRI) and Upper Intake Level (UL) were retrieved retrieved using the "Dietary Reference Values (DRV) Tool" by EFSA. To characterize the potential benefits the percentage contribution to the DRV was calculated by employing the following formula:

%DRV = Daily Exposure / DRV × 100

2.5 Cumulative risk assessment

The quantification of the non-carcinogenic cumulative risk from the chronic intake of potentially toxic metals and metalloids was carried out through the calculation of the Target hazard quotient (THQ) and Hazard Index (HI). THQ was calculated individually for each element through the following formulas:

$$THQ = \frac{EDI}{Reference\ Dose(RfD)} \times Lifespan\ of\ exposure$$

Hazard Index was calculated by the sum of all the THQ values:

$$HI = THQ_{As} + THQ_{Al} + THQ_{Ni} + THQ_{Pb} + THQ_{Zn} \ldots$$

2.6 Data Analysis

All computations for data processing, statistical analysis, and visualization were performed in RStudio (Version 2024.09.0+375) with R (Version 4.3.2; R Core Team, 2023). The calculations followed a probabilistic approach, accounting for the inherent variability in the available data. Input parameters were retrieved, when possible, as probability distributions or given as means with associated standard deviations (Sd). To propagate this uncertainty through the analysis, Monte Carlo simulations were performed with 100,000 iterations, allowing for a robust statistical characterization of the results, using the R studio package "stats" (R Core Team, 2023).

3. Main Results

3.2 Bovine samples study

Multi-elemental analysis results from the bovine samples study are currently being processed, since my doctoral studies will end in January 2026, and will be presented directly at the event during oral communication.

3.1. Descriptive analysis of multi-elemental concentration in botanical extracts samples

A great variability of concentration values of all the 49 analyzed elements was observed across the 29 botanical extracts samples, as well as within the different samples of each botanical group, divided by the plant part used to obtain the final product, i.e., fruits, fungi, leaves, peels, roots, seeds, spirulina. This scenario can be attributed to the wide range of species used to obtain botanical preparations, the diversity of plant parts utilized, and their geographical origins. Indeed, biological, geochemical, and climatic factors influence the process of mobility and availability of elements, impacting the final chemical composition of plant biomass (Kabata-Pendias, 2004). In all 29 analyzed botanical samples, the most abundant elements resulted to be K, Ca, Na, P and Mg. Toxic heavy metals and metalloids As, Cd, Hg, and Pb were all found as ultra-trace elements (<1 mg kg^{-1}). In the present work, none of the 29 analyzed samples exceeded the maximum limits (MLs) limits set by the European Commission for other food products, although the levels of Pb and Cd in some botanical groups can be considered relatively high and close to the MLs (Commission Regulation (EU) No. 2023/915).

3.1.1. Probabilistic dietary assessment and characterization of potential risks and benefits

Within the first assessment which targeted all age groups (infants, toddlers, children, adolescents, adults and elderlies) within the European population: the analysis revealed worrying intake levels of Al, As, and Ni, primarily stemming from the consumption of spirulina-, peel-, and leaf-based botanicals by younger age groups. The intake of As from all analyzed botanicals posed a significant risk for infants, yielding margins of exposure (MOEs) below

1, while those deriving from peel-based botanicals raised concerns across all age groups (MOEs = 0.04–2.3). The consumption of peel-based botanicals contributed substantially (13–130%) also to the tolerable daily intake of Ni for infants, toddlers, and children, while that of spirulina-based botanicals raised concerns related to Al intake also among adults, contributing to 11–176% of the tolerable weekly intake of this element. Instead, the second assessment, targeted to the Italian adult population, showed intakes of As from botanicals alone raised potential health concerns, displaying great variability of margins of exposure (MOEs), with $p5^{th}$-$p95^{th}$ ranges of 0.26-410 for carcinogenicity and 6.94-10935 for skin lesions. While, considering a total diet scenario in the target population, exposure levels to Pb also emerged as a concern, and probability distributions of MOEs resulted below 10 for both cardiovascular and nephrotoxic endpoints. Cumulative risk assessment, in the Italian adult population, revealed that combined exposure to As, Ni, Al, Pb, and Zn resulted in Hazard Index values exceeding 100% only when considering the total diet, indicating potential non-carcinogenic health risks under this scenario. These results suggest that the toxicological risks linked to botanical consumption may outweigh their presumed health benefits, highlighting the need for evidence-based regulatory actions targeting these products and a cautious consideration of their inclusion in the diet.

Table 5, Mean, standard deviation, median and percentile range (5th - 95th) values for percentage contributions to the DRVs or HBGVs, and MOE values, through the exclusive chronic consumption of botanical extracts by the Italian adult population.

Element	Mean	Sd	Median	Percentiles (5th - 95th)	Health metric
Ca	1.20	4.94	0,25	(0.01 – 4.68)	%PRI
K	4.07	19.6	0,76	(0.04 – 15.4)	%AI
Mg	2.23	8.61	0,49	(0.03 – 8.75)	%AI
P	2.41	8.55	0,58	(0.04 – 9.33)	%AI
Fe	4.11	12.95	0,98	(0.03 – 16.8)	%PRI
Zn	0.64	1.99	0,15	(0.01 – 2.59)	%PRI
Al	2.34	8.15	0,54	(0.02 – 9.32)	%TWI
Ni	2.05	14.8	0,24	(0.01 – 7.34)	%TDI
As^a	121	832	10,4	(0.26 – 410)	MOE
As^b	3232	22195	277	(6.94 – 10935)	MOE
Pb^a	1087	4625	218	(11.5 – 4225)	MOE
Pb^b	2588	11012	519	(27.3 – 10059)	MOE

As^a is the MOE associated with the BMDL01 for carginogenic effects; As^b is the MOE associated with skin lesions; Pb^a is the MOE associated with nephrotoxicity; Pb^b is the MOE associated with cardiovascular diseases.

Finally, findings revealed that botanicals consumption contributed minimally to the recommended daily intakes of essential nutrients (Ca, K, P, Mg, Fe and Zn), resulting in median contributions <1% and percentile ranges ($p5^{th}$-$p95^{th}$) of 0.01% - 15.4% of dietary reference values (1), suggesting a limited support in nutritional adequacy. While individually modest and unable to compensate for poor dietary patterns, this incremental gain may help bridge minor dietary shortfalls especially in individuals with marginal deficiencies. Overall, in the total diet scenario of the Italian adult population, Sd values indicated substantial variability in intake among individuals, ranging from potentially concerning deficiency outputs

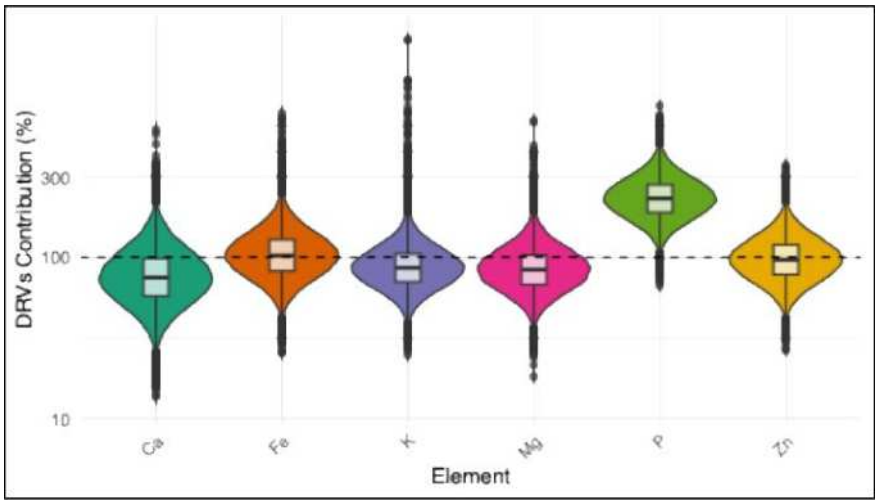


Figure 1, Probability distribution for contributions (%) to the Dietary Reference Values, for several essential nutrients by the combined of exposure deriving from botanical extracts and total diet in the Italian adult population.

4. Conclusions

Comparing the results of this study with existing literature is difficult due to differences in consumption data and risk assessment methodologies, even for the same food products. Nonetheless, prior research has advanced understanding of botanical preparations used in food supplements and contributed to methodological progress in dietary exposure assessments. Findings from this study suggest that the toxicological risks associated with botanical product consumption may outweigh the presumed benefits of essential nutrient intake particularly for vulnerable groups like children, who should only use such products under medical supervision. This underscores the need for evidence-based regulatory measures and cautious dietary inclusion of botanicals. Despite yielding important insights, the study has limitations. Exposure variability due to differing diets, lifestyles, and geographic regions may not be fully captured. Temporal and spatial variations in contaminant concentrations, as well as susceptibility among sensitive subgroups (e.g., pregnant women, individuals with health conditions), were also insufficiently addressed due to data limitations. To protect public health, producers must enhance quality control, given the chemical variability and potential contamination of botanical products. A clearer regulatory framework in the EU is urgently needed, incorporating comprehensive exposure assessments. Additional evaluation and targeted regulatory actions are essential to reduce risks. Notably, toxic metals with similar health impacts not included in this study may further increase risk. These results pertain to adults—the intended consumer group—and are based on probabilistic Monte Carlo simulations, which allowed for robust, uncertainty-sensitive exposure estimates. Overall, this research contributes to understanding the trade-offs between nutritional benefits and toxicological risks of botanical extracts. It supports regulatory development, risk management, and consumer guidance, offering a dietary exposure assessment relevant to the Italian adult population.

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Oral presentation sessions
PhD students – 3rd year
Abstracts

PARALLEL SESSION 4
Advanced Approaches in Wine Science

Resilient wineries
Enological strategies for adaptation to climate change, reduction of inputs, reuse of waste, valorization of native grape varieties

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The aim of this PhD project was to improve the quality of Apulian grape varieties, highlighting local traditions and promoting more sustainable winemaking practices throughout: I) Study on prolonged maceration of Minutolo and Verdeca wines; II) Influence of amphorae and different types of wood during Negroamaro wine ageing; III) review on premature ageing of red wines; IV) evaluation of pectolytic enzyme impact on Primitivo and Bombino nero wines quality V) investigating the impact of withering on Negroamaro wines; VI) Mapping Apulia's rosé wines according to their colors.

Cantine resilienti
Strategie enologiche di adattamento al cambiamento climatico, riduzione degli input, riutilizzo degli scarti, valorizzazione di varietà di uva tradizionali

L'obiettivo di questo progetto di dottorato è stato quello di migliorare la qualità dei vitigni pugliesi, valorizzando le tradizioni locali e promuovendo pratiche enologiche più sostenibili attraverso: I) Studio della macerazione prolungata sui vini Minutolo e Verdeca; II) Influenza delle anfore e dei diversi tipi di legno durante l'affinamento del Negroamaro; III) Review sull'invecchiamento prematuro dei vini rossi; IV) Utilizzo di enzimi pectolitici sui mosti di Primitivo e Bombino nero; V) Impatto dell'appassimento sui vini Negroamaro; VI) Mappatura dei vini rosati pugliesi in base ai loro colori.

Key words: Cultivar Valorisation, Sustainability, Withering, Defoliation, Pectolytic Enzymes, Enzymes Side-activities

1. Introduction

In accordance with the objectives of the PhD thesis outlined above, this oral communication presents the main findings of an experimental study conducted during the third year of the doctoral program, aimed at enhancing the quality and typicity of the Apulian wine Negroamaro through the application of withering and defoliation techniques.

2. Autochthonous grape valorization: withering and pectolytic enzymes

In recent years, there has been growing interest in enological strategies aimed at enhancing the varietal expression and quality of wines produced from local grape cultivars, in order to preserve regional identity, promote biodiversity, and improve wine quality through innovative and sustainable practices (Prezioso et al., 2023). Withering is a traditional post-harvest technique used to concentrate grape components through dehydration, allowing the production of sweet or fortified wines. Through the progressive dehydration of grapes, this process leads to an increase in sugar content, modulation of acidity, and significant enrichment of phenolic and aromatic compounds (Rolle et al., 2013; Scalzini et al., 2021). Historically, different withering methods have developed in response to environmental conditions, local grape varieties and technical practices, becoming integral to the cultural and viticultural heritage of many winemaking regions (Slaghenaufi et al., 2020). In Italy, the most emblematic example is Amarone, produced from Corvina, Corvinone and Corvinella grape varieties that are subjected to dehydration, resulting in wines with high extract, alcohol and complexity (Barbanti et al., 2008). Withering can be performed on-vine, by delaying harvest or employing techniques like cane cutting and peduncle twisting, or off-vine, where harvested grapes are dried under controlled or semi-controlled conditions. Off-vine withering allows for greater control over key environmental parameters (temperature, humidity and air flow), which directly influence the rate and extent of dehydration as well as the biochemical transformations occurring in berry (Scalzini et al., 2021; Slaghenaufi et al., 2020). Simultaneously, enzyme-based technologies, particularly pectolytic enzyme preparations, have gained traction in winemaking for their ability to improve must clarification and enhance the extraction of varietal aromas and structural components (Osete-Alcaraz et al., 2022). While primarily applied for technical purposes, these enzymes often carry side activities (e.g., glycosidases, esterases), that can modulate the aromatic complexity and varietal expression of wines (Claus and Mojsov, 2018).

3. Experimental Procedure

In accordance with the PhD thesis project, a two-step experimental procedure was set up by performing the following independent test: the pilot-scale experiment carried out on Negroamaro grapes harvested from defoliated and non-defoliated vines, subjected to withering either on-vine or in a *pajara*; ii) the study of the side activity of three pectolytic enzymes preparations on the quality of Bombino nero and Primitivo wines.

4. Materials and Methods

I) Negroamaro grapes were cultivated in defoliated and non-defoliated vines. After reaching maturity, the grapes were either left on-vine or harvested and placed on nets within traditional stone structures (*pajara*) for withering. After 20 days of withering, the dehydrated grapes from each treatment were vinified in triplicate at Menhir wineries using 50 L steel tanks. Four wine samples were produced and analyzed: i) wines from defoliated vines withered in *pajara* (P-DEF); ii) wines from non-defoliated vines withered on-vine (P-NDEF); iii) wines from defoliated vines withered on-vine (V-DEF); iii) wines from non-defoliated vines withered on-vine (V-NDEF). During withering, average weight of five berries (g), average weight of five peels (g), and sugar content (°Brix) were monitored. Wines were analyzed for ethanol content (% vol) using a WineScan FT 120 (FOSS, Hillerød, Denmark). pH and total acidity with an automatic titrator (Flash Automatic Titrator, Steroglass, Perugia, Italy). Phenolic composition and color parameters were determined using a modified Somers assay (Mercurio et al., 2007), and CIELab color analysis was performed according to the CIE 1976 (L*a*b*) color space, using the MSCV® software. The volatile composition of the wines was assessed according to Tufariello et al. (2012). Quantitative sensorial analysis was conducted by a trained panel of eight expert judges. Each wine sample was evaluated three times across two separate tasting sessions. All statistical analyses were performed using Origin Pro 2022 software (OriginLab, Northampton, MA, USA).
II) The experiment consisted of pilot-scale trial using real musts from *Primitivo* and *Bombino nero* grapes. Three commercial pectolytic enzyme preparations (E1, E2 E3) provided by Enolife s.r.l. were evaluated. Grapes were harvested at full ripeness and processed following a short maceration phase. The musts were treated with the selected enzymes and underwent static cold clarification, followed by fermentation in stainless steel tanks. Resulting wines were analyzed for phenolic and chromatic properties as well as for their volatile compound profiles. Statistical analysis included ANOVA, Tukey's post-hoc tests, and multivariate clustering to evaluate the effects of each enzyme on wine composition.

5. Results and Discussion

I) Influence of withering on Negroamaro wines

5.1.1 Withering monitoring

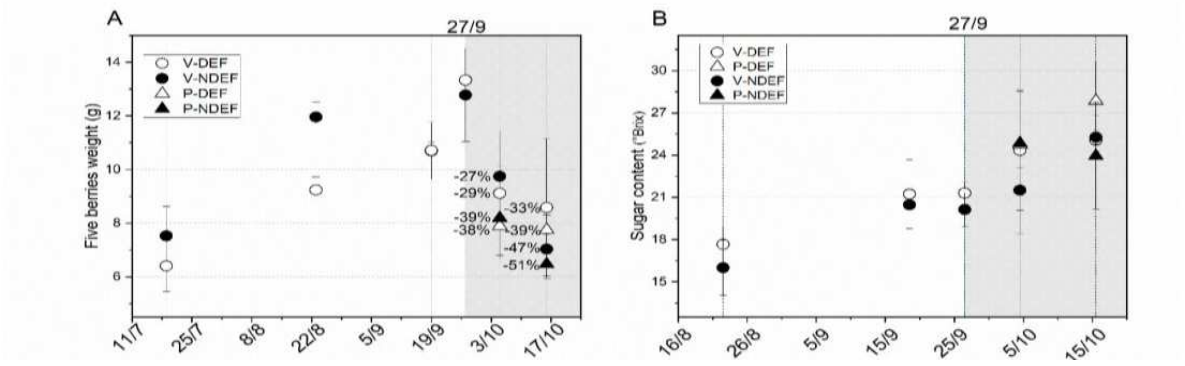


Figure 1: Panel A: trend of five berries weight registered during withering in defoliated and non-defoliated samples, withered in *pajara* and on-vine. Panel B: trend of sugar content registered during withering in defoliated and non-defoliated samples, withered in *pajara* and on-vine.

The results of withering monitoring based on five-berry weight and sugar content are shown in Figure 1, panels A and B. Initially ranging from 12 to 14 g at the beginning of the withering, berry weight decreased progressively. This decline was more rapid in samples withered in the *pajara* during the first eight days. By the end of the process, significant differences in weight loss were observed among the treatments. Total water loss ranged from 33% to 51%, with the highest reductions found in non-defoliated samples: 47% for those withered on-vine and 51% in the *pajara.* As expected, water loss was accompanied by an increase in sugar concentration across all samples. Starting from 20–22 °Brix at harvest, sugar levels peaked at 27.09 °Brix in grapes from defoliated vines withered in the

pajara.

5.1.2 Chemical composition and phenolic indices

All samples resulted in high total acidity and low pH (Figure 1, A). Without defoliation the total acidity was higher, reaching a maximum of 11.6 in V-NDEF. The high values of total acidity registered in all the thesis are ascribable to concentration phenomena, which compensated the biological deacidification determined by dehydration process (Xi et al., 2024). pH levels were higher in samples withered in *Pajara* than on-vine, similarly to a study conducted on Corvina grapes by Slaghenaufi et al., (2020), while they were not affected by vine management. These results are particularly important in the context of climate change where the pH of wines tends to increase and the acidity to decrease (Sadras et al., 2013). Significant differences in chemical and phenolic composition were observed among the samples. Figure 1 (B) presents the results for two key parameters related to wine color stability. Withering in the *pajara* appeared to better preserve color compared to on-vine withering, as indicated by higher levels of SO_2-resistant pigments. Notably, in non-defoliated samples, the *pajara* method also resulted in the highest color intensity. This may be attributed to reduced oxidation, leading to lower formation of brown pigments and a greater accumulation of red-colored compounds (Figueiredo-González et al., 2013).

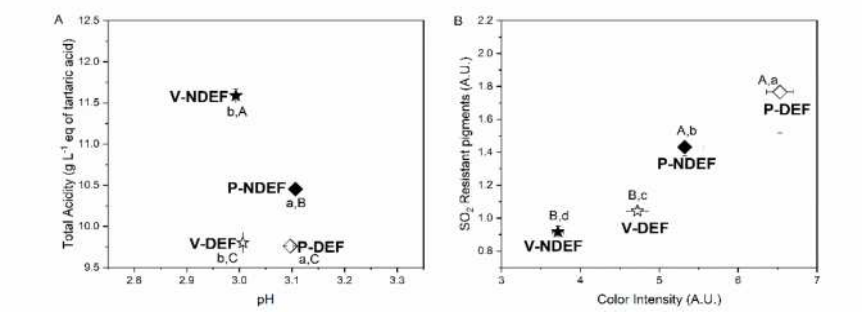


Figure 2: Total Acidity and pH in withered wines (Panel A). Capital letters indicate statistical different means among sample's Acidity; lower letters indicate significant different means among sample's pH. Panel B: color intensity and SO2 resistant pigments in withered wines. Different capital letters indicate significant different means among sample's color intensity; lower letters indicate significant different means among sample's SO2 resistant pigments.

5.1.3 Volatile composition and sensorial profile

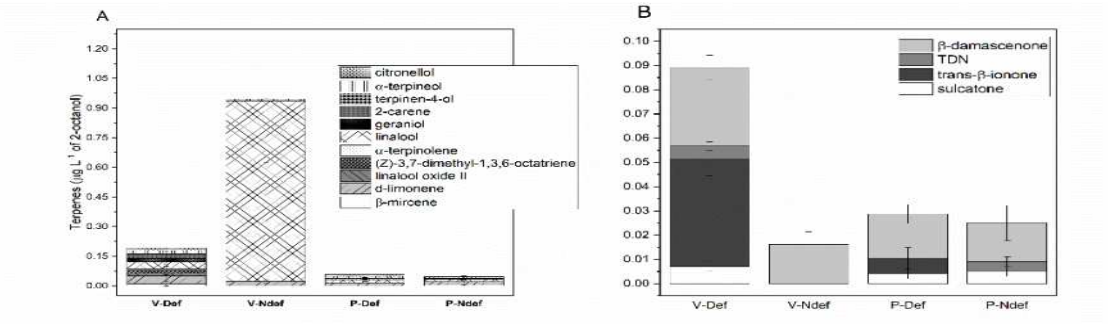


Figure 3: Terpenes concentrations (Panel A) and Norisoprenoids concentrations (Panel B) of withered wine samples. Different letters indicate statistical different means.

The volatile composition was strongly influenced by the different techniques applied in this study, with notable effects on primary aroma compounds. Figure 3 displays two stacked bar charts illustrating the concentrations of terpenes (panel A) and norisoprenoids (panel B) concentrations revealed in the four samples. The highest terpene concentration was observed in the non-defoliated samples withered on-vine, reaching 0.946 µg L^{-1} equivalent of 2-octanol. This is likely due to the lower temperatures around non-defoliated clusters, which favor terpene accumulation (Shmuleviz et al., 2023). The terpene profile of the V-NDEF sample was dominated by linalool, which is characteristic of Negroamaro wines (Tufariello et al., 2012), accounting for nearly 100% of the composition. In contrast, the other samples displayed a more diverse terpene profile. Withering in *pajara* resulted in the lowest concentration of terpenes, and their profile was dominated from d-limonene (36-27%), followed by linalool (30-27%). Regarding norisoprenoids, V-DEF exhibited the highest concentration, that can be attributed to higher sun exposure, which favors the degradation of carotenoids and the subsequent formation of norisoprenoids (Alessandrini et al., 2018). Sample withered on-vine, in absence of defoliation, had the lowest concentration and

presented the lowest diversity of norisoprenoids. β-damascenone was present in high concentrations in all samples. It is associated to flowery and exotic flowery notes and is typically found in several dehydrated wines (Giordano et al., 2009). Trans-β-ionone, typically associated to violet odor, was found exclusively in defoliated samples. During sensory analysis, differences emerged between samples for fruity, toasty and color intensity attributes (data not shown). Samples withered on-vine were fruitier than pajara withered ones, in accordance with the higher concentration of primary aroma compounds resulting from aroma analysis. Confirming chemical analysis results, panelists scored a higher color intensity in samples withered in pajara.

II) Study of three pectolytic enzymes on Primitivo and Bombino nero musts

5.2 Phenolic indices and volatile profile

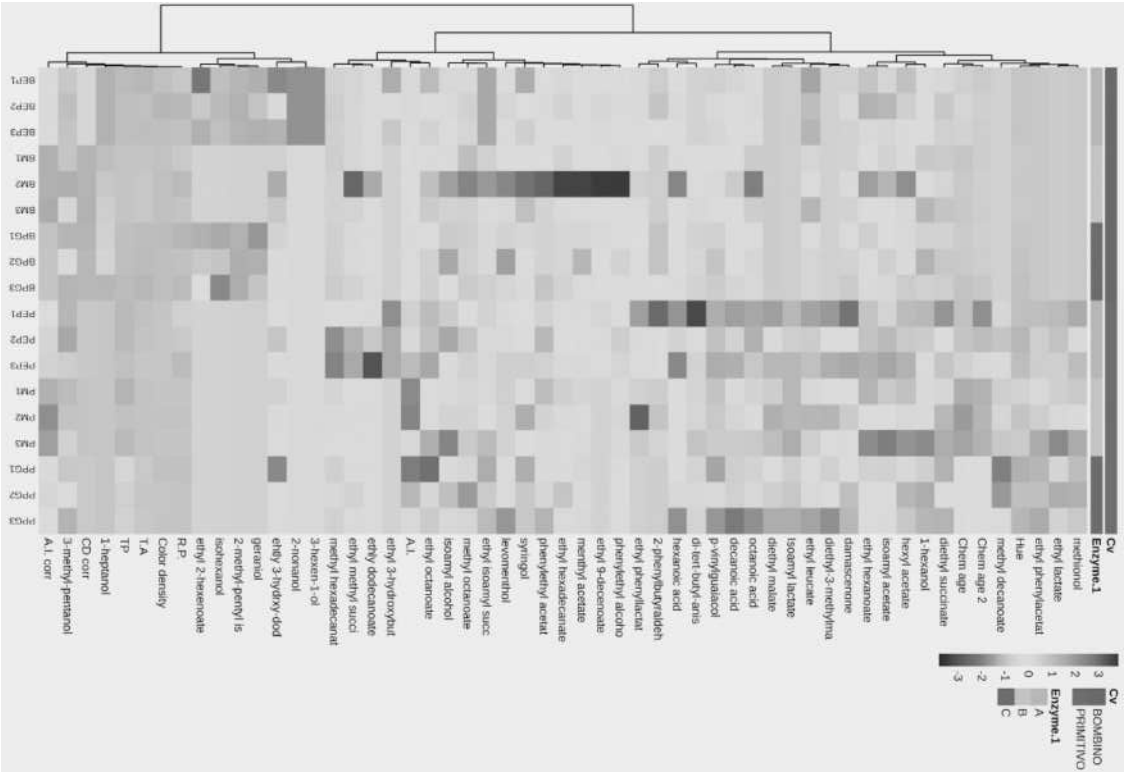


Figure 4: heatmap of volatile compounds and phenolic indices of Primitivo e Bombino wines obtained with different pectolytic enzymes preparations.

Chemical and volatile analysis of wines treated with enzymes revealed distinct clustering of wines based on grape cultivar, clearly separating *Bombino nero* and *Primitivo* samples (Figure 4). Wines made from *Bombino nero* were characterized from color density, total phenols, anthocyanins, and SO_2-resistant pigments, indicating a richer and more stable color. In contrast, *Primitivo* wines were marked by higher hue and chemical age, suggesting differences in color evolution and stability. Regarding aroma, *Primitivo* wines were generally richer in esters, contributing to fruity notes. Among the enzymes tested, enzyme E1 significantly increased certain volatile compounds in both grape varieties. In *Bombino nero* wines, E1 enhanced levels of 3-hexen-1-ol, 2-nonanol, and ethyl 3-hydroxy-dodecanoate (associated with fresh, green, or floral aromas). It also increased geraniol and several other esters in both cultivars, suggesting a broader impact on the wine's aromatic complexity. Importantly, the study highlights how clarification enzymes, originally intended to improve must clarity, can have notable side effects on wine volatilome (Espejo, 2021). The presence of certain volatile compounds like C6 alcohols and esters suggests that these enzymes may enhance the activity of natural grape enzymes involved in lipid metabolism (e.g., lipoxygenase and β-oxidation pathways), contributing to the formation of aroma precursors and aroma relevant thiols, whose conversion can be achieved during fermentation (Clark and Deed, 2018). Furthermore, the increased levels of compounds like ethyl 3-hydroxybutyrate and ethyl dodecanoate is oenologically relevant, as these esters contribute to the perception of red-fruit and fruity notes (Gracia-Moreno et al., 2015).

6. Conclusions and Future Perspectives

The results obtained in this PhD project allowed us to investigate the influence of withering and defoliation on the quality of Negroamaro wine and the influence of three pectolytic enzymes preparations on the quality of Bombino Nero and Primitivo wines. In both cases the techniques modulated aromatic composition, valorising in particular

primary aroma compounds, that are strongly correlated to the grape identity. Withering determined wines with low pH and high total acidity, key attributes in the context of climate change. The combination of withering techniques and canopy management allowed us to modulate key wine characteristics, reaching good results in terms of color stability with *pajara* withering and primary aromatic compounds valorisation with on-vine withering. The employment of *pajara* proved to be well-suited for the withering process and may offer added value to this Apulian cultivar by reinforcing its connection to the local terroir. The results obtained, particularly those related to pH and total acidity, could serve as the basis for further studies on the development of blends aimed at correcting wines with high pH and low total acidity. The use of clarification enzymes, particularly those with specific side activities, represents a promising tool to enhance the aromatic and phenolic expression of Apulian cultivars, supporting their differentiation and oenological valorization.

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Assessment of quercetin precipitation risk in Italian red wines

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The goal of this Ph.D. thesis project is to study the chemical and physical phenomena responsible for the instability and reduced longevity of red wines caused by imbalances in phenolic composition due to climate change. Specifically, the study will analyse how the chemical composition of red wine affects quercetin's behaviour in solution over time. This analysis will examine several representative Italian red wines with different phenolic profiles to identify the main risk factors associated with instability.

Valutazione del rischio di precipitazione della quercetina nei vini rossi italiani

Questo progetto di tesi di dottorato si propone di studiare i fenomeni chimico-fisici responsabili dell'instabilità e della riduzione della longevità dei vini rossi, causate dagli squilibri nella composizione fenolica dovuti al cambiamento climatico. In particolare, lo studio analizzerà come la composizione chimica del vino rosso influenzi il comportamento della quercetina (Q) in soluzione nel tempo, esaminando diversi vini rossi rappresentativi di varietà italiane con profili fenolici differenti, e si concentrerà sull'identificazione dei principali fattori di rischio associati alla sua instabilità.

Key words: Quercetin; anthocyanins; solubility; red wine; co-pigmentation; precipitation risk.

In accordance with the PhD thesis project previously described (Luciano, 2023), this oral communication reports the main results of the following four activities directed to:
A1) Evaluation of the impact of anthocyanins on quercetin solubility in a model solution.
A2) Understanding whether the addition of quercetin powder can act as nucleation sites and facilitate the further precipitation of quercetin in solution.
A3) Development of an experimental protocol to identify wine parameters that influence quercetin solubility.
A4) Select and analyze wines with different phenolic compositions to identify the main risk factor for precipitation.

1. Introduction

Quercetin (Q), a phenolic compound released from grape skins during red wine maceration, has been identified as a potential source of instability in bottled red wines, especially Sangiovese varieties (Lanati *et al.,* 2014). In grapes, Q is synthesized as glycosides, wherein sugar molecules are attached to the aglycone. During the early stages of vinification or wine aging, these glycosides undergo enzymatic or acidic hydrolysis, releasing the free aglycone into the medium. The aglycone is significantly less soluble than the glycosides in the hydroalcoholic matrix of wine, which increases the likelihood of precipitation and sediment formation. Crystal formation is thermodynamically favored due to two phenomena: first, Q molecules aggregate in π-stacking configurations. Second, a network of intermolecular hydrogen bonding forms among the Q molecules (Filip *et al.,* 2013; Campo & Corral, 2022). The precipitation of Q can result in sedimentary deposits of typical, needle-like, yellow crystals (Patel *et al.,* 2012), which negatively affect the wine's visual clarity and perceived quality (Lanati *et al.,* 2014; Gambuti *et al.,* 2020).
Unfortunately, there is no defined solubility range for Q in red wines in the literature due to the numerous factors that affect it. The phenolic composition of wines is probably one of the most important factors. Therefore, an in-depth understanding of the chemical behavior of Q in red wine is a prerequisite. The content of Q and its glycosides in wine is strongly influenced by grape variety (Mattivi *et al.,* 2006), climatic conditions (Price *et al.,* 1995; McDonald *et al.,* 1998), and winemaking practices applied during vinification (Gambuti *et al.,* 2004) and aging (Fang *et al.,* 2007; Picariello *et al.,* 2023). One of the first goals was to evaluate the key factors influencing Q solubility and precipitation. Regarding Q solubility, it is important to note that Q's electronic configuration favors π–π interactions with anthocyanins, forming copigmentation complexes in red wines. Therefore, anthocyanins are expected to play a key role in Q solubility. A previous study investigated how copigmentation improves Q solubility (Lanati *et al.,* 2022). The interaction between Q and anthocyanins appears to stabilize Q in solution, reducing its tendency to precipitate.
The impact of increasing the concentration of anthocyanins in the medium has never been evaluated. This knowledge gap implies the need for a more comprehensive investigation of the relationship between anthocyanins and Q solubility.
Regarding the precipitation of Q, it is a process that results in the rapid formation of solids. Because of its planar conformation, it is known that Q gives rise to crystals, especially when hydrated, in which molecules tend to stack closely on top of each other by strong π-π interactions. Since the precipitation process begins at elevated

supersaturation levels, introducing nucleation seeds can accelerate solid phase formation (McGinty *et al.,* 2020). Therefore, we conducted a second experiment in which three important crystallization factors were investigated: temperature, time, and the addition of nucleation seeds (Boulton *et al.,* 1999). Red wines were stored at two different temperatures (2 °C and 20 °C) and supplemented with a known amount of Q to act as a seed crystal and promote crystal growth. Thus, the addition of a seed crystal may speed up the time required to stabilize the wine. Based on the results obtained, another goal was to study how all the main wine parameters impact the content and evolution of Q in red wine over time. To this end, red wines representative of various Italian grape varieties and their different phenolic compositions were analyzed. Additionally, the study aims to identify the primary risk factors for flavonol instability in red wine.

2. Materials

Frist goal: In this trial four experimental samples containing increasing concentrations of anyhocyanins and 88.70 µM of Q were prepared in a model solution. The solutions were prepared by adding anthocyanins to volumetric flasks, resulting in the following final concentrations: 0 µM (A0); 101.3 µM (A1); 405.3 µM (A2); 1.013 mM (A3). Then, Q was added to all the aforementioned flasks to achieve a final concentration of 88.70 µM, and the volume was adjusted with the model solution. The experimental solutions were mixed by delivering the Q stock solution into the stirred A1, A2, and A3 solutions held in the volumetric flasks. Stirring was achieved using a magnetic stirrer and a Teflon stirring bar for 60 seconds at 20 °C. For each sample, a corresponding control with the same anthocyanin concentration but without Q was prepared. All samples were stored in the dark at 20°C, filtered, and analyzed at the following times: 2 hours (time 0); 24 hours (time 1); 144 hours (time 2); 312 hours (time 3); and 840 hours (time 4).

The second goal of the experiment was to evaluate the effect of Q seeds on three Sangiovese red wines (Wa, Wb, and Wc), which were selected because they had the highest concentrations of Q (ranging from 44.3 µM to 65.0 µM). To evaluate the effect of Q seeds, Q powder was added to the wine in dark 10 mL glass vials. The air in the vials was thoroughly displaced with nitrogen, and the Q powder concentration was greater than the usual concentration found in wines (around 2.07 mM). Two replicates were prepared for each sampling point and stored at two different temperatures (2 °C and 20 °C) in the dark. Three sampling times were considered: 0, 3, and 10 days. Prior to analysis, samples were filtered using 0.45 µm Durapore membrane filters.

Third goal: In this study, to explore a broad range of red wine matrices influencing Q solubility, different grape cultivars (16 Italian; 1 French) cultivated in various regions of Italy in 2023, were used to produce the 32 100% single-cultivar wines used in the study. These wines were sourced from several wine producers. All wines were fermented in stainless steel vats at a commercial scale and sampled before malolactic fermentation (MLF) and wood ageing (4 months after harvest). Each sample was preserved with SO_2 and bottled. Bottles were sealed with Select Green 100 corks (Nomacorc, Rivesaltes, France) and stored at a constant cellar temperature (16 ± 2°C) until analysis (after 8 months of bottle aging). The oenological parameters of the selected wines are reported in Supplementary Table 1. Due to the diversity of grape varieties and vinification conditions, oenological parameters varied among the samples. For the experiment two sampling times were considered: time 0 and time 1, corresponding to the moment of bottling and after 8 months of bottle ageing, respectively. Before HPLC analysis, all samples were filtered using polyvinylidene difluoride PVDF filters 0.45 µm (Durapore, Millipore, Ireland) membrane.

3. Methods

3.1 Wine main compositional parameters: (OIV Compendium of International) Methods of Wine and Must Analysis 2017.
3.2 HPLC-DAD analysis of Q and Q-Gs: (Luciano et al*.,* 2025)
3.3HPLC-DAD analysis of anthocyanins:(OIV Compendium of International) Methods of Wine and Must Analysis 2017.
3.4 Spectrophotometric analyses of phenolic compounds: (Harbertson et al., 2003); (Gambuti *et al*., 2018).
3.5 HPLC-DAD analysis of acetaldehyde: (Han *et al.* 2015).

4. Results and Discussion

4.1 effect of anthocyanins on Q solubility

Figure 1 shows the levels of Q in experimental samples with different concentrations of anthocyanin. In sample A0, only 14.78 µM of Q remained in solution right after 88.70 µM of Q was added (time 0), which is consistent

with Q's limited solubility in a 15% hydroalcoholic solution and tartrate buffer at pH 3.20 (Lanati *et al.,* 2022) When Q was added to samples with increasing anthocyanin content, initial solubility improved (see Fig. 1).

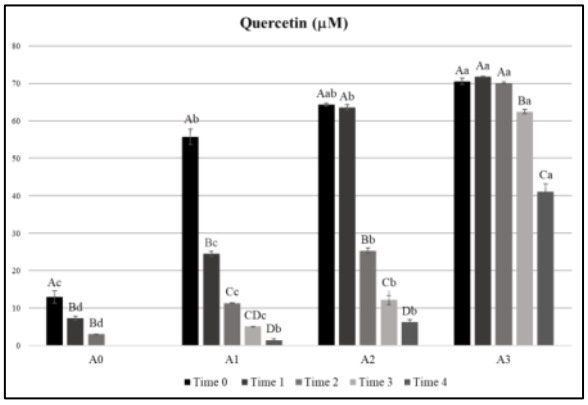


Figure 1. *Content of Quercetin in experimental samples at different concentrations of anthocyanins. A0, A1, A2, A3: samples obtained by mixing Q (88.687 M) with increasing amounts of anthocyanins (101.3, 405.3 and 1013 M respectively) in model solution. Time 0=2h. Time1=24h. Time 2=144h. Time 3=312h and Time 4=840h.*
Different capital letters indicate the significant differences (p<0.005) of each experimental sample (A0, A1, A2 and A3) over time.
Different lowercase letters indicate the significant differences (p<0.005) among samples added with increasing concentration of anthocyanins at the same time.

However, as in A0, Q levels declined over time in A1 and A2 after 24 and 144 hours, respectively. In A3, precipitation began later (after 312 hours) and continued until 840 hours. Notably, higher anthocyanin concentrations increased Q's solubility. This is relevant because anthocyanins decrease during wine aging, which increases the risk of Q precipitation. The enhanced solubility in A1–A3 may be due to copigmentation between the anthocyanins and Q. Q is a strong copigment thanks to its extended π-conjugation and its ability to form π–π stacking and hydrogen bonds (Waterhouse *et al.,* 2024)

4.2 precipitation trial

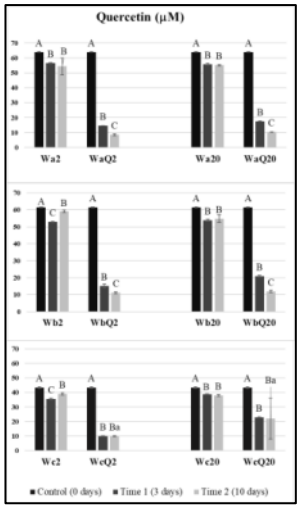


Figure 2. *Content of quercetin in experimental wines (Wa2, WaQ2, Wa20, WaQ20, Wb2, WbQ2, Wb20, WbQ20, Wc2, WcQ2, Wc20, WcQ20) at different time: 0 days (control),3 days (time 1), 10 days (time 2). W and WQ codes refer to control wines (W) and wines added with Q seeds (WQ). Different capital letters indicate significant differences (p<0.005) of each experimental sample over time. The same lowercase letter indicates no significant differences between control wine and the same wine after the addition of nucleation seeds.*

In Figure 2. are shown data on the effect of Q powder addition on red wine containing Q. Immediately after the addition of the Q seeds, a substantial reduction over 75% of the initial Q value was observed at 2°C for all the investigated wines. A similar trend was observed at 20°C, albeit to a lesser extent, with a reduction over 47.3% of the initial Q value. Subsequently, the decrease slightly intensified after 10 days for both Wa and Wb wines.

4.3 factors influencing precipitation risks

Before bottling, the wines did not show any sediment, while after 8 months of bottle aging 9 wines showed sediment and for all of them Q was found in the extract obtained from the sediment. The wines were then divided into two groups based on the presence or absence of Q sediment and the Spearman rank correlation test, a non-parametric test that measures the strength of association between two variables, was used to examine the associations between Qsol and Qsed and the initial chemical and phenolic parameters of red wines. To optimize the test, only the variables highly correlated with Qsol and Qsed were selected for correlation analyses, as shown in Figs. 3 and 4

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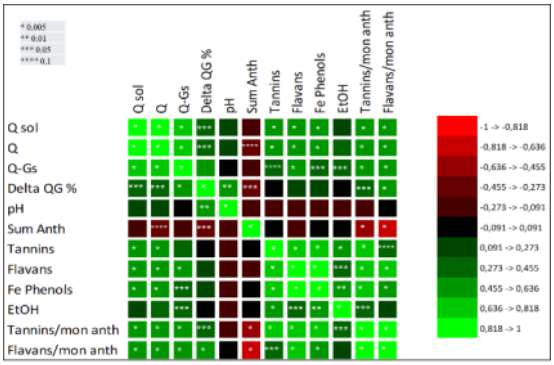


Figure 3. Correlation between Qsol and parameters of stable red wines (not showing any sediment after 8 months) before ageing (Spearman test: ****p level < 0.1; ***p level < 0.05; **p level < 0.01; *p level < 0.005).

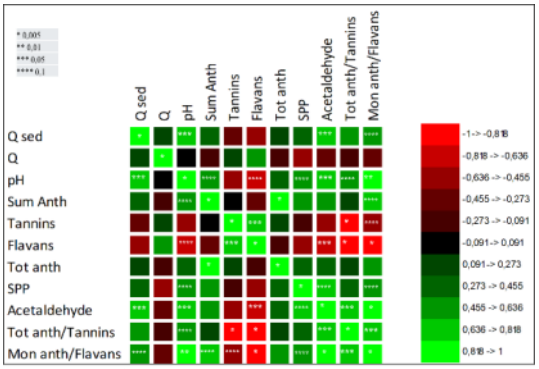


Figure 4. Correlation between Qsed and parameters of unstable red wines (showing a sediment after 8 months) before ageing (Spearman test: ****p level < 0.1; ***p level < 0.05; **p level < 0.01; *p level < 0.005).

As expected, a correlation was found between quercetin solubility (Qsol) and the initial levels of Q and Q-Gs in red wines. In contrast, no significant correlation was observed between Qsol and pH, ethanol content, or anthocyanin levels. Significant correlations emerged with other phenolic compounds, especially flavans, tannins, and their ratios with monomeric anthocyanins.

These findings suggest that specific wine components may affect Q crystallization by interacting with nucleation sites, potentially inhibiting crystal growth—similar to how phenolic compounds impact potassium bitartrate crystallization in white wine (Waterhouse *et al.,* 2024).

Regarding Q sedimentation (Qsed), it was strongly correlated with pH, acetaldehyde content, and the ratio of monomeric anthocyanins to flavans. A predictive PLS model was built, explaining 79.8% of the original variance and indicated that the strongest factors impacting the content of Qsed are pH, acetaldehyde, and Tot Anth/Tannins. Instead Qsed is negatively correlated to the sum of monomeric anthocyanins, tannins, flavans and total anthocyanins

Overall, wines with higher pH, acetaldehyde, and pigment/tannin imbalance showed a greater risk of quercetin precipitation, while higher levels of tannins, flavans, and monomeric anthocyanins reduced this risk.

5. Conclusions:

The results of this study provide new insights into the behaviour of Q in wine-like systems, highlighting how anthocyanin concentration can significantly impact its solubility. Copigmentation effects appear to impact Q stability, but these interactions are unstable, underscoring the importance of time in such molecular interactions. Analysis of real wines revealed numerous factors involved in Q solubility, with the phenolic richness of the wines being the most important factor, especially regarding tannins rather than anthocyanins. Furthermore, multivariate analysis (PLS) revealed an increased risk of quercetin (Q) precipitation at elevated pH levels. Among the key factors affecting crystallization, nucleation seeds were the most effective in accelerating precipitation. This finding suggests the possibility of developing strategies to control and potentially limit Q precipitation in bottled wine. Further microscopic and chemical analyses could help us understand the nature of Q in the deposit and continue the search for strategies to stabilize wine.

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Wild yeasts as source for designing specific "synthetics microbiota" to be used for wine fermentation.

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This PhD project aims to highlight the importance of grape and other natural environment microflora in increasing the diversity and improving quality of wines from different *terroirs*. The use of commercial starter cultures (mainly *S. cerevisiae*) reduces the potential of the native microbiota to contribute to the terroir effect. The project involves the development of a protocol for mix starter culture (synthetic microbiota) design made up from different selected species from specific *terroirs*. The synthetic microbiota could bring improvements from a sustainability point of view by exploiting the individual characteristics of each selected strain to achieve greater complexity, stability and natural protection of wine.

Lieviti selvatici come fonte per la progettazione di uno specifico "microbiota sintetico" da utilizzare per la fermentazione del vino.

Questo progetto di dottorato mira a evidenziare l'importanza della microflora dell'uva e degli ambienti naturali nell'aumentare la diversità e migliorare la qualità dei vini provenienti da diversi *terroirs*. L'uso di colture starter commerciali (principalmente *S. cerevisiae*) riduce il potenziale del microbiota nativo di contribuire all'effetto *terroir*. Il progetto prevede lo sviluppo di un protocollo di progettazione di colture starter miste (microbiota sintetico) costituito da diverse specie selezionate provenienti da specifici *terroirs* e da ambienti naturali. I microbiota sintetici potrebbero portare miglioramenti dal punto di vista della sostenibilità sfruttando le caratteristiche individuali di ciascun ceppo selezionato al fine di ottenere una maggiore complessità, stabilità e protezione naturale del vino.

Key words: yeast microflora, metataxonomic, CRISP-Cas9, wine fermentation, terroir

1. Introduction

Wine fermentation is not only a biochemical conversion of sugars into ethanol, but rather a microbial ecosystem. Each grape berry harbors a lot of yeast species, lactic acid bacteria, and filamentous fungi, whose relative abundance is influenced by soil composition, climate, grape cultivar, vineyard management practices, and cellar equipment (Wei et al., 2023). In the last two decades, meta-barcoding and meta-transcriptomic approaches have revealed that this "microbial signature" contributes significantly to the sensory definition of *terroir*, influencing both the accumulation of aroma precursors and the modulation of the wine's acid-phenolic balance. Microbial biodiversity should thus not be considered a marginal element, but rather a strategic asset for market differentiation, especially for wines with strong territorial identity. Since the 1980s, the introduction of selected *Saccharomyces (S.) cerevisiae* starter cultures has ensured reliable fermentation kinetics and minimized microbiological risks. However, the systematic use of pure cultures has progressively been directed to aromatic homogenization and the loss of regional specificity. Furthermore, the strong competitive behavior of *S. cerevisiae* toward non-*Saccharomyces* species reduces the biochemical complexity of must, limiting the formation of terpenes, fruity esters, and sensory-relevant polysaccharides (Castrillo and Blanco 2022). Grapes as well as natural environments as oak forests, tree bark, floral nectar, and humus-rich soils host wild *Saccharomyces spp*. and many underexplored non-*Saccharomyces* species. Other work has described unique metabolic pathways in *Metschnikowia (M.) pulcherrima* leading to the synthesis of floral esters. Exploring these ecological niches enables the recovery of phenotypic traits lost in conventional enology, such as cryotolerance, enhanced glycerol production, and reduced SO_2 formation.

Within the overall objective mentioned above this PhD thesis project can be subdivided into:

1) **Evaluation of the microbial diversity**, in particular from grapes and wine of different *terroirs* by culture dependent and meta-taxonomic analyses as well as natural environments (oak bark, acorns, forest soil, olives, and olive wastewater).

2) Molecular (identification and bio typing) and technological characterization of yeasts isolates.

3**) Bottom-up,** metabolic activities of individual strains and their interactions (using the CRISPR-Cas9 technique) in synthetic must.

4) **Top-down,** design of the synthetic microbiota by lab-scale experiments and use by cellar-scale trials.

2. Materials and Methods

Yeast strains were isolated from various natural environments and identified by internal transcribed spacer (ITS1-5.8S-ITS2) analysis (Esteve-Zarzoso et al., 1999) and sequencing. Molecular biotyping of *Saccharomyces* isolates was performed by six different molecular markers (Legras and Karst, 2003; Marinangeli et al., 2004). All isolates were characterized by technological traits such as ethanol and sulfur dioxide resistance, utilization of alternative carbon sources, enzymatic activities, secondary metabolite production, oxidative stress tolerance, and antifungal activity against *Botrytis cinerea*. For metataxonomic analysis, 3 different rDNA regions (ITS1, ITS2, and 26S),

considered (Bokulich & Mills 2013; Toju et al., 2012; White et al., 1990; Mota-Guiterrez et al., 2019) and sequencing was performed on an Illumina MiSeq platform. Bioinformatic processing of the was performed using the QIIME2-amplicon pipeline (version 2024.8) (Bolyen et al., 2019). Taxonomic classification of ASVs was conducted using the UNITE database (version 8.3) (Kõljalg et al., 2020).

To evaluate the interactions of *S. cerevisiae* with non-*Saccharomyces*, the strain designated F14-72 (herein isolated from Fiano grape must) was genetically modified using CRISPR-Cas9 genome editing technology. A DSB (double-strand break) at the *HO* locus was induced by Cas9 nuclease, expressed from the plasmid pL39-kanHO (Tellini et al., 2024). Simultaneously, a donor repair template was supplied via the plasmid pL42 (p426 yGam) (Tellini et al., 2024), containing a GFP (yGamillus) flanked by 5' and 3' homology arms corresponding to the *HO* locus. *S. cerevisiae*/non-*Saccharomyces* interaction experiments were carried out in 250 mL Erlenmeyer flasks containing 120 mL of synthetic must (SM) (Rinaldi et al., 2016), in triplicates at 18 °C under static conditions. A total of six different combinations were tested: *S. cerevisiae* (F14-72-GFP) at 1×10^4 cells/mL alone and in combination with five non-*Saccharomyces* yeast at 1×10^6 cells/mL (*M. pulcherrima, L. thermotolerans, H. guilliermondii, T. delbrueckii, Star. bacillaris*) isolated during this study. Fermentation progress was monitored daily by weight loss (CO_2 release). Samples were collected at regular intervals and analyzed CytoFLEX (Beckman Coulter) cytometer equipped with a 488 nm blue laser for excitation and a 525/40 nm bandpass filter for GFP detection. Data acquisition was carried out using CytExpert software (version X), and at least 10,000 events were recorded per sample. For each time point the cells were diluted 1:10 in PBS, Propidium iodide (1 µg/mL) was added to distinguish live (PI^-) from dead (PI^+) cells.

To validate laboratory-scale observations, pilot-scale fermentations *S. cerevisiae/M. pulcherrima* were carried out under winery conditions. Three different combinations were tested in 120 L of Falanghina grape must at 14 °C in stainless steel tanks. Particularly, *S. cerevisiae* B3 1×10^6 cells/mL, co-inoculum *S. cerevisiae* B3/*M. pulcherrima* B12 (1×10^4/1×10^6cells/mL) and co-inoculum *S. cerevisiae* B3/*M. pulcherrima* B24 (1×10^4/1×10^6cells/mL).

Fermentation kinetics were monitored by standard chemical-physical and microbiological analyses. Colonies isolated from count plates were subsequently subjected to molecular identification to confirm the persistence and dominance of the inoculated strains. Chemical parameters (residual sugars, ethanol, acidity, and volatile compounds) were assessed at the end of fermentation using standard oenological methods (enzymatic assays and HPLC analysis). Produced wines were subject of sensorial analyses.

3. Results and Discussion

The first aim of the study was the analysis of yeast biodiversity associated with both vineyard and natural environments. Thirteen different yeast species isolated from natural environments not directly associated with grapevine ecosystems, specifically oak bark, acorns, olive pomaces, and olive processing residues (Figure 1A). Figure 1A shows the clear predominance of *S. cerevisiae*, but also the occurrence of wine related yeast. This result is consistent with prior literature (Liti, 2015) suggesting that *S. cerevisiae* can establish a stable ecological niche on oak bark, likely due to its osmotolerance, ethanol resistance, and competitive metabolic profile. Moreover, as also shown during this study, *T. delbrueckii, M. pulcherrima, L. thermotolerans* harbor interesting oenological trains of strains as good fermentation power, low acetic acid production, lactic acid production and β-glucosidase activity. Their presence in olive-derived matrices suggests a broader ecological distribution and confirms their ability to thrive in substrates rich in phenolic compounds and low pH, conditions analogous to must or wine. From an ecological and biotechnological perspective, this analysis highlights that non-viticultural environments represent valuable reservoirs of microbial diversity.

From grape and related grape-musts, Fiano, Falanghina, Aglianico Lasco, Camaiola cultivars, 17 distinct yeast species were isolated (Figure 1B). Remarkably, three species rarely or never previously reported in southern Italian vineyards, *Nakazawaea spp.*, *S. kudriavzevii*, and *S. paradoxus* were identified, highlighting the potential of grapes to harbor novel yeast biodiversity. A focused investigation was conducted on Falanghina grapes from six distinct *terroirs* in the Sannio region (Figure 2). In this context, 142 yeasts were isolated, including 70 *S. cerevisiae*. Molecular typing of the *S. cerevisiae* isolates using six distinct markers revealing 16 different biotypes. Biotype 1 emerged as the most widespread, present in all fermentations. The greatest intraspecific diversity was recorded in sample 6 (Bonea), likely due to the unique environmental and grape compositional conditions that may have promoted microbial diversification. While *S. cerevisiae* was consistently recovered across all *terroirs*, the composition of non-*Saccharomyces* species showed marked variability. In many samples, *M. pulcherrima* was even more abundant than *S. cerevisiae*. Other non-*Saccharomyces* yeasts, including *Star. stellata*, *C. dosseyi H. guilliermondii*, *H. pseudoguilliermondii*, *H. uvarum*, and *P. terricola*, exhibit substantial variations in distribution, suggesting terroir-specific influences. Overall, Figure 2 clearly underscores significant diversity and variability in yeast composition across the different terroirs of the Sannio region, suggesting that environmental and geographic factors markedly influence the microbiota involved in spontaneous fermentations of Falanghina wines.

To complement the culture-based approach, a metataxonomic analysis was conducted on the Falanghina samples. As visualized in the Venn diagram (Figure 3), ITS2 primer set detected the highest number of genera (82), compared to ITS1 (49) and 26S (28). Only eight genera were common by all three primer sets, emphasizing the primer-dependent nature of fungal diversity assessments by meta-taxonomic. Figure 4 presents the alpha diversity analysis of fungal communities assessed through Shannon and Simpson diversity indices in samples analyzed.

With both diversity indexes ITS2 demonstrates significantly higher values compared to other two markers, clearly suggesting that this region is the most effective and sensitive for fungal metataxonomic studies focused on diversity assessment. A heatmap based on ITS2 data (Figure 5) illustrated fungal succession during must fermentation. At T0 (0dd), a mixed mycobiota including *Hanseniaspora* and *Cladosporium* was observed, likely introduced from grape skins. At T7 (7dd) and T14 (14dd), *S. cerevisiae* became predominant, reflecting its ethanol tolerance and fermentative capacity. However, in specific zones like sample 6, molds such as *Aspergillus* and *Rhizopus* were also detected, possibly due to contamination or conducive environmental conditions.

Figure 6 shows the cellular viability dynamics during fermentation in mixed cultures consisting of GFP-tagged *S. cerevisiae* (F14-72-GFP) and various non-*Saccharomyces* yeast species, monitored at different sampling times. Despite initial differences in inoculum size (10^6 vs 10^4 cells/mL), *S. cerevisiae* populations converged to similar densities over time, indicating ecological compensation. In co-fermentations with *S. bacillaris*, *S. cerevisiae* exhibited low abundance, suggesting antagonistic inhibition. By contrast, *M. pulcherrima*, *L. thermotolerans*, and *T. delbrueckii* showed moderate competitive behavior but permitted *S. cerevisiae* persistence. At the end of fermentation, plate counting, and colony sequencing confirmed the survival of *S. cerevisiae* in most mixed cultures, validating the flow cytometry data and highlighting species-specific interactions that modulate fermentative outcomes in synthetic microbial communities. In conclusion, Figure 6 clearly shows that the presence and type of non-*Saccharomyces* yeasts have profound impacts on the viability and competitive performance of GFP-tagged *S. cerevisiae* during fermentation. These findings strongly suggest that selecting specific non-*Saccharomyces* partners can directly influence fermentation dynamics and thus could be strategically employed to modulate wine composition and sensory characteristics derived from mixed-culture fermentations. Furthermore, an additional fermentation is planned using the GFP-tagged *S. cerevisiae* in combination with a more complex yeast consortium, in order to simulate a synthetic microbiota model. This approach will allow for detailed monitoring of population dynamics and interspecies interactions within a controlled multi-strain ecosystem (work in progress).

Pilot-scale fermentations (120 L at 14 °C) were conducted with *S. cerevisiae* (B3) alone and in co-inoculation with two *M. pulcherrima* strains (B12, B24). Sugar consumption proceeded uniformly across all trials, completing by day 18 without fermentation delays or faults. pH and total acidity showed slight but significant differences, with co-inoculated samples exhibiting higher acidity. Microbial counts confirmed rapid yeast growth by day 2 and stabilization thereafter. *S. cerevisiae* dominated fermentation, while *M. pulcherrima* populations declined over time, suggesting strain-specific dynamics. Ethanol levels were similar across samples (~13.3% v/v), indicating robust fermentative performance. Overall, co-inoculation with *M. pulcherrima* subtly modulated acidity without impairing fermentation, supporting its enological potential. A trained panel evaluated the three wines (B12+B3, B24+B3, B3), revealing subtle but significant differences in aroma profiles. Fruity notes were stronger in the B3 sample, with banana as the dominant descriptor, while B12+B3 was characterized by pear, and both showed hints of tropical fruit. Citrus notes, particularly grapefruit, were more pronounced in B24+B3 and B3. B24+B3 also exhibited more intense floral and vegetal aromas, suggesting that co-inoculation influenced the aromatic complexity of the final wines.

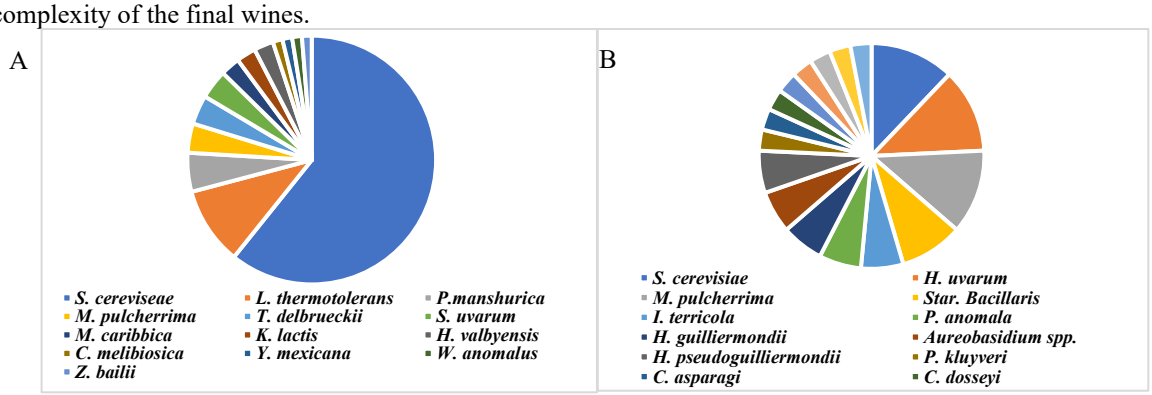


Figure 1. A- yeasts isolate from oak, olive pomace and olive env; B -Yeast isolate from grape and wine environment (Fiano, Falanghina, Aglianico lasco, Camaiola).

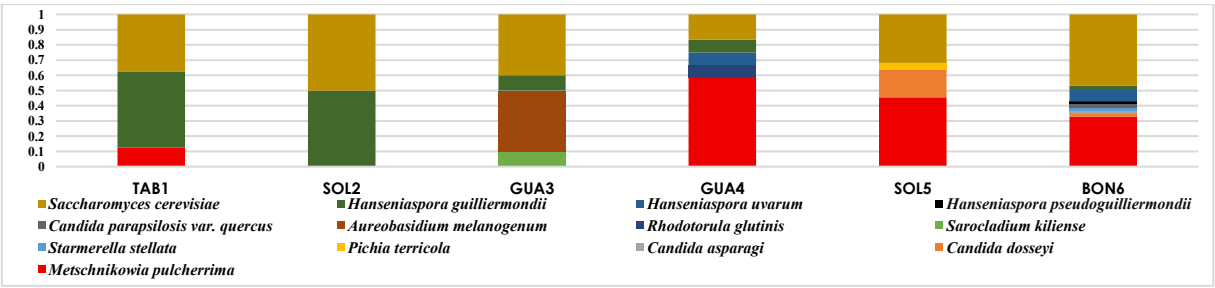


Figure 2: Occurrence of yeast species during Falanghina wine fermentations from 6 different *terroirs* of Sannio.

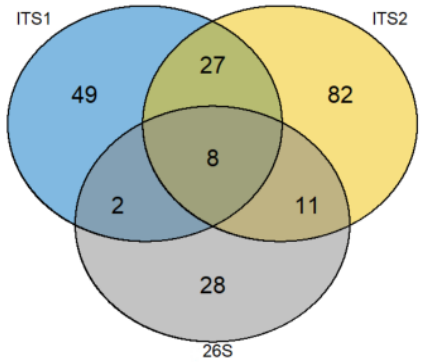


Figure 3. Venn diagram of genera with different primers.

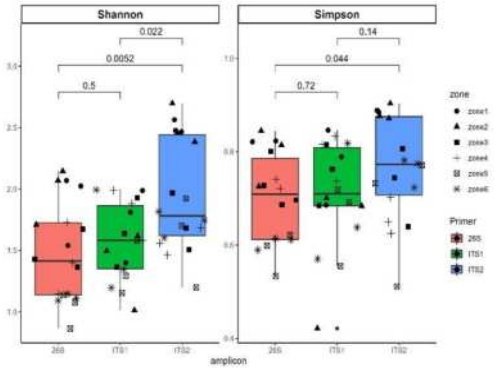


Figure 4. Alpha diversity with Shannon and Simpson index.

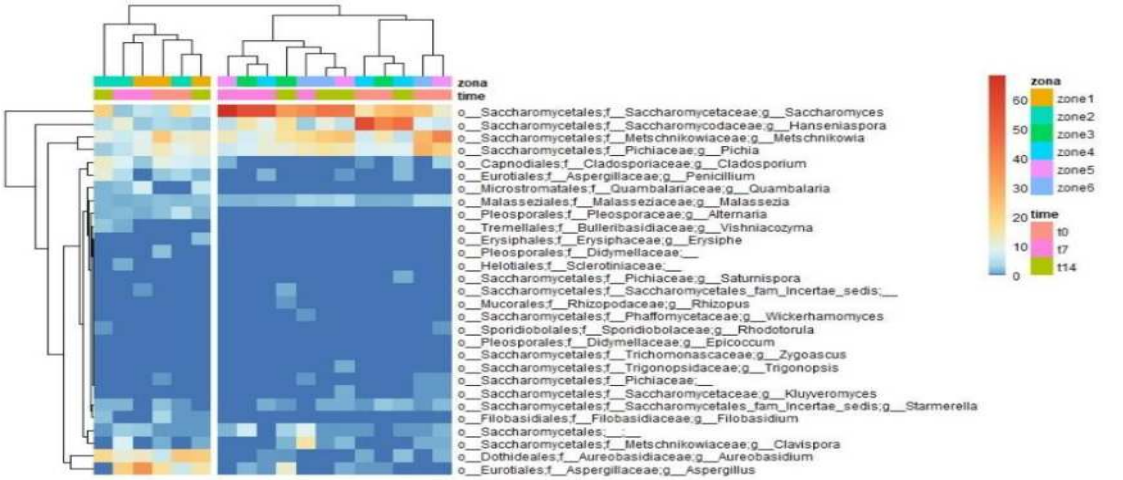


Figure 5. Heatmap with primer ITS2 of Falanghina samples at different fermentation times (0, 7, 14 dd)

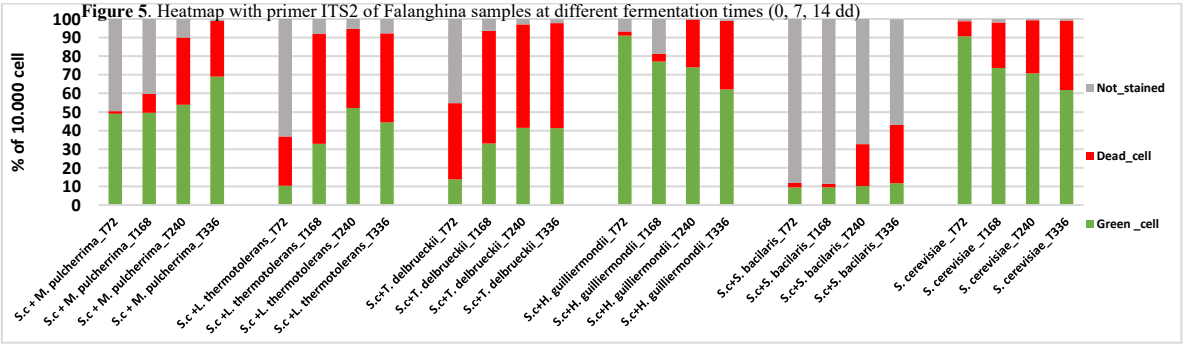


Figure 6. Yeast behavior during fermentation: Green_cell (*S. cerevisae* GFP), Not_stained (non-*Saccharomyces*); Dead_cell (dead *S. cerevisae* GFP + non-*Saccharomyces*).

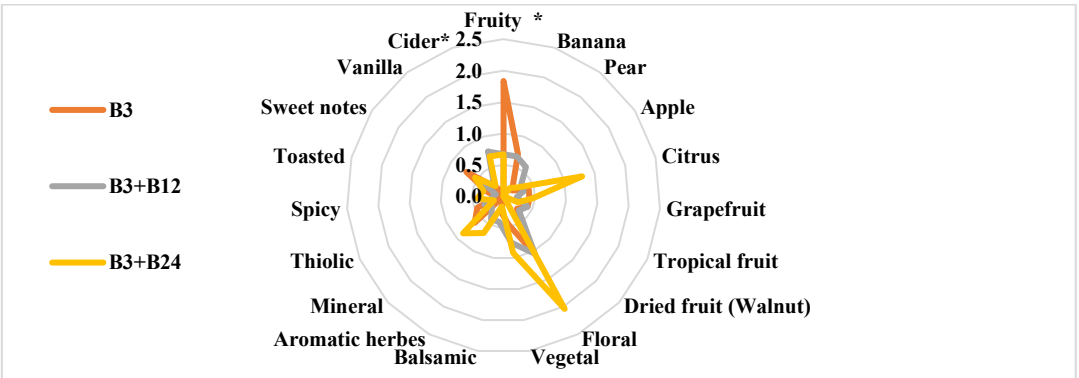


Figure 7. Sensorial analysis of wines from pilot scale fermentations.

4. Conclusions and Future Perspectives

This work highlights the potential of natural yeast biodiversity as a reservoir for the design of synthetic microbiota aimed at improving wine fermentation. By combining classical microbiological isolation with meta-taxonomic profiling and molecular biotyping, a rich and diversified yeast collection was assembled from both vineyard and surrounding natural environments. The detection of rare and underexplored species, such as *S. paradoxus*, *S. kudriavzevii*, and *Nakazawaea*, confirms that non-cultivated ecosystems represent valuable niches for the identification of strains with desirable oenological traits. The species diversity and the intraspecific diversity of *S. cerevisiae* across different terroirs further supports the concept of a microbial terroir that contribute to region-specific wine identity. The application of the CRISPR-Cas9 system for fluorescent tagging of *S. cerevisiae* enabled real-time tracking of strain behavior during mixed fermentations, offering a powerful tool for evaluating yeast interactions. Flow cytometry analysis confirmed the viability, competitiveness, and survival dynamics of *S. cerevisiae* when co-inoculated with different non-*Saccharomyces* species. The antagonistic effect observed with *S. bacillaris*, and the more balanced coexistence with *M. pulcherrima*, *L. thermotolerans*, and *T. delbrueckii*, exemplify how synthetic microbial communities can be rationally assembled to enhance fermentation complexity. The findings provide a conceptual and technical framework for designing starter cultures that retain the aromatic richness of spontaneous fermentations, while ensuring process control and reproducibility. Synthetic microbiota assembled from natural isolates could be customized for different grape varieties and winemaking goals, aligning microbial ecology with modern enological practices. Building on this foundation, future research should aim to functionally characterize the metabolic outputs (ester, acid, polysaccharide profiles) of individual and consortia-level fermentations through metabolomics and transcriptomics. Validate synthetic microbiota under pilot- and industrial-scale fermentations across different vintages and grape varieties. Evaluate the functional stability of microbial consortia, assessing the impact of environmental stressors (temperature, SO_2, osmotic pressure) on community stability and fermentation outcomes. Ultimately, this work sets the stage for a more sustainable, *terroir*-driven, and ecologically informed approach to wine microbiology.

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Yeast Derivatives for Precision Oenology: Emerging and Sustainable Application for Wine Production (WInnY)

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This PhD thesis aims to identify potential relationships between the chemical composition of yeast derived products (YDPs) and their functional properties. In this work, various commercial products were characterized and evaluated both as nutrient sources for fermentation and as stabilizing agents. The findings of this research may provide valuable insights for YDP manufacturers, enabling them to improve their production methods and enhance the specificity and effectiveness of their products. Consequently, winemakers will be able to benefit from more targeted and efficient YDPs.

Derivati di lievito per l'enologia di precisione: applicazione sostenibile ed emergente per la produzione enologica (WInnY)

Questa tesi di dottorato mira a trovare le possibili relazioni tra la composizione chimica dei derivati di lievito (YDPs) e la loro funzione. In questa tesi, diversi prodotti commerciali sono stati caratterizzati e testati come nutrienti per le fermentazioni e come agenti stabilizzanti. I risultati di questa ricerca potranno fornire importanti informazioni ai produttori di YDPs che potranno perfezionare i metodi di produzione e la precisione di tali prodotti. In questo modo, gli enologi potranno usufruire di YDPs più specifici ed efficaci.

Key words: Yeast derivative products, alcoholic fermentation, malolactic fermentation, wine stabilization, yeast nutrition

1. Introduction

In accordance with the PhD thesis project previously described (Altomare, 2024), this oral communication reports the main results of the following activities (A) which concern:

A1) Characterization of yeast derivative products (YDPs)
- *A1.1* Chemical characterization of YDPs

A2) YDPs as source of nutrients for fermentation
- *A2.1* Alcoholic fermentations (AF) trials
- *A2.2* Malolactic fermentation (MLF) trials

A3) Improvement of wine stabilization in a sustainable perspective
- *A3.1* YDPs as tartaric stabilization enhancers
- *A3.2* YDPs as protein stabilization enhancers
- *A3.3* YDPs as color stabilization enhancers

2. Insights into the use of yeast derivative products in winemaking

Over the past decades, the use of YDPs in the wine industry has become increasingly widespread, not only as effective substitutes for traditional sur lie aging (Rigou *et al.,* 2021; Pozo-Bayón *et al.,* 2009), but also due to their multifunctional roles throughout the various stages of winemaking. The OIV has approved several categories of YDPs for oenological use, including inactivated yeasts, yeast autolysates, yeast protein extracts, yeast hulls, and mannoproteins.

Depending on their specific physicochemical properties and composition, YDPs can be applied at different stages of the winemaking process to serve a wide range of technological purposes. These products are reported to enhance both AF and MLF, they could help to prevent sluggish or stuck fermentations. Furthermore, they can modulate wine colloidal stability and support tartaric and protein stabilization (Rigou *et al.,* 2021).

The employment of YDPs during wine fining could have different effects: they can reduce turbidity by precipitating suspended particles, preserve the chromatic attributes of wines, eliminate the excess of tannins, and enhance wine filterability (Resolution OIV-OENO 417-2011). Moreover, YDPs have been widely studied and applied for their protective effects on the aromatic, sensory, and color characteristics of wines (Lambert-Royo *et al.,* 2022). Their impact on the colloidal matrix of wine can also affect the volatility and release of aroma compounds, ultimately influencing the sensory profile.

The main goal of this PhD project is the selection of YDPs with specific functions for the further improvement of precision oenology. This goal will be achieved by means of investigating the use of YDPs for specific applications and their mechanisms of action. Therefore, the specific objectives of the WInnY proposal will concern the use of YDPs as (i) nutrient source for both alcoholic and malolactic fermentations, (ii) stabilizing agent and (iii) antioxidant, considering their impact on the overall composition of wine. Moreover, (iv) a crossover activity

regarding a deep and novel characterization of YDPs will be performed in order to evidence, for the first time, a possible correlation existing between their composition and their specific ability.
The expected increase of knowledge will regard the composition and mechanisms of action of YDPs, in order to improve the accuracy of commercial YDPs and to provide directions to winemakers on their properties. These recommendations can also support the YDP producers being able to adequately set up their production methods allowing to obtain YDPs with more specific functions and improved performance.

3. Materials and Methods

A1.1: Twenty-eight commercial yeast derivative products (YDPs), including 9 inactivated yeasts (IY), 6 yeast autolysates (YA), 2 yeast hulls (YH), 5 yeast extracts (YE), and 6 mannoproteins (MP), were characterized based on their content of glutathione (GSH), cysteine (Cys), readily assimilable nitrogen (RAN), antioxidant capacity and amino acid profile. GSH and Cys concentrations were quantified using UPLC-UV following derivatization with *p*-benzoquinone, according to Tirelli *et al.* (2010). RAN content was determined through enzymatic assays, while the amino acid profile was analyzed by UPLC-HRMS. Antioxidant activity was assessed using three radical scavenging assays: DPPH, ABTS and FRAP. Significant differences among the YDP categories were evaluated using the Kruskal-Wallis test ($p < 0.05$). Pearson correlation coefficients were calculated, with a critical threshold of 0.479 ($df = 26$; $p = 0.01$).
A2.1: Five YDPs selected by means of data obtained in activity *A1.1* and preliminary fermentation trials were used for alcoholic fermentation. They were either added in the *pied de cuve* or directly in Riesling must (40 g/hL). The fermentations have been performed by *Saccharomyces cerevisiae* strain EC1118, inoculated at a concentration of 20 g/hL, at 22±1°C. The fermentation kinetics were monitored through weight loss. At the end of the fermentation the wines were sensory evaluated, and the volatile organic compounds (VOCs) profile was determined (Fracassetti *et al.,* 2020).
A2.2: Twenty-two out of 28 YDPs were screened as MLF enhancers (mannoproteins were excluded); the trials were carried out in triplicate in 15 mL tubes at 18° C. YDPs were added at 40 g/hL without inoculum. MLF trend was monitored through the measurement of DL lactic acid, determined by enzymatic assays.
A3.1: Three YDPs were selected through a previous screening on the ability of YDPs as tartaric stabilization enhancers. The selected YDPs were added at 10, 20, 30 and 40 g/hL in an unstable Traminer wine; the YDPs treatments were compared with treatments that involve metatartaric addition at 2 and 5 g/hL. Twenty-four hours after the addition, tartaric instability was measured through the mini-contact test, which measures the conductivity drop after potassium bitartrate addition and exposition to low temperatures.
A3.2: Three potentially effective YDPs were selected after a screening performed on Traminer and Pinot Noir rosé wines. The trials were carried out on unstable Traminer wine; YDPs were added at different concentrations (10, 20, 30 and 40 g/hL). To check wine stability, a heating test has been performed 24 hours after the addition; turbidity was measured before and after heating at 80° C for 1 hour and cooling at 22° C for 24 hours.
A3.3: The twenty-eight YDPs were screened for color stabilization trials. The trials were conducted in duplicate on Cabernet Franc wine, the addition of YDPs was performed at 40 g/hL in 100 mL bottles and the wines were stored at 25° C for 8 weeks, in order to speed up wine color evolution. Samples were collected at 5 and 8 weeks after the addition. CIELab coordinates, Glories indexes (Glories *et al.,* 1984) and free anthocyanidins (Somers *et al.,* 1977) have been determined for each sample.
The statistical analysis was performed using one-way ANOVA; significant differences were evaluated by Fisher's LSD test at $\alpha=0.05$ (activity A2.1). Principal Components Analysis (PCA) were performed for the data of activity *A2.1* combining the concentration of VOCs as active variables and the sensory descriptors as supplementary variables.

4. Results and Discussion

4.1 Chemical characterization of YDPs (*A1.1*)

Cys and GSH were detected at concentrations below 10 mg/g in all YDPs. Among the different product categories, yeast extracts and yeast lysates exhibited significantly higher levels of these sulfur compounds compared to other YDP types (*Table 1*). Based on an application concentration of 40 g/hL, the maximum contribution of Cys and GSH to wine would be approximately 4 mg/L, indicating a potentially limited role of these compounds in enhancing antioxidant capacity. Nevertheless, both Cys and GSH showed a positive correlation with antioxidant capacity values ($r = 0.683$ for Cys; $r = 0.499$ for GSH), suggesting some contribution despite their low concentration. Regarding the content of readily assimilable nitrogen (RAN), considerable variability was observed both among and within the different YDP categories (*Table 1*). The lowest RAN concentrations were found in inactivated yeasts, yeast hulls, and mannoproteins. At an addition rate of 40 g/hL, these products would provide less than 10 mg/L of RAN, which is generally insufficient to meet the nitrogen requirements of yeasts during alcoholic fermentation. The beneficial effects of YDPs on fermentation performance may be attributed to other compositional factors, such as lipids and vitamins concentration (Soubeyrand *et al.,* 2005). Yeast extracts also

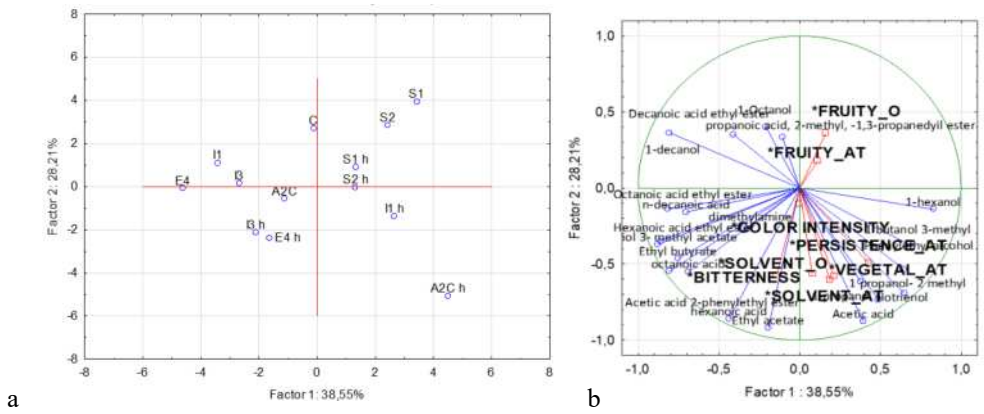
exhibited significantly higher antioxidant capacity than the other YDP categories. This finding highlights their potential application during wine aging and storage, where they could contribute to improved oxidative stability, enhanced aging potential, and extended shelf life.

Table 1 *Average (*Av*), maximum (*Max*) and minimum (*Min*) concentration for the parameters measured in all the YDPs categories investigated. Different letters mean significant differences (Kruskal-Wallis test, $p < 0.05$). Legend:* RAN*: readily assimilable nitrogen;* AC*: antioxidant capacity.*

YDPs	Cys (mg/g)			GSH (mg/g)			RAN (mg/g)			AC (mM Trolox/g)		
	Min	Max	Av	Min	Max	Av	Min	Max	Av	Min	Max	Av
Inactivated Yeasts	0,00	0,10	0,08^{a}	0,00	4,88	8,12^{ab}	3,48	14,72	8,12^{ab}	0,39	10,28	7,30^{a}
Yeast hulls	0,04	0,04	0,04^{a}	0,04	0,13	4,58^{a}	3,06	6,10	4,58^{a}	0,32	2,51	1,42^{a}
Lysate yeasts	0,08	1,20	0,49^{b}	0,09	4,74	9,29^{b}	6,60	14,92	9,29^{b}	2,52	19,03	11,48^{b}
Yeast extracts	0,11	1,11	0,52^{b}	0,08	8,99	12,97^{b}	5,35	21,69	12,97^{b}	16,42	44,31	28,06^{c}
Mannoproteins	0,00	0,16	0,03^{a}	0,00	4,98	6,44^{ab}	1,39	15,62	6,44^{ab}	2,08	17,21	9,88^{b}

4.2 Alcoholic fermentation (AF) trials (*A2.1*)

Although slight differences in fermentation kinetics were observed among the trials with different YDPs, with the control wine showing a slightly slower fermentation rate, no statistically significant differences were found (data not shown). The addition of YDPs did not improve fermentation capacity of yeasts, this results discord with previously found by other authors (Soubeyrand *et al.,* 2005). Statistical analysis of the sensory data revealed significant differences for fruity aroma perception, color intensity, solvent-like aroma perception, vegetal note, bitterness, and persistence. The PCA explained 66.8% variance (*Figure 1*). Wines did not cluster neither according to the type of YDP added nor the timing of addition. However, it is noteworthy that the addition of yeast hulls consistently resulted in wines with enhanced fruity character, both olfactory and mouthfeel features. In contrast, the use of yeast lysate led to wines with increased persistence, but also with more pronounced vegetal and solvent-like notes. Under these conditions, the addition of yeast hulls appears to yield the most promising sensory outcomes.



a b

Figure 1 *Projection of the cases (a) and projection of the scores (b), obtained for the volatile compounds (VOCs) concentration and sensory evaluation scores in Riesling samples. VOCs were considered as active variable (in blue) and sensory evaluation score as supplementary variable (in red).*

4.3 Malolactic fermentation (MLF) trials (*A2.2*)

Figure 2 shows the evolution of DL lactic acid after the addition of YDPs to the wine. It can be observed that most of the assayed YDPs did not promote a faster progression of MLF. Only after 37 days a slight increase in DL lactic acid was observed for most of the YDPs. Only the addition of yeast extracts and one yeast hull led to a faster occurrence of MLF, in particular E1A and E2 showed significantly higher concentration *($p<0.001$)* of DL lactic acid at the last sampling time. As previously discussed in activity *A1.1* (paragraph 4.2), yeast extracts were found to be the richest in RAN among the YDP categories. However, these differences in MLF kinetics cannot be solely attributed to nitrogen

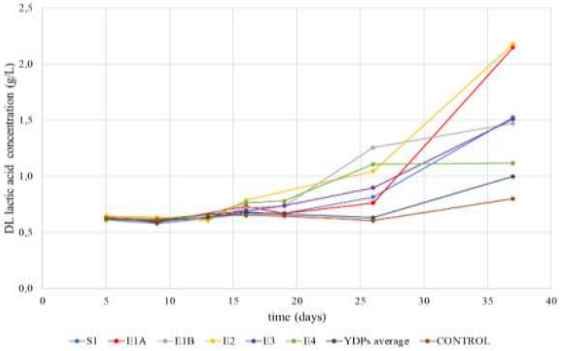


Figure 2 *DL lactic acid (g/L) produced during the malolactic fermentation in the presence of yeast derivatives products.*

content. Despite the absence of statistically significant differences in average RAN concentrations among yeast lysates, inactivated yeasts and yeast extracts, only the extracts led to a noticeable increase in MLF rate. Moreover, a yeast hull, characterized by significantly lower RAN levels, significantly enhanced MLF kinetics ($p<0.001$) compared to the control trial. These findings suggest that factors other than RAN content, such as the presence of vitamins and lipids, but also a possible adsorption of anti-fermentative compounds (i.e., middle chain fatty acid) may play a key role in influencing the activity and growth of lactic acid bacteria, thereby modulating the progression of MLF.

4.4 YDPs as tartaric stabilization enhancers (A3.1)

Three mannoproteins were found to limit the tartrate precipitation in a screening assay (data not shown). The untreated wine proved to be highly unstable, exhibiting a conductivity drop (Δ µS) greater than 100 µS. The decrease in Δ µS was proportional to the concentration of added mannoproteins ($r^2>0.9$ for all the mannoproteins). As it can be observed in *Table 2*, MP2 and MP5 exhibited similar behavior: at a dosage of 30 g/hL, the wine showed instability risk (Δ µS>40), despite a significant decrease in conductivity drop to approximately 50 µS. Increasing the dosage to 40 g/hL resulted in a further decrease to around 60 µS. MP6 demonstrated much higher efficacy, yielding a stable product already at a dosage of 30 g/hL. Under these conditions and with this contact time, as expected, metatartaric acid produced the best performances, making the wine highly stable with an addition of 2 g/hL. However, it should be noted that metatartaric acid is subject to hydrolysis over time, potentially compromising long-term stability (Gerbaud *et al.,* 2010). Therefore, it is necessary to monitor the stability of these wines over extended periods and at varying storage temperatures, in order to determine whether mannoproteins can confer greater long-term tartrate stability.

Table 1 *Δ µS measured after treatment and tartaric stability of the resultant wines. Legend: MP, mannoprotein; MT, metatartaric acid*

Trial	YDP concentration (g/hL)	Δ µS	Stability
CONTROL	-	102,6±1,4	UNSTABLE
MP2	10	78±1,4	UNSTABLE
	20	63,3±3,8	UNSTABLE
	30	54,95±2	AT RISK
	40	41,75±0,5	AT RISK
MP5	10	78,05±3,9	UNSTABLE
	20	60,9±0,4	UNSTABLE
	30	51,6±1	AT RISK
	40	48±0,6	AT RISK
MP6	10	73,6±4,3	UNSTABLE
	20	48,5±0,2	AT RISK
	30	37,6±2,8	STABLE
	40	27,1±0,4	STABLE
MT	2	14,9±2,7	VERY STABLE
	5	10,2±0,8	VERY STABLE

4.5 YDPs as protein stabilization enhancers (A3.2)

After a screening conducted with all the YDPs considered in this study (data not shown), three inactivated yeasts were identified as potential candidates for achieving protein stability. However, the trials run with increasing concentrations of YDPs revealed that the turbidity reduction was little (up to 4 NTU), and none of the three products were capable of achieving protein stabilization in wine. The concentration added did not influence the reduction of turbidity, which remained in all the cases very little, reaching a maximum with the addition of I8 at 20 g/hL (4,3 NTU). Further studies will aim to determine whether the addition of these products could reduce the amount of bentonite required to achieve protein stability.

4.6 YDPs as color stabilization enhancers (A3.3)

The addition of most inactive yeast lysates led to an increase in chroma after 8 weeks (*Figure 3a*), attributable to an increase of both a* and b* color coordinates. Conversely, the addition of mannoproteins generally resulted in a decrease in chroma, indicating a reduction in color saturation following mannoprotein supplementation (*Figure 3a*). The ΔE values suggest that, in most cases, the addition of mannoproteins produced only little color differences, while inactive yeasts and yeast lysates led to noticeable and even strongly noticeable color differences (*Figure 3b*). Nevertheless, mannoproteins seem to interact and exert a protective effect on anthocyanins. Lower absorbance values at 520 nm were found in the presence of certain mannoproteins (namely MP4 and MP5) even if an acidification (with HCl) led to an increase in absorbance at 520 nm (data not shown). An interaction between mannoproteins and anthocyanins could be hypothesized and it could be destabilized under strong acidic conditions with the consequent release of the anthocyanins. Such interactions could protect free anthocyanins from oxidation or degradation and limit their interaction with other molecules involved in copigmentation (i.e., tannins). Therefore, the addition of mannoproteins could lead to a slower evolution of wine color as well as to a slower

decay of free anthocyanin content.

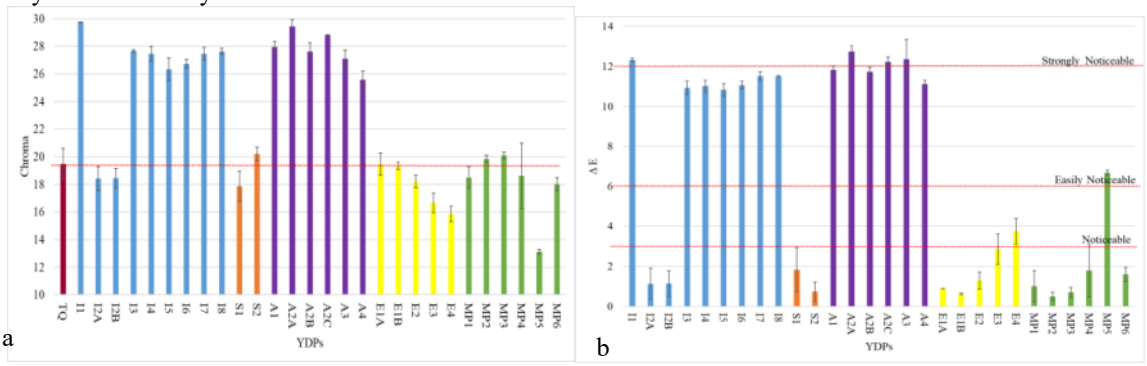


Figure 3 *Chroma (a) and ΔE (control-YDP) (b) measured in Cabernet Sauvignon 8 weeks after the addition of yeast derivatives products.*

6. Conclusions and Future Perspectives

The addition of YDPs during winemaking can exert various effects on the quality of the final product. These studies have shown that the addition of YDPs during alcoholic fermentation can influence the sensory characteristics of the wine, either positively or negatively, although no effects were observed on fermentation kinetics under the experimental conditions adopted. In contrast, YDPs can favor the occurrence of malolactic fermentation. Among the YDPs, primarily mannoproteins have proven effectiveness for tartrate stabilization, while none of the tested compounds showed efficacy in achieving protein stabilization. However, it is necessary to assess the sensory impact of mannoprotein addition for the tartrate stabilization, as well as their potential ability either to reduce the duration of cold stabilization or to limit the amount of bentonite required to achieve the protein stability desired. The effects on wine color can vary greatly depending on the specific product used. It is essential to investigate whether these products behave similarly in wines with different polyphenolic compositions, and to evaluate the degree of copigmentation and anthocyanin polymerization during aging.

YDPs may represent valuable tools in the development of precision enology, given the wide range of potential effects. The assessment of effectiveness of YDPs towards specific oenological objectives is crucial in relation to the wine matrices, as each product may behave differently depending on the wine in which it is applied.

7. Nomenclature

YDPs, yeast derivative products; OIV, International Organization of Vine and Wine; AF, alcoholic fermentation, MLF, malolactic fermentation; Cys, cysteine; GSH, reduced glutathione; PCA, principal component analysis; VOCs, volatile organic compounds.

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Study of combined stress factors on shelf-life of rosé wines: interaction of oxygen, light and temperature on color and aroma evolution

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This study investigated the combined effects of temperature, light, oxygen, and time on rosé wine evolution using a Design of Experiments (DoE) approach. Significant interactions were observed on color parameters and key aroma compounds, highlighting the complexity of wine aging mechanisms under real storage conditions.

Key words: Wine shelf-life; Rosé wine; Photo-oxidation; Norisoprenoids; Sulfur dioxide; Riboflavin

1. Introduction

The chemical stability of bottled wine during storage and transport represents a key challenge in modern winemaking, particularly in view of the growing internationalization of the wine market. During bottle aging, wines undergo complex chemical transformations that can alter their composition and sensory profile, sometimes leading to the development of defects that compromise product quality and marketability (Díaz-Maroto et al., 2021; Gabrielli et al., 2021).

Among the main factors influencing SL are temperature, oxygen exposure, and light. While oxidative and thermal degradation mechanisms affect all wine types, photo-oxidation is particularly relevant for white and rosé wines, especially given the trend towards packaging in clear glass bottles (Carlin et al., 2022; Ugliano, 2013). This configuration offers little protection from light, encouraging the development of defects such as the light-struck aroma. Traditionally, these factors have been studied individually, an approach which fails to capture their interactions, limiting the real-word application of the obtained results (Fisher R.A., 1935).

The aim of this work was to systematically investigate how oxygen, light, temperature, and time interact to influence the color and volatile profile of rosé wines, through the application of a Design of Experiments (DoE) methodology.

2. Materials and Methods

A Central Composite Design (CCD) was implemented with four factors, oxygen, light, temperature, and time, generating 31 experimental conditions. The experimental domain is reported in Table 1. Two Italian rosé wines were selected to minimize the influence of matrix effects, and data were normalized and analyzed as a single dataset.

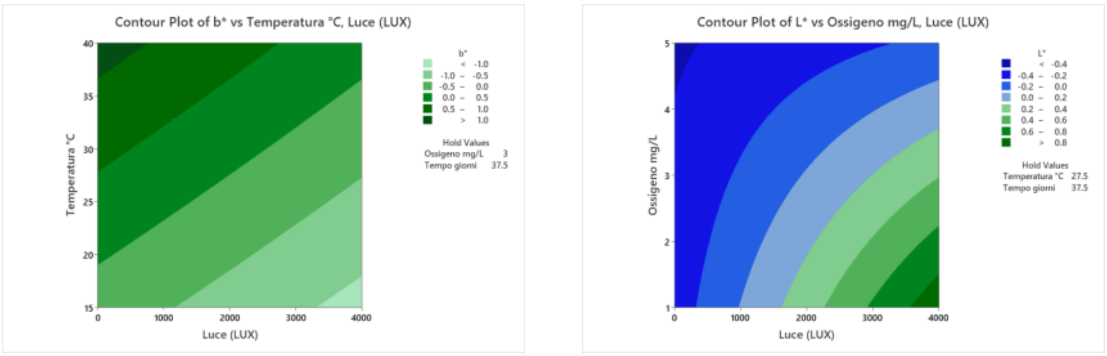
	O2 (mg/L)	**Light (lux)**	**Temperature (°C)**	**Time (days)**
Min	1	0	15	15
Max	5	4000	40	60

Colorimetric changes were monitored through CIELAB parameters, while the volatile profile was analyzed by GC-MS (for terpenes and volatile sulfur compounds) and LC-QqQ-MS (for thiols and norisoprenoids). Analyses of riboflavin and SO_2 (free and bound) were also performed.
Follow-up experiments were conducted to clarify two key aspects observed during the main study: the role of riboflavin in norisoprenoid photodegradation, and the interaction between β-damascenone and SO_2. Both the experiments were performed by adding the pure volatile compound of interest in model wine (pH 3.2, 12% EtOH, tartaric acid 3.6g/L).

3. Results and Discussion

Of the 45 parameters analyzed, 35 were significantly affected by at least one of the experimental factors or their interactions. Oxygen exposure caused a clear reduction in both free and bound SO_2 levels. Riboflavin was completely degraded in all light-exposed samples, irrespective of light intensity or duration, confirming its extreme sensitivity to light.

Color evolution (Fig 1) showed a complex behavior under the combined action of the stress factors. Lightness (L*) increased under light exposure at low oxygen levels, indicating a brightening effect. Redness (a*) increased in response to oxygen exposure, while yellowness (b*) decreased with light but was enhanced by the combined effect of higher temperature and prolonged storage time. These results demonstrate the importance of considering interactive effects when evaluating wine color stability.



The volatile profile revealed distinct and interpretable trends. Dimethyl sulfide (DMS), a compound linked to truffle-like aromas, was strongly modulated by the interaction of temperature and time, with higher levels observed under prolonged storage at elevated temperatures. Methanethiol (MeSH) and dimethyl disulfide (DMDS) showed a significant increase with light intensity, consistent with known light-induced release pathways.

Among norisoprenoids, TDN and vitispirane, responsible for aged notes such as petroleum and tobacco, increased with temperature, while light exposure led to a marked reduction of these compounds. β-Damascenone and β-

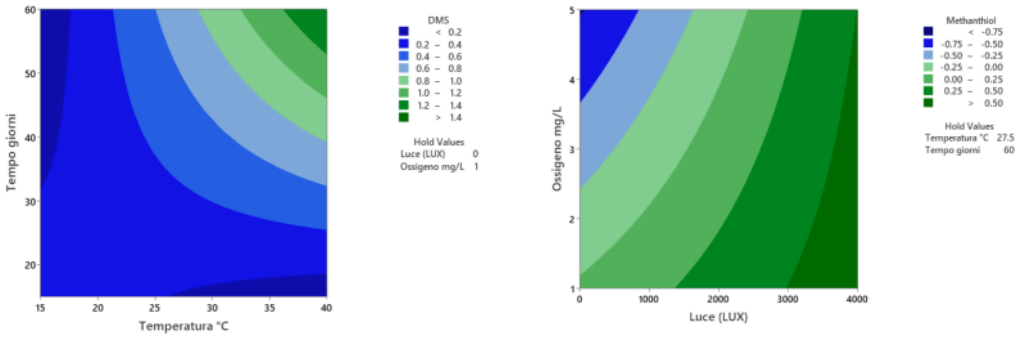


Figure 2 a: *Contour Plot of DMS vs Time and Temperature; b: Contour Plot of methanethiol vs oxygen and light (normalized concentrations)*

myrcene, on the other hand, increased in response to oxygen exposure, suggesting an interaction with SO_2 chemistry.

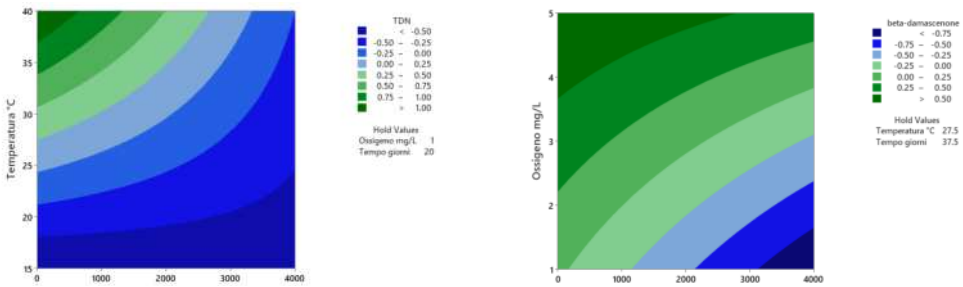


Figure 3 a: *Contour Plot of TDN vs Light and Temperature; b: Contour Plot of β-damascenone vs Light and Oxygen (normalized concentrations)*

The dedicated follow-up experiments confirmed that photodegradation of norisoprenoids is mediated by riboflavin. Light exposure in the presence of riboflavin resulted in up to 90% degradation of these compounds. In contrast, in riboflavin-depleted wines, β-damascenone remained stable under light, highlighting the key role of riboflavin in driving this photodegradation pathway.

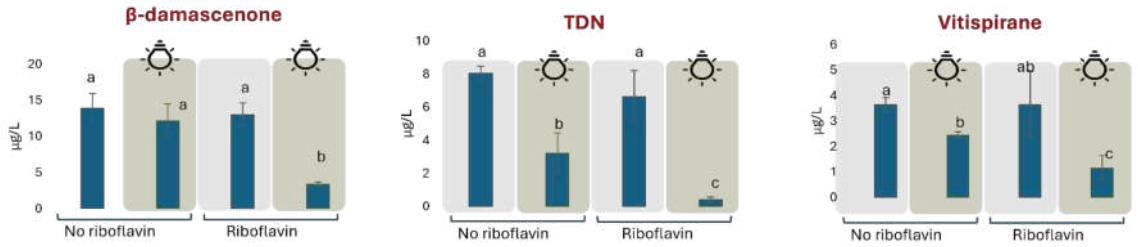
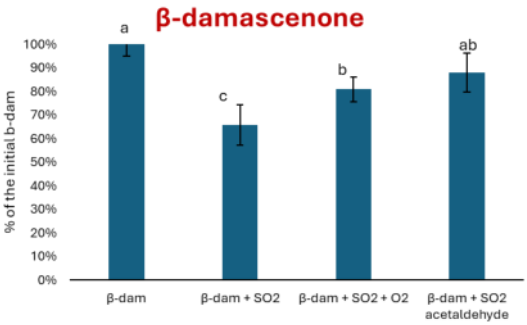


Figure 4 a: *Concentrarion of beta-damascenon, TDN and vitispirane in model wine with and without riboflavin stored in dark and light conditions*

A second experiment demonstrated that β-damascenone binds reversibly to SO_2. After SO_2 addition, a sharp decrease in headspace β-damascenone was observed (HS-SPME-GC-MS), followed by a gradual reappearance as SO_2 was consumed through oxidation. Similar behavior was noted with the addition of acetaldehyde, confirming the dynamic equilibrium between bound and free β-damascenone and its modulation by SO_2 availability.



Altogether, these results provide a comprehensive picture of the chemical processes underlying the evolution of rosé wines under realistic storage and transport conditions. The use of a factorial design enabled the identification of key interactions that govern the stability of both color and aroma components, offering new insights beyond what could be obtained with traditional single-factor studies.

4. Conclusions

This study has provided a detailed understanding of how oxygen, temperature, and light interact to affect the stability of rosé wines during bottle aging. It demonstrates that these factors act in a highly interactive and non-linear fashion, influencing both the visual and aromatic attributes of wine. The identification of riboflavin as a key mediator of norisoprenoid photodegradation, together with the elucidation of the dynamic behavior of β-damascenone in relation to SO_2 binding, represents a significant advancement in the understanding of wine stability mechanisms. Overall, the results of this project contribute original knowledge to the field of wine chemistry and offer a rational basis for improving wine shelf-life through targeted control of packaging choices, oxygen management, and SO_2 use. The study also validates the experimental strategy adopted, demonstrating the value of applying DoE approaches to complex oenological systems.

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Oenological characterization of ancient Sicilian cultivars cv *Vitis vinifera* L. and evaluation of grape ripening heterogeneity at harvest

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This PhD thesis focused on the oenological characterization of ancient Sicilian grape varieties, with the aim of preserving the Island's agricultural biodiversity. The research also sought to identify cultivars resilient to extreme climate conditions, in response to climate change, and to promote the development of new wines strongly rooted in their territory of origin, with lower alcohol content, capable of appealing to modern consumers and emerging market trends. These ancient varieties were studied through an innovative approach based on the assessment of ripening heterogeneity at harvest, a growing challenge in modern viticulture due to climate change.

Caratterizzazione enologica di antichi vitigni siciliani e valutazione dell'eterogeneità dell'uva attraverso parametri tecnologici e fenolici

Questa tesi di dottorato ha riguardato la caratterizzazione enologica di varietà antiche Siciliane, con l'obiettivo di contribuire al mantenimento della biodiversità vitivinicola dell'Isola. Il lavoro ha inoltre mirato a individuare vitigni resilienti a condizioni climatiche estreme, in risposta agli effetti del cambiamento climatico, e a valorizzare nuove tipologie di vino fortemente legate al territorio e caratterizzate da una bassa gradazione alcolica, in linea con i nuovi trend di mercato. Queste varietà antiche sono state studiate attraverso un approccio innovativo basato sullo studio dell'etereogeneità di maturazione delle uve alla raccolta, un problema oggi sempre più frequente a causa del cambiamento climatico.

Key words: grape variability, grape maturity, density sorting, wine quality, phenolic compounds, ancient winegrapes, aromas

1. Introduction

In accordance with the PhD thesis project, this oral communication reports the main results of the following four activities directed to:

A1) determine the grape heterogeneity distribution at the harvest of different ancient grape varieties and from different growing environments;
A2) assess the most important qualitative parameter of the juice, the phenolic compounds of skin and seeds extract such as total flavonoids, total anthocyanins and non-anthocyanic flavonoids and analysis of monomer anthocyanins, hydroxycinnamoyl tartaric acids (HTCAs) and flavonols;
A3) assess the grape's aroma profile.

Table 1 Berry color classification of the analyzed ancient Sicilian grape cultivar

Code	Cultivar	berry color
LUC	*Lucignola*	rose-pale berry cultivar
AN	*Anonima*	red berry cultivar
BR	*Bracau*	red berry cultivar
CN	*Catanese Nera*	red berry cultivar
VIT	*Vitrarolo*	red berry cultivar

2. Material and methods

2.1 Grape berries sampling and density sorting by means of densiometric flotation

Grape samples from seven ancient Sicilian cultivars of Vitis vinifera L. were collected during the 2023 and 2024 vintages in Marsala (TP), Italy. These included four red (*Anonima, Bracau, Catanese Nera, Vitrarolo*) and one rose-pale (*Lucignola*). *Vitrarolo* was also sampled from two additional Sicilian regions: Contessa Entellina and Sclafani Bagni. Berries were harvested at technological maturity and sorted by apparent density using flotation in NaCl solutions of increasing concentration. This method allowed the classification of grapes by ripeness level. Damaged berries were discarded, and the remaining ones were counted and weighed by density class. Subsamples were used to prepare skin and seed extracts for phenolic analysis. The rest were crushed and centrifuged to obtain

juice for chemical-physical characterization. Parameters measured included reducing sugars, pH, titratable acidity, tartaric and malic acid content, potassium, and assimilable nitrogen (APA), analyzed using Winescan™.

2.2 Grape skin and seeds extraction

Phenolic extracts from skins and seeds were obtained following Squadrito et al. (2007), with modifications. Skins and seeds from 20 berries were manually separated and macerated in a tartaric acid buffer (pH 3.2) containing ethanol and sodium metabisulfite ($Na_2S_2O_5$) to prevent oxidation. After a 4-hour room temperature extraction, samples were homogenized (5,000 rpm), sonicated (3 min), and centrifuged (4,000 rpm). The supernatants were collected, and residual pellets were washed and centrifuged three additional times. All fractions were combined and diluted to 50 mL. This method ensured efficient phenolic extraction while minimizing enzymatic browning.

2.3 Phenolic compounds analysis

2.3.1 Analysis of total flavonoids and total anthocyanins

Total flavonoid content in both skins and seeds, as well as total anthocyanin content in skins, was quantified using UV–Vis spectrophotometry. Measurements were performed with a UV-1800 spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA). Prior to analysis, extracts were diluted in hydrochloric ethanol (ethanol:water:hydrochloric acid 37%, in a 70:30:1 v/v/v ratio) following the protocol described by Bambina et al. (2024). The dilution factors applied were 50 for seed extracts and 25 for skin extracts.

2.3.2 Analysis of monomer anthocyanins, hydroxycinnamoyl tartaric acids (HTCAs) and flavonols

To analyze the profiles of monomeric anthocyanins, hydroxycinnamic tartaric acid esters (HCTAs), and flavonols, skin extracts were first acidified by adding 0.5 mL of 1 M phosphoric acid (H_3PO_4) to 4.5 mL of extract. The acidified solutions were then filtered through 0.45 µm nylon membrane filters and transferred into 1.5 mL vials for analysis. Chromatographic separation and identification were performed using a high-performance liquid chromatography (HPLC) system (Agilent Series 1200, Milan, Italy) equipped with a diode array detector (Hewlett-Packard 1100 D.A.D.). The separation was achieved on a C18 reverse-phase column (Econosphere™ C18, 5 µm, 250 × 4.6 mm i.d., Lokeren, Belgium, part no. 70066). An injection volume of 20 µL was used, in accordance with the method described by Squadrito et al. (2007).

2.3.3 Monomeric Anthocyanins Analysis

Monomeric anthocyanins were analyzed by reverse-phase HPLC using a binary solvent system consisting of solvent A (formic acid:water, 10:90 v/v) and solvent B (formic acid:methanol:water, 10:50:40 v/v/v). The elution was performed using a multi-step gradient ranging from 45% to 99% B, with re-equilibration at the end of the run. The column temperature was maintained at 45 °C, with a flow rate of 0.48 mL/min. Detection was carried out at 520 nm using a diode array detector. Anthocyanins were identified based on retention times and UV–Vis spectra, as described by López et al. (2009), and quantified by peak integration. Results were expressed as the relative percentage of each monomeric anthocyanin with respect to the total anthocyanin content.

2.4.2 HCTAs and Flavonols Analysis

Hydroxycinnamic tartaric acid esters (HCTAs) and flavonols were analyzed using a gradient elution method with a binary solvent system composed of 10^{-3} M phosphoric acid in water (solvent A) and methanol (solvent B). The elution gradient started at 5% B for 5 minutes, increased linearly to 10% B over 5 minutes, then to 30% B in the next 10 minutes, followed by a ramp to 60% B in another 10 minutes, and finally to 100% B over the last 10 minutes. The column was re-equilibrated by returning to 5% B in 5 minutes. The flow rate was set at 0.48 mL/min, and the column temperature was maintained at 40 °C. Compounds were identified based on retention times and UV–Vis spectra, using both literature data (Di Stefano & Flamini, 2008) and authentic standards previously isolated in-house. Quantification was performed by external calibration with chlorogenic acid for HCTAs and quercetin for flavonols, and results were expressed in mg per kg of fresh berry weight (mg kg^{-1}).

2.4 Volatile compounds

Approximately 150 g of grape berries were homogenized using a blade mixer, and 100 g of homogenate were centrifuged at 4000 rpm for 15 minutes. The juice and the pellet, resuspended in tartaric buffer (pH 3.2), were treated with pectolytic enzyme and kept at 4 °C for 24 hours. After centrifugation, supernatants were combined, filtered, and subjected to solid-phase extraction on C18 cartridges. Free volatile compounds were eluted with

dichloromethane and analyzed by GC-MS. Glycosidic fractions were eluted with methanol, enzymatically hydrolyzed, and the released volatiles were extracted and analyzed using the same method. An internal standard (1-heptanol) was added prior to each extraction.

3. Results and Discussion

3.1 Density classes distribution

The distribution of grape berries across density classes was analyzed to assess ripening heterogeneity in relic varieties (Figure 1). Berry density reflects sugar content and ripeness stage, with class distributions forming bell-shaped curves characterized by mode, skewness, and kurtosis. In 2023, most varieties showed high kurtosis and better ripeness uniformity, while in 2024, distribution was broader with lower kurtosis, indicating greater heterogeneity. Vitrarolo showed similar ripeness trends across vineyard sites in both years. A higher percentage of underripe berries (<1075 kg/m³) was observed in 2023, contributing to potential astringency and bitterness in wine. This percentage declined in 2024, suggesting improved overall ripeness.

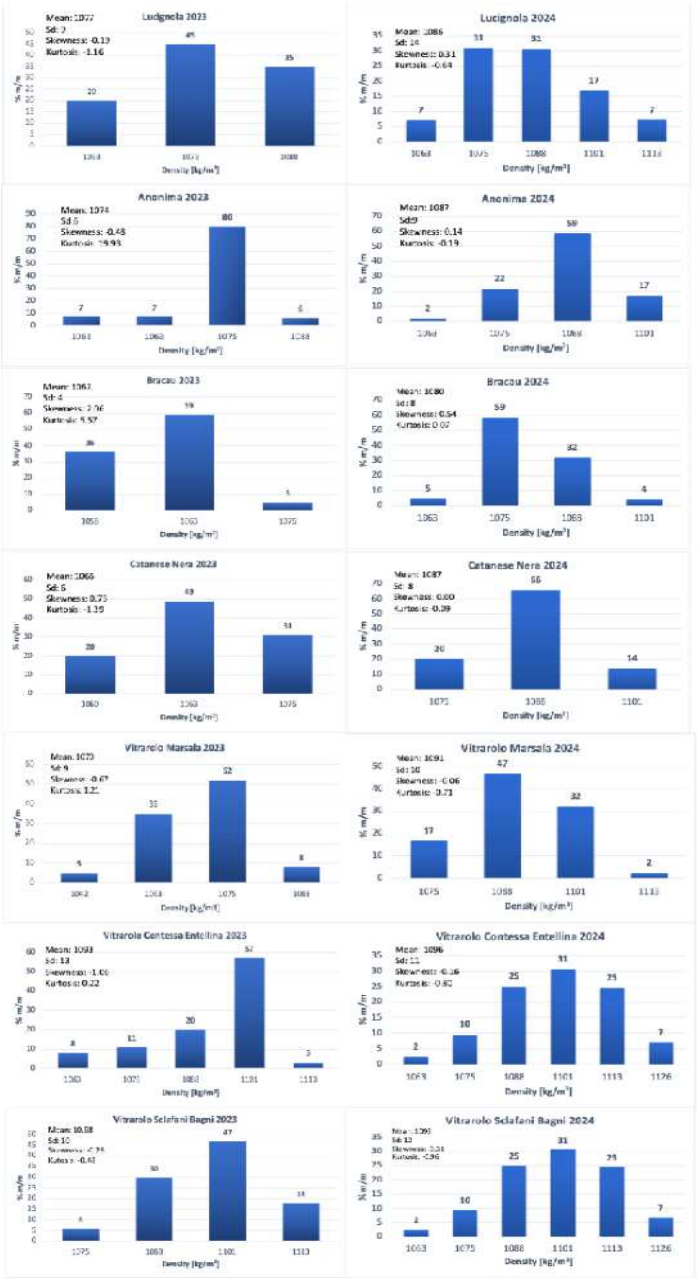


Figure 1 *Density class distributions of ancient grape berries grown in Marsala and Vitrarolo berries grown in different environments in 2023 and 2024 vintages.*

3.2 Multivariate statistical analysis

As shown in Figure 2, the PLS-DA combining the 2023 and 2024 vintages does not display a clear separation between samples. This may be attributed to the markedly different environmental conditions that characterized the two vintages. In the 2023 vintage alone (Figure 3), samples clustered distinctly according to grape variety, with quercetin-3-glucoside, kaempferol-3-glucoside, and acetylated anthocyanins emerging as the main discriminant compounds. In contrast, in 2024 (Figure 4), greater dispersion was observed among the varieties, and key contributors to sample differentiation included glycosylated terpenes, caftaric acid, and trans-ferulic acid. Overall, the results suggest that vintage-specific environmental conditions strongly influence the metabolic profiles of the studied grape genotypes.

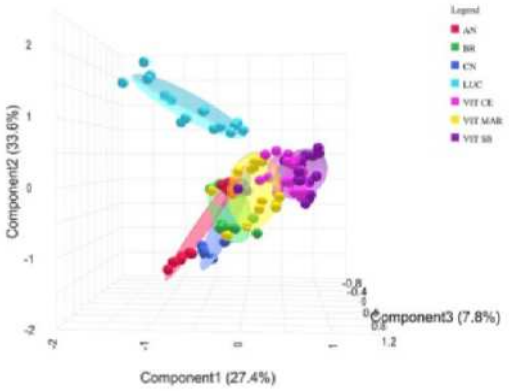


Figure 2 *3D PLS-DA score plot (A) shows the separation of ancient Sicilian grape cultivars and Vitrarolo from different environments (Contessa Entellina – CE, Marsala – MAR, and Sclafani Bagni – SB) based on phenolic and aromatic profiles in 2023 and 2024 vintage, simultaneously.*

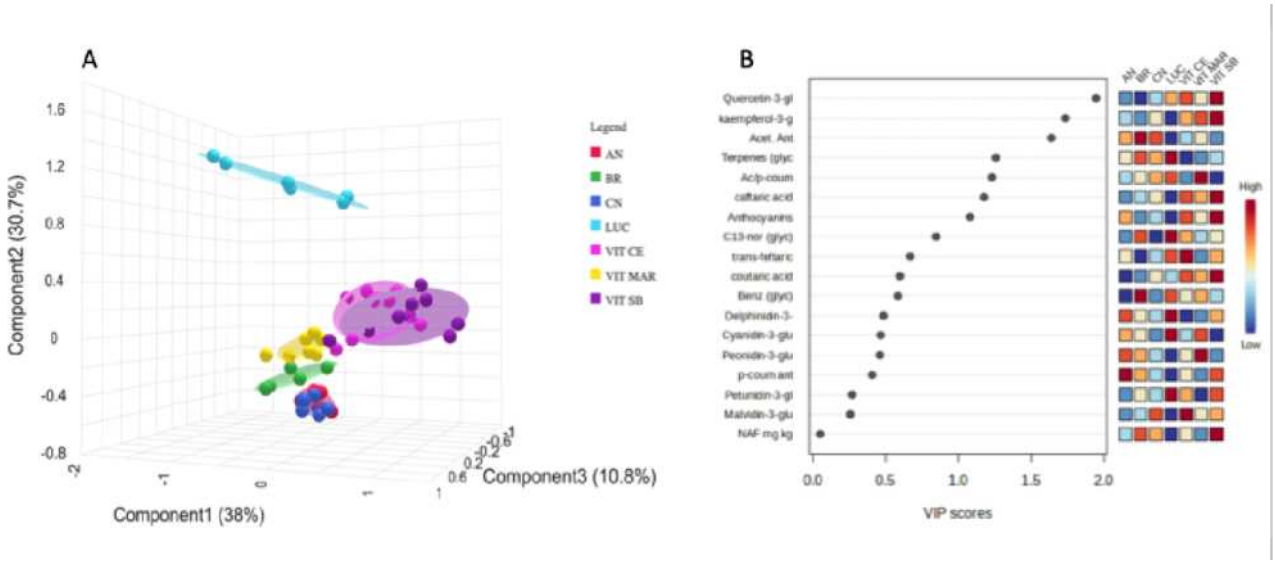


Figure 3 *3D PLS-DA score plot (A) shows the separation of ancient Sicilian grape cultivars and Vitrarolo from different environments (Contessa Entellina – CE, Marsala – MAR, and Sclafani Bagni – SB) based on phenolic and aromatic profiles in 2023 vintage. VIP scores (B) highlight the key compounds driving group separation, with a heatmap indicating their relative abundance across samples. The heatmap on the right shows the relative abundance of each variable across samples, with a color gradient from low (blue) to high (red).*

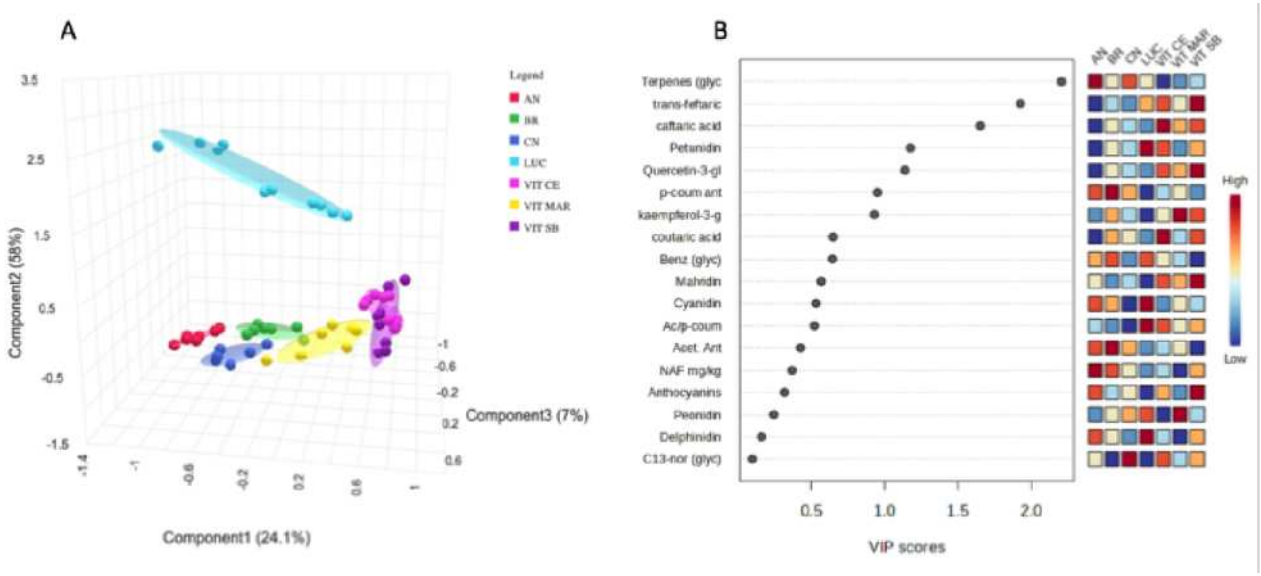


Figure 4 *3D PLS-DA score plot (A) shows the separation of ancient Sicilian grape cultivars and Vitrarolo from different environments (CE, MAR, SB) based on phenolic and aromatic profiles in 2024 vintage. VIP scores (B) highlight the key compounds driving group separation, with a heatmap indicating their relative abundance across samples. The heatmap on the right shows the relative abundance of each variable across samples, with a color gradient from low (blue) to high (red).*

4. Conclusions and Future Perspectives

This study highlights the significant role of ancient Sicilian grape cultivars in preserving viticultural biodiversity and enhancing wine quality under changing climatic conditions. Through comprehensive chemical and statistical analyses, clear varietal and environmental influences were observed on the phenolic and aromatic profiles of the grapes. The variability in ripening stages and metabolite composition across vintages reinforces the importance of tailoring harvest and winemaking strategies to specific genotypes and terroirs. In particular, cultivars like *Vitrarolo* showed consistent responses across environments, while others revealed greater sensitivity to vintage effects. Key compounds such as flavonols, anthocyanins, and glycosylated terpenes emerged as reliable markers for genotype and site differentiation. Future research should focus on the sensory validation of these findings, long-term monitoring of vintage effects, and exploring the winemaking potential of these minor cultivars.

5. References

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Oral presentation sessions
PhD students – 3rd year
Abstracts

PARALLEL SESSION 5
Functional Ingredients from By-Products

Fostering Sustainability in the Olive Oil Supply Chain: Valorization of Typical Extra Virgin Olive Oils and Olive Mill By-products

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This PhD thesis aims to foster sustainability in the olive oil supply chain by valorizing typical extra virgin olive oils with different geographical origins and several olive mill by-products. Three case studies were selected, namely Albania, Morocco, and Portugal. The valorization of olive mill by-products focused mainly on biogas production through co-digestion of olive leaves and olive mill wastewater.

Promozione della sostenibilità nella filiera dell'olio d'oliva: valorizzazione di oli extravergini di oliva tipici e sottoprodotti del frantoio

Questa tesi di dottorato mira a promuovere la sostenibilità nella filiera dell'olio d'oliva attraverso la valorizzazione di oli extravergini tipici con diverse origini geografiche e dei sottoprodotti del frantoio. Sono stati selezionati tre casi studio per la caratterizzazione degli oli: Albania, Marocco e Portogallo. La valorizzazione dei sottoprodotti del frantoio si è concentrata principalmente sulla produzione di biogas mediante co-digestione di foglie di olivo e acque di vegetazione.

Key words: Olive oil, by-products, sustainability, geographical indication, circular economy.

1. Introduction

In accordance with the PhD thesis project previously decribed (Zantedeschi, 2024), this oral communication reports the main results of the following four activities directed to:

A1) Characterization of Albanian virgin olive oils: instrumental and sensory analysis of two oils produced by two Albanian mills from monovarietal Kalinjot olives.
A2) Characterization of Portuguese virgin olive oils: instrumental and sensory analysis of six samples.
A3) Characterization of Moroccan virgin olive oils: instrumental and sensory analysis of fifteen samples.
A4) Valorization of olive mill by-products: characterization (lipid and moisture content) of olive pomace, olive mill wastewater, olive pits, and olive leaves. Biogas production tests using mixtures of olive mill wastewater and olive leaves.

2. Socio-Economic Sustainability in the Olive Oil Supply Chain: Typical Olive Oil Characterization Towards a Geographical Indication

Olive oil is a product of great economic value within the Mediterranean diet, and its production is continuously increasing worldwide (Guasch-Ferré *et al*., 2014). It is important to ensure and promote the sustainability of the olive oil supply chain, as this provides a competitive advantage for producers and an incentive for consumers, representing both added value and a key element of product differentiation (Lombardo *et al*., 2021).

To support the socio-economic sustainability of the olive oil sector, the European Union's rural development policy encourages the production of geographical indications (GIs), including Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) (Menozzi, 2014). These certifications promote food quality linked to geographical origin and generate positive economic, social, and environmental impacts that strengthen over time through the preservation of local resources involved (Vandecandelaere, 2010). Consumers are generally willing to pay a premium price for GI-certified products compared to non-certified ones (Cei *et al*., 2018). Moreover, GIs can help safeguard traditional methods and cultural heritage through the use of traditional production techniques (WIPO, 2021). The valorization of GI-certified food products can also contribute to job creation and stimulate economic growth, particularly in rural areas (Deselnicu *et al*., 2013).

Ensuring sustainability in the olive oil sector provides competitive advantages for producers and added value for consumers (Lombardo *et al*., 2021). EU rural development policy supports the production of Geographical Indications (GIs) - Protected Designations of Origin (PDO) and Protected Geographical Indications (PGI) - which enhance product quality linked to geographical origin and promote positive socio-economic and environmental effects (Vandecandelaere, 2010; Cei *et al*., 2018). GIs help preserve traditional production methods and cultural heritage (WIPO, 2021), stimulate economic growth, and create employment, particularly in rural areas (Deselnicu *et al*., 2013).

3. Environmental Sustainability in the Olive Oil Supply Chain: Valorization of Olive Mill By-products

Achieving sustainability in olive oil production requires interventions across all stages of the supply chain. In addition to the valorization of high-quality locally produced extra virgin olive oils, a critical aspect is the large quantity of by-products and waste generated in olive mills, such as olive pits, pomace, wastewater, and olive leaves. Olive pits are primarily used as a renewable and sustainable bioenergy source through combustion processes and, potentially, for biofuel production (García Martín, 2020). Pomace can be sent to pomace oil extraction plants or used as raw material for composting, energy production through combustion or biogas processes, and the extraction of bioactive compounds such as polyphenols (Dermeche *et al*., 2013).
A particularly large amount of olive mill wastewater is generated, which poses an environmental challenge due to its high pollutant potential that can negatively affect soil and groundwater (Fleyfel *et al*., 2024). However, these wastewaters are rich in bioactive compounds and can be valorized, for instance, through the extraction of phenolic compounds to be used as functional ingredients in food, cosmetics, or animal feed (Comandini *et al*., 2015). Additionally, olive leaves, which are separated from the olives during oil production, represent another by-product that is rarely valorized. One potential approach involves the use of olive leaves and wastewater for biogas production (Romero-Garcia *et al*., 2014). However, the high lignin content in olive leaves poses a challenge for their conversion into biogas (Espeso *et al*., 2021).
Given the importance of developing a sustainable olive oil supply chain and promoting virtuous olive mills, it is necessary to further investigate the valorization of underutilized by-products and wastes, such as olive leaves. These practices support resource sustainability and reduce pollution from the olive oil sector, while also representing economic opportunities (D'Adamo *et al*., 2019).

4. Experimental Procedure

The experimental design comprised two complementary research lines. The first focused on the characterization of virgin olive oils from Albania, an emerging producer, Morocco, an important producer with national geographical indications, but not yet recognized by the European Union, and Portugal, a well-established producer with 6 PDOs whose specifications are outdated and in need of revision. The VOOs characterization comprised quality parameters (acidity, peroxide value, and UV indices), stability (OSI), fatty acid and phenolic profiles, total phenolic content, volatile compounds profile, and sensory analysis (Panel test). The second line addressed the characterization of olive mill by-products such as olive stones, pomace, wastewater, and olive leaves. Additionally, biomethane potential analysis tests were performed on OMWW and olive leaves mixtures (4% and 10%) to evaluate the possibility of the co-digestion and assess impact on biogas yield and composition.

5. Materials and Methods

5.1 Virgin olive oils characterization

Vorgin olive oils (VOO) from Albania (two samples of monovarietal Kalinjot EVOO), Morocco (15 samples of VOO with at least 80% Picholine Marocaine) and Portugal (three samples of azeite de Moura and three samples of Trás-os-montes) were instrumentally and sensory characterized. Peroxide values, free acidity, fatty acid profile, spectrophotometric investigation in the ultraviolet and sensory analysis were performed according to the EU official methods (EU Reg. 2022/2105). Oxidation stability was measured with the 892 Rancimat instrument (Metrohm, Filderstadt, Germany). Total phenolic content was measured following the Folin-Ciocalteau procedure as reported by Singleton and Rossi (1965) and phenolic compound analysis was carried out by HPLC (Shimadzu, Kyoto, Japan) according to the method proposed by the International Olive Council (2017). Additionally, Moroccan virgin olive oils and Portuguese olive oils free radicals were measured with microESR benchtop spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) and an autosampler (SampleBench Automation) (Bruker, Germany). To investigate the volatile profile of the two olive oil samples, solid-phase microextraction followed by gas chromatography-mass spectrometry method (SPME-GC-MS) was performed with a polar-phase column (TG-WAXMS: length 60 m, internal diameter 0.25 mm, and coating 0.50 µm; Thermo Fisher Scientific, Waltham, MA, USA). Moreover, the two Albanian VOO samples volatile profiles were also determined with gas chromatography coupled with ion-mobility spectrometry (HS-GC-IMS) Flavourspec® instrument (G.A.S. Dortmund, Dortmund, Germany) with a nitrogen generator (Microprogel, Pordenone, Italy) provided with an autosampler unit, HT2000H (HTA s.r.l., Brescia, Italy).

5.2 Olive mills by-products valorization

Olive mill wastewaters (OMWW) and olive leaves were collected from a local olive mill, C.A.B. - COOP. AGRICOLA BRISIGHELLESE, in Brisighella (Ravenna). The samples were frozen on the day of collection and stored at -20°C until use. OMWW, the mixtures OMWW+4 and OMWW+10 were characterized for total solids (TS), volatile solids (VS), pH, and lipid content. Total solids and volatile solids were measured drying and incinerating the samples at 105°C and 550°C, respectively, according to the related standard methods (APHA-

AWWA-WPCF, 2005). The pH was measured with a pH meter (Crison, Barcelona, Spain). Finally, lipid content was determined with Soxhlet extraction. Biomethane Potential (BMP) analysis was performed in batch mode using the Methan tube digester (Biological Care, Bologna, Italy). Student's t-test ($p< 0.05$) was carried out to evaluate significant differences in the results obtained on the two samples, both in their characterization and BMP.

6. Results and Discussion

6.1 Albanian virgin olive oils characterization

Two Albanian monovarietal olive oils (100% Kalinjot), produced by two mills located in the southwest of the country, were analyzed. The study shows the characteristics of these oils, in line with what has been previously reported in the literature for the Kalinjot variety (Topi *et al*., 2019; Velo & Topi, 2017; Morina & Kongoli, 2022). Both oils complied with the limits established for extra virgin olive oils under EU Regulation 2022/2104. The oils exhibited a medium phenolic content (7.00 ± 0.12 mg GA/20 g and 5.36 ± 0.10 mg GA/20 g), exceeding the threshold required for the health claim related to polyphenols on the product label. They also showed an oxidative stability index (OSI) exceeding 20 hours, a key parameter for assessing oxidative stability over time and providing an indication of the product's shelf life. Higher OSI values correspond to greater resistance to oxidation, thus suggesting a longer shelf life.

SPME-GC-MS analysis identified 33 volatile compounds, predominantly aldehydes, alcohols, and ketones. In particular, the main compounds detected and quantified included the aldehydes (*Z*)-2-hexenal, hexanal, and (*E*)-2-pentenal, the alcohols (*Z*)-3-hexen-1-ol and 1-hexanol, and 1-penten-3-one - all associated with the characteristic "green" sensory notes. Volatile compounds typically linked to sensory defects, such as butanoic acid and acetic acid, were detected at significantly lower concentrations.

In the heatmaps generated from HS-GC-IMS analysis, twelve volatile compounds were identified, including those associated with fruity notes as reported in the literature. Moreover, sensory analysis revealed two minor positive secondary attributes - grassy and tomato notes - which are also relevant for the potential future application for a protected designation of origin (PDO). In fact, secondary sensory attributes may reflect the specific production area of the oil and can be included in PDO specifications, as they depend on factors such as pedoclimatic conditions and olive cultivar (Deiana *et al*., 2019).

6.2 Moroccan virgin olive oils characterization

All fifteen Moroccan virgin olive oil samples met the quality criteria established by the EU Regulation 2022/2104 for extra virgin olive oils for free acidity, peroxide values and spectrophotometric investigations in the ultraviolet range. Moreover, their fatty acid composition fell within the regulatory limits for extra virgin classification (EU Regulation 2022/2104). The free radical levels showed notable variability across the samples, ranging between low and moderate concentrations, particularly after 220 minutes of heating at 80°C. OSI time varied between 15.9 and 23.6 hours, reflecting a moderate resistance to oxidation. However, both the free radical levels and OSI times may have been impacted by shipment conditions from the place of production to the laboratory which could have influenced the oils' stability profiles. Moreover, their fatty acid composition fell within the regulatory limits for extra virgin classification.

Finally, the panel test classified 9 out of 15 oils as extra virgin, while the remaining 6 samples exhibited low-intensity defects, including fusty, rancid, and winey. Further investigation into the causes of these defects could help improve overall quality. The presence of defects such as fusty and winey is typically linked to improper post-harvest handling, including delays in processing or suboptimal storage conditions that favor fermentation (Barbieri *et al*., 2021). These issues highlight the need for targeted technical support and training for cooperatives, particularly in optimizing harvesting protocols, ensuring timely fruit delivery to mills, and maintaining hygienic conditions throughout the processing chain. In addition, four samples exhibited a rancid defect, indicative of oxidative degradation (Barbieri *et al*., 2021).

6.3 Portuguese PDO olive oils characterization

Two Portuguese PDO olive oils, Azeite de Moura and Azeite de Trás-os-Montes, were characterized. For Azeite de Moura, three samples were collected: an extra virgin olive oil from the cooperative (M-AVE), a virgin olive oil from the cooperative (M-AV), and an extra virgin olive oil from a local private producer (M-C). For Azeite de Trás-os-Montes, three extra virgin olive oils were collected from different private producers: Acushla (TOM-A), Rosmaninho (TOM-R), and Marca Branca (TOM-MB).

All samples, with the exception of TOM-MB, met the European Union quality standards for extra virgin olive oil (or virgin olive oil in the case of M-AV) in terms of free acidity, peroxide value and UV spectrophotometric indices (K_{232}, K_{268}, ΔK). TOM-MB exceeded the extra virgin limits for K_{232} and ΔK. Fatty acid composition fell within the regulatory limits for extra virgin classification (EU Regulation 2022/2104) for all samples.

Table 1 *Results, as an average of three replicates of the basic quality parameters (free acidity, peroxide values, induction period, K268, K232, ΔK) and related EU legal limits for EVOO, when applicable (EU Reg. 2022/2104)., and total phenolic content for the herein analyzed samples.*

Sample	Free acidity (%) *	POV (mEq O_2/kg)*	K_{268}*	K_{232}*	ΔK*	OSI time (h)	Total phenols (mg GA/kg)
M-AVE	0.51 ± 0.7	7.1 ± 0.0	0.15 ± 0.01	0.66 ± 0.06	-0.08 ± 0.00	25.7 ± 0.2	414.83 ± 12.80
M-AV	0.99 ± 0.14	4.8 ± 0.1	0.13 ± 0.00	1.75 ± 0.04	-0.08 ± 0.00	27.4 ± 0.2	350.89 ± 1.71
M-C	0.3 ± 0.04	8.8 ± 0.0	0.14 ± 0.0	1.97 ± 0.09	-0.08 ± 0.00	29.9 ± 0.1	570.93 ± 2.22
TOM-A	0.2 ± 0.03	8.4 ± 0.1	0.15 ± 0.00	1.64 ± 0.07	-0.07 ± 0.00	19.0 ± 0.1	400.62 ± 2.56
TOM-R	0.4 ± 0.05	7.1 ± 0.1	0.15 ± 0.00	1.66 ± 0.07	-0.09 ± 0.00	21.9 ± 0.0	324.53 ± 9.09
TOM-MB	0.53 ± 0.07	17.4 ± 0.0	0.23 ± 0.01	2.92 ± 0.08	-0.11 ± 0.00	7.2 ± 0.0	88.18 ± 5.25

Oxidative stability was assessed through both OSI time and the presence of free radicals. Moderate OSI values were observed for all samples, with the exception of TOM-MB, which exhibited a markedly low OSI of 7 hours. This sample also showed a high peroxide value still within the limit for extra virgin olive oil but approaching it closely. Free radical levels across all samples were found to be in low to moderate concentrations.
Sensory analysis classified all Azeite de Moura samples as virgin olive oils, due to the presence of slight defects such as fusty and winey. Among the Trás-os-Montes samples, two (TOM-A and TOM-R) were classified as extra virgin olive oils, whereas TOM-MB exhibited significant sensory defects (2.5 for fusty and 1 for rancid) along with very low fruitiness, bitterness, and pungency, in stark contrast to TOM-A and TOM-R.
Further analyses, including phenolic compound profiling via HPLC and volatile compound analysis, are currently in progress.

6.4 Olive mill by-products valorization: evaluation of OMWW and olive leaves co-digestion

Samples were characterized in terms of lipidic content and pH. Moreover, total solids and total volatiles were measured to set correctly the BMP analysis (Table 2).

Table 2 *Samples characterization of total solid (TS), volatile solids (VS), lipidic content, and pH.*

Sample	TS (% w/w)	VS (% w/w)	Lipidic content (% w/w)	pH
OMWW	20.56 ± 0.15	16.58 ± 0.67	0.75 ± 0.03	5.2
OMWW+4	18.31 ± 0.12	14.17 ± 1.02	1.40 ± 0.08	5.2
OMWW+19	23.20 ± 0.04	4.48 ± 0.54	1.43 ± 0.06	5.2

The results showed that the addition of 4% olive leaves did not lead to a statistically significant increase ($p > 0.05$) in cumulative biogas production compared to the OMWW alone. In contrast, the addition of 10% resulted in a significant reduction in biogas production ($p < 0.05$).

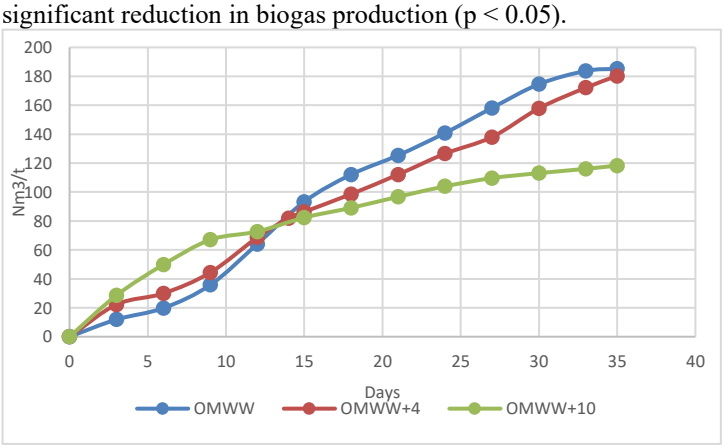


Figure 1 Cumulated biogas production of the BMP test. Comparison between OMWW, OMWW+4, and OMWW+10.

However, in both formulations containing leaves, an improvement in biogas quality was observed: the methane (CH_4) content increased significantly compared to the control, reaching 52.6% with OMWW+4 and 53.8% with OMWW+10, compared to 50.7% in the OMWW alone.

7. Conclusions and Future Perspectives

The characterizations conducted in this PhD work, although based on a limited number of samples, provided valuable insights into the potential of typical virgin olive oils - such as Kalinjot from Albania and Picholine Marocaine from Morocco - to support the application of geographical indications. These results contribute to

strengthening product differentiation and promoting local socio-economic development.
Preliminary trials on biogas production from olive mill wastewater and olive leaves demonstrated the feasibility of co-digestion. While low percentages of olive leaves did not significantly affect biogas yield, an improvement in methane content was observed, suggesting a promising direction for optimizing biogas quality and supporting a circular approach to olive mill by-product valorization.
Taken together, these findings contribute to the broader goal of fostering a more sustainable olive oil supply chain by promoting both product quality and the efficient use of resources. Future research should further expand the characterization of typical olive oils to include a larger number of producers and harvest seasons and explore advanced pre-treatment strategies to enhance the biogas potential of lignocellulosic by-products such as olive leaves.

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Development of functional ingredients using byproducts from agro-industrial process

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This PhD thesis addresses the issue of food loss occurring during the processing of vegetables. The aim of the research is to convert vegetable byproducts (VBs) into value-added ingredients for food development, leading to process and product innovation.

Sviluppo di ingredienti funzionali utilizzando sottoprodotti di processi agroindustriali

Questa tesi di dottorato ha affrontato il problema degli scarti agro-alimentari generati durante la lavorazione di vegetali surgelati. L'obiettivo della ricerca era trasformare i sottoprodotti vegetali in ingredienti con valore aggiunto per lo sviluppo di alimenti, portando innovazione di processo e di prodotto.

Key words: byproduct; food formulation; green process; nutritional quality; techno-functional properties.

1. Introduction

This oral communication reports the main results of the following four activities directed to:

A1) chemical characterization of selected agro-industrial VBs (proximate composition and chemical profiling of bioactive compounds, e.g., carotenoids, tocopherols, polyphenols, glucosinolates)

A2) Definition and optimization of the technological process (air drying, fermentation) to obtain powdered functional ingredients rich in bioactive compounds

A3) Evaluation of the techno-functional and rheological properties of functional ingredients and determination of their shelf-life.

A4) Formulation of bakery products (bread, biscuits, salty muffins) enriched with functional ingredients from agro-industrial VBs.

This PhD thesis is part of WINFOOD ("Vegetable byproducts from industrial processing: form Waste to functional Ingredient for innovative FOOD formulation"), which is a PSR Marche project. The project involved the participation of 5 partners, including 2 Universities (Marche Polytechnic University and University of Udine) and 3 local companies (Covalm, OrtoVerde and Gasteghini).

2. Background: from byproducts to value-added food ingredients

VBs are a source of nutrients and bioactive compounds, such as polyphenols, carotenoids and tocopherols. Therefore, the valorisation of agro-industrial VBs is important to ensure efficient and sustainable agrifood systems. VBs are represented by leaves, stalks, seed coats and broken seeds, pods, press-cake, as well as non-compliant products with defects (Nartea et al., 2023). Considering the high perishability of these products, they must be stabilized to avoid spoilage. Solar-drying, air-drying and freeze-drying are commonly used techniques to remove the water content and stabilize VBs. However, air drying is the easiest, cost- and energy-effective strategy and it allows to control time and temperature parameters. The temperature is usually kept below 40 °C to avoid the degradation of thermolabile bioactive compounds (Darko et al., 2024).
Later, dried VBs can be ground and sieved to obtain a homogeneous flour which can be directly employed for food formulation or further processed to obtain a functional ingredient such as a concentrate of nutrients (e.g., proteins, fibres) or bioactive compounds (e.g., polyphenols, carotenoids, glucosinolates). Dry fractionation and wet extraction are the main methods to obtain a protein concentrate ingredient (Kottage et al., 2024). On the other hand, green and food-grade solvents (e.g., water, ethanol) can be employed to extract phytochemicals (Fanesi et al., 2023). Additional strategies can be adopted to improve extraction yield, nutritional, sensory and techno-functional properties of the ingredients such as ultrasounds (US), microwave (MW), high pressure processing (HPP), and fermentation. Specifically, the fermentation process has been successfully applied to reduce the level of antinutrients (e.g., phytate, raffinose-family oligosaccharides, saponins, enzyme inhibitors) as well as off-flavours typically present in plant matrices (Molina et al., 2025). Table 1 summarizes target compounds usually present in VBs, extraction methods, and food enriched with VBs ingredients.

Table 1. *Summary of target compounds, extraction methods and food formulation.*

Target Compounds	Extraction method	Enriched food
Proteins/Fibers	Dry fractionation	Pasta
	Alkaline extraction-isoelectric precipitation	Extrudates
	Fat removal	Emulsions (mayo, ice cream, spread)
	Fermentation	Bakery products (bread, biscuits, muffins)
		Protein bars and beverages
		Gluten-free products
		Liquid product (beverages, soups)
Polar phytochemicals (e.g., polyphenols, glucosinolates)	Solvent extraction (+US/MW/maceration/fermentation)	Bakery products (cookies, biscuits)
		Beverages (spirits, smoothie)
		Oil and emulsions
		Meat products (sausages)
		Dairy products (cheese, yoghurt)
		Hydrogels
		Nanoparticles
Non-polar phytochemicals (e.g., carotenoids, tocopherols)	Solvent extraction (+US/ MW/HPP/fermentation)	Oil and emulsions
	Pressing	Pasta
		Beverages (juice)

3. Experimental Procedure

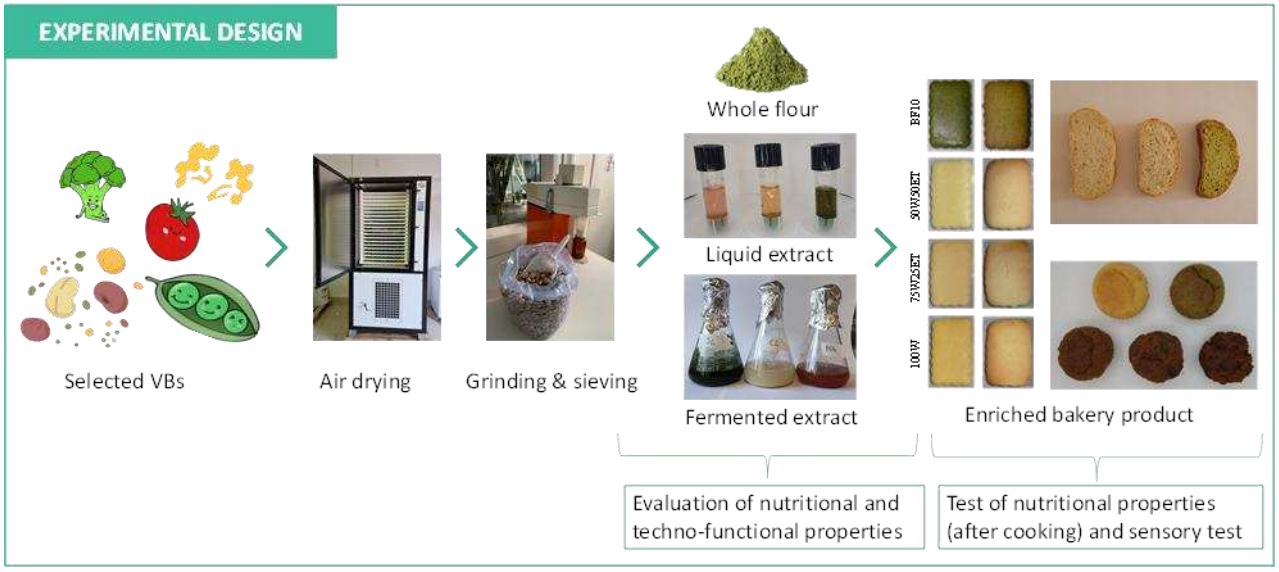


Figure 1. *Experimental design: from agro-industrial vegetable byproducts to enriched bakery products.*

4. Materials and Methods

VBs (broccoli, cauliflower, pea, beans, tomatoes) were provided by local companies, air dried at 40 °C for 24 h, grinded and sieved. The whole flour was used as it is, extracted with food-grade solvents (water, ethanol) or fermented with lactic acid bacteria starter cultures (ongoing experiments) to obtain functional ingredients. Each ingredient was used to formulate bakery products, specifically 1) biscuits were enriched with broccoli (whole flour, hydroethanolic extracts), 2) bread with pea and bean (whole flour), 3) Salty muffins with tomato and cauliflower byproducts (whole flour). Ingredients and developed food were then subjected to chemical, rheological and sensory analysis. As regards chemical analysis, proximate composition and chemical profiling of polyphenols, glucosinolates, carotenoids, and tocopherols were performed by UHPLC-PDA-MS. Texture analysis was performed on bakery products pre- and after cooking according to official AACC methods. Sensory analysis was conducted by untrained panellists (26 female and 24 male) recruited from staff and students at Marche Polytechnic University.

5. Results and Discussion

5.1 Biscuits enriched with broccoli byproducts

Hydroethanolic extracts (especially 50:50, ethanol:water) from broccoli byproducts were rich in polar

phytochemicals (i.e., glucosinolates and polyphenols). However, the addition of liquid extracts, substituting oil fraction, in biscuit formulation did not guarantee proper enrichment because of the enhanced stickiness and poor workability of the doughs (**Figure 2**.1). On the other hand, the incorporation of 10% broccoli byproduct flour resulted in a cohesive and easy-to-work dough and the final biscuit resulted in enhanced nutritional quality displaying significant differences compared to the control biscuit (without broccoli byproducts) in terms of the glucosinolate (33 µg/g dw, ***Table 2***), phenolic (2 mg GAE/g dw, ***Figure 2***.2) and carotenoid content (3 µg/g dw β-carotene, 8 µg/g dw lutein, ***Figure 2***.3).

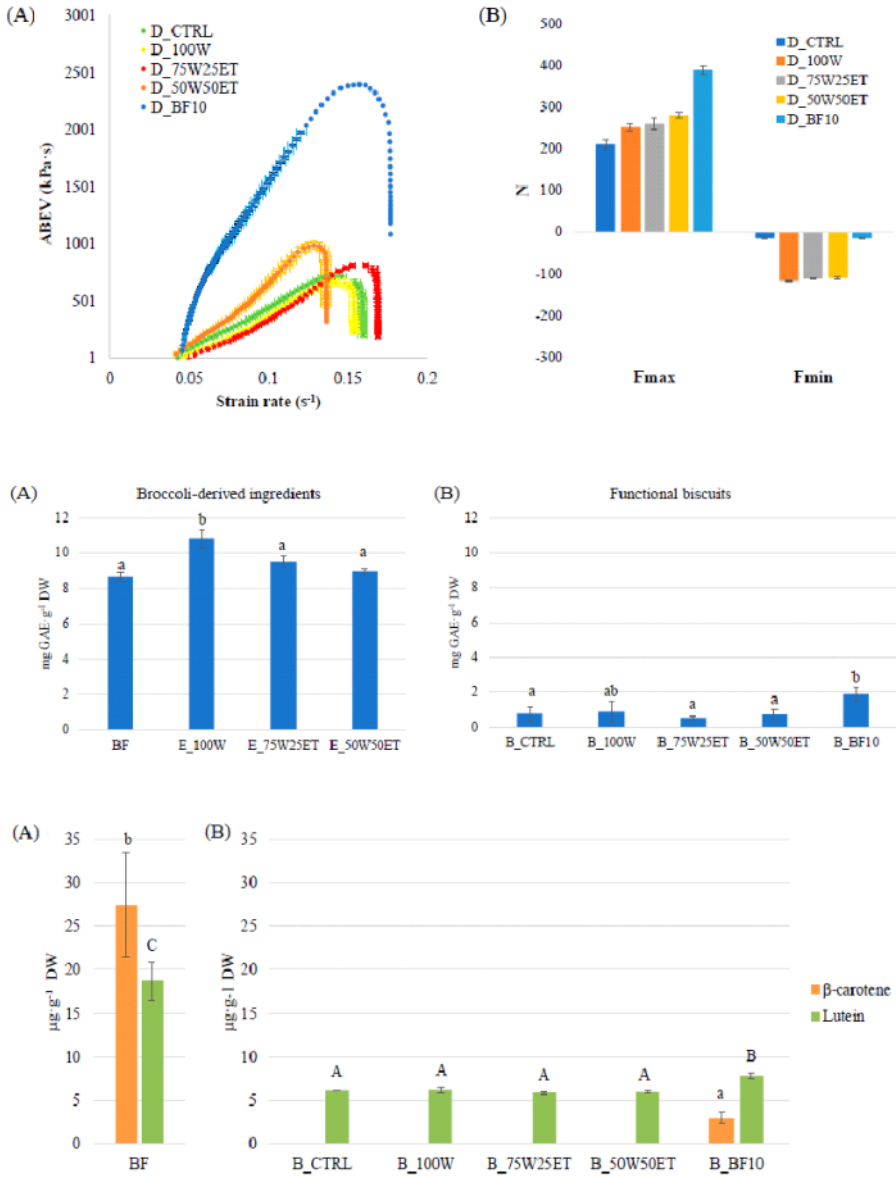


Figure 2. *1)(A) biaxial extension of the doughs under large strain lubricated compressive conditions (B)spread and stickiness of the doughs under large-strain conditions. 2) Total phenolic content of broccoli-derived ingredients (A) and formulated biscuits (B). 3) Carotenoid content in broccoli flour (A) and formulated biscuits (B).*

Table 2. *Concentration of GLSs in biscuits with 10% broccoli flour (expressed as µg/g dw).*

GLSs	B_BF10
Glucoraphanin	18.1 ± 1.6
Gluconapin	0.7 ± 0.4
4-Hydroxyglucobrassicin	0.6 ± 0.2
Glucobrassicin	5.7 ± 1.1
Gluconasturtiin	0.5 ± 0.1
4-Methoxyglucobrassicin	2.0 ± 0.4
Neoglucobrassicin	3.9 ± 0.4
Total	33.2 ±

3.4

5.2 Salty Muffins enriched with cauliflower and tomato byproducts

Muffins were formulated with two byproduct sources, specifically 20% of cauliflower and 0%, 10%, 20%, and 30% of tomato flour (C20, C20T10, C20T20, C20T30). The higher the substitution level of wheat flour, the lower the specific volume of muffins. The addition of VBs led to an increase in phenolic, carotenoids (i.e., lutein, lycopene, and β-carotene), and tocopherol content compared to the control muffin. The muffin C20T30 provided about 56 µg RAE per portion, corresponding to ~14% of the recommended intake for children and 6-8% for adults (male and female) (***Table 3***). The sensory analysis revealed that C20 and C20T30 were overall more appreciated compared to the non-enriched control ***Figure 3***. C20 was preferred for appearance, colour and texture, while C20T30 was appreciated for its strong tomato and acidic taste.

Table 3. *Total phenolic content (TPC) and Retinol Activity Equivalent (RAE) of enriched muffins.*

	TPC mg GAE/g dw	µg RAE/ portion
CTRL	1.3 ± 0.2	0.0
C20	1.8 ± 0.9	2.0
C20T10	2.6 ± 0.6	18.3
C20T20	2.6 ± 0.9	35.8
C20T30	4.0 ± 0.4	56.2



Figure 3. *Sensory scores for enriched muffins.*

5.3 Bread enriched with bean and pea byproducts

Pea and borlotti bean displayed a similar nutritional composition in terms of proteins (~26%), fat (~3%), carbohydrates (~41%), fibers (~19%), and ashes (~3%), except for the sugar content mainly accounted by sucrose (~18% in pea and ~2.5%). A standard direct bread-making method was used to enrich bread with borlotti beans and pea flour. Butter (3%), sugar (6%), and salt (1%) were included in the formulation. Wheat flour was substituted with 10% and 15% bean flour (BOR-10, BOR-15). Enriched breads were characterised by fine and regular alveolation. However, the higher the amount of BOR, the higher the stickiness and humidity of the final bread, thus was not possible to achieve higher substitution levels.

In the case of pea flour, 10% was the maximum enrichment level achievable because of dough stickiness. The high sucrose content (18%) of pea flour caused tumultuous fermentation resulting in a non-homogeneous alveolation. Therefore, the amount of sucrose in one formulation was reduced until 4% (PSP+Z) and in another one was totally removed (PSP─Z) (**Figure 4**). PSP─Z showed a specific volume comparable to control and PSP+Z bread, suggesting that pea flour is suitable for free-sucrose formulations.

*Chemical and sensory analysis are still ongoing.

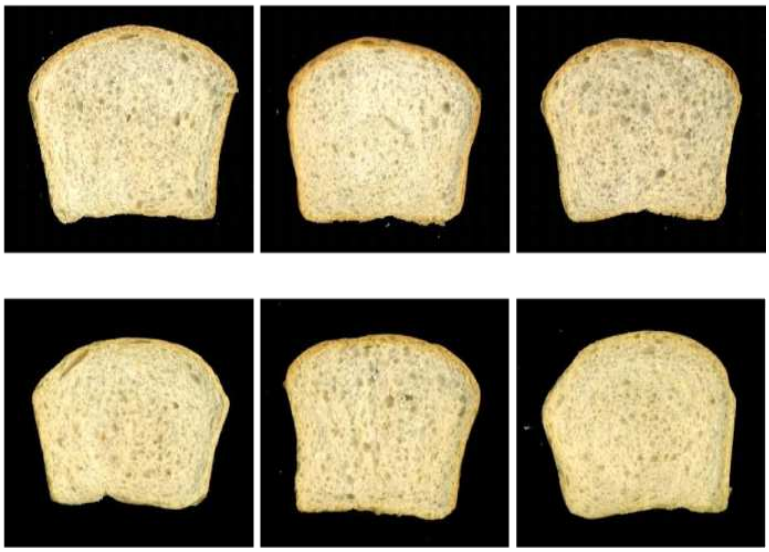


Figure 4. *Bread formulations enriched with 10% and 15% borlotti beans (BOR-10, BOR-15) and with pea flour with and without the addition of sugar (PSP-Z, PSP+Z).*

6. Conclusions and Future Perspectives

Vegetable processing generates a huge number of VBs which are rich in micro- and macro-nutrients. Therefore, VBs can be valorized by transforming them into functional food ingredients. Air drying is the most sufficient way to stabilize them. VBs can be converted into whole flour and be employed as food ingredients, for example for the formulation of bakery products. This allows for minimizing food waste and increasing the nutritional quality of enriched food, as the matrix helps to prevent the thermal degradation of sensitive compounds, such as polyphenols, carotenoids and tocopherols. However, the maximum enrichment level must be evaluated in terms of rheological and sensory properties, to optimize the food formulation and test it on an industrial scale, while also assessing consumer acceptance. The knowledge gained through this research activity will be used to implement technological processes and develop new marketable products on an industrial scale.

7. Nomenclature

VBs: vegetable byproducts; UHPLC: ultra-high performance liquid chromatography; PDA: photo-diode array; MS: mass spectrometer; dw: dry weight; GAE: gallic acid equivalent; TPC: total phenolic content; GLS: glucosinolates; RAE: retinol activity equivalent.

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Technological treatments to obtain high-quality vegetable products

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Bergamot (*Citrus bergamia* Risso et Poiteau) processing generates significant amounts of pomace (BP), an agro-industrial by-product rich in antioxidant and antimicrobial compounds. Pulsed electric fields (PEF) technology has emerged as a promising assisted-extraction technique to enhance the recovery of such compounds from plant by-products. This study investigated the feasibility of applying PEF treatment to BP before maceration, aiming to optimize the extraction process. The extract obtained under optimal PEF-conditions was subsequently evaluated for its antioxidant and antimicrobial activities against *Listeria Innocua*.

Trattamenti tecnologici per ottenere prodotti vegetali di alta qualità

La trasformazione di bergamotto (*Citrus bergamia* Risso et Poiteau) genera quantità significative di pastazzo (BP), un sottoprodotto agroindustriale ricco di composti antiossidanti e antimicrobici. L'applicazione dei campi elettrici pulsati (PEF) si è affermata come una promettente tecnica di estrazione assistita, in grado di migliorare il recupero di tali composti bioattivi da sottoprodotti vegetali. Questo studio ha avuto l'obiettivo di valutare la fattibilità dell'applicazione del trattamento PEF al BP prima della macerazione, con l'obiettivo di ottimizzare il processo di estrazione. L'estratto ottenuto in condizioni ottimali è stato successivamente valutato per le sue attività antiossidanti e antimicrobiche contro *Listeria Innocua*.

Key words: Bergamot pomace; PEF; *Listeria Innocua*.

1. Introduction

In accordance with the PhD thesis project previously described (Cilea, 2023), this oral communication reports the main results from two interconnected activities:

A1) Evaluation of PEF treatment as an assisted-extraction technique for BP, using a Box Behnken Design (BBD) and response surface methodolgy (RSM), to optimize the recovery of polyphenols and antioxidant compounds.

A2) Assessment of the antimicrobial activity of the optimized BP extract against *Listeria Innocua*, through two-hour microbial kinetics.

2. Materials and methods

2.1 PEF treatment, experimental design and bergamot pomace extraction

BP mixed with ethanol solution was PEF treated in a laboratory-scale system (EPULSUS®-LBM1A-15, Energy Pulse Systems, Lisbon, Portugal) using a parallel-plate treatment chamber in batch mode. PEF processing was applied at different electric field strength (E, 2–8 kV/cm), pulse width (τ, 5–15 μs), and treatment time (t, 300–1500 μs) using bipolar pulses at a frequency of 3 Hz. A Box-Behnken Design (BBD) was used to evaluate the effect of these three independent variables on the extraction of antioxidant compounds from BP. 17 experimental runs were performed in duplicate, including 10 replicates at the central point corresponding to samples O, O2, O3, O4, O5, O6, O7, O8, O9 and O10 (5 kV/cm, τ 10 μs, t 900 μs) to monitor experimental error. The sample set included treatments with low field intensity and short treatment time, A (2 kV/cm, 5 μs, 900 μs), E (2 kV/cm, 10 μs, 300 μs), and G (2 kV/cm, 10 μs, 1500 μs). Intermediate conditions included samples C (2 kV/cm, 15 μs, 900 μs), I (5 kV/cm, 5 μs, 300 μs), M (5 kV/cm, 5 μs, 1500 μs), and L (5 kV/cm, 15 μs, 300 μs). High-intensity conditions were applied in B (8 kV/cm, 5 μs, 900 μs), F (8 kV/cm, 10 μs, 300 μs), D (8 kV/cm, 15 μs, 900 μs), N (5 kV/cm, 15 μs, 1500 μs), and H (8 kV/cm, 10 μs, 1500 μs). The untreated sample was used as the control (CTR), processed only by maceration. Dried BP (12% moisture) was mixed with an ethanol (Food Grade)-water solution (50:50 w/w) at a solid-to-liquid ratio of 1:4 and subjected to conventional maceration (MC) at 70 °C for 30 minutes (Gattuso et al., 2023). The obtained extracts were characterised for total phenols content (TPC) (González-Molina et al. 2009), compared with a gallic acid calibration curve and were expressed as mg of gallic acid $g^{-1}$of the dry weight of pomace (mg GAE g^{-1} dw); total antioxidant activity (DPPH and ABTS) (De Bruno et al., 2021) were reported as μmol TE g^{-1} of the dry weight of pomace (μmol TE g^{-1} dw). The CTR was directly macerated, while the other samples were pre-treated with PEF before MC. TPC, DPPH and ABTS assays of the extracts were selected as response variables.

2.2 *Listeria Innocua* inactivation

After model optimisation, the extract obtained from the PEF treatment corresponding to optimal conditions, was used against *Listeria Innocua* strain IPL 1.17 with a final concentration of 10^8 CFU/mL. PEF-BP extract was concentrated via rotary evaporation (40 °C, 15 min) to remove ethanol. The antimicrobial efficacy was tested using a modified method based on Ferreira et al. (2010). Bacterial inactivation kinetics were assessed over 2 hours at 20°C.

2.4 Statistical analysis

The statistical evaluation of BBD was conducted using Design Expert Software (version 7.00, Stat-Ease Inc., Minneapolis, MN, USA). The suitability and validity of the selected model terms were assessed through analysis of variance (ANOVA). The significance of individual model terms and their interactions was determined based on F-values and associated p-values, allowing for the identification of the most influential factors and validation of the model's predictive capacity. By using RSM to generate three-dimensional surface plots and contour plots, variables could be visualized, and optimal treatment conditions could be determined.

Following model, obtained extracts from PEF treatment were compared to CTR, using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons, with significance set at $p < 0.05$. These analyses were carried out using SPSS software (version 20.0, SPSS Inc., Chicago, IL, USA). Pearson's correlation test was performed to determine correlation coefficients (r) among total phenolic content and antioxidant assays.

3. Results and discussion

3.1 Effect of PEF Treatment on TPC and Antioxidant Activity

As seen in Table 1, PEF-treated BP extract under condition H (8 kV/cm, τ 10 µs, t 1500 µs) showed the highest TPC (20.13 ± 0.15 mg GAE g^{-1} dw), significantly higher ($p < 0.05$) than the CTR (17.33 ± 0.24 mg GAE g^{-1} dw) and all other samples. Samples I (19.59 ± 0.16), F (19.58 ± 0.49), E (19.32 ± 0.13), N (19.09 ± 0.16), and B (18.95 ± 0.20 mg GAE g^{-1} dw) also had TPC ≥ 19 mg GAE g^{-1} dw, significantly above CTR ($p < 0.05$). Samples G (17.79 ± 0.11) and A (18.34 ± 0.17 mg GAE g^{-1} dw) showed values close to CTR without significant difference ($p > 0.05$), suggesting low field strength or short treatment times may not enhance extraction.

Antioxidant activity via ABTS assay was 54.38 ± 0.42 µmol Trolox equivalents (TE) g^{-1} dw in CTR, with sample H showing the highest activity (60.24 ± 0.34 µmol TE g^{-1} dw), significantly different ($p < 0.05$) from most others. Samples I (56.95 ± 0.34), F (56.86 ± 0.42), and D (57.28 ± 0.60 µmol TE g^{-1} dw) also had significantly higher values than CTR ($p < 0.05$), while samples O (56.95 ± 0.28) and L (56.43 ± 0.51 µmol TE g^{-1} dw) showed intermediate values with no significant difference from CTR ($p > 0.05$), indicating that those specific PEF conditions did not markedly affect antioxidant capacity.

DPPH assay showed antioxidant activities of 11.86 ± 0.15 µmol TE g^{-1} dw in CTR and 14.15 ± 0.13 µmol TE g^{-1} dw in sample H, significantly higher than CTR and most other treatments ($p < 0.05$). Samples I (13.42 ± 0.12), B (12.88 ± 0.11), F (13.88 ± 0.14), and M (13.39 ± 0.14 µmol TE g^{-1} dw) also had significant increases ($p < 0.05$). Samples G (12.59 ± 0.10), E (13.12 ± 0.18), and D (13.30 ± 0.06 µmol TE g^{-1} dw) showed no significant difference from CTR ($p > 0.05$), indicating less enhancement under those conditions. This suggests that these samples did not enhance antioxidant activity as much as the control group under these specific conditions.

Data confirmed by the determination of the correlation coefficient (r). A positive relationship was observed between TPC and antioxidant activity. In particular, the DPPH assay showed a significant correlation coefficient of r = 0.864 with TPC, confirming that the overall antioxidant potential of the extracts was substantially due to by higher phenolic concentrations. Similarly, the ABTS assay exhibited a correlation with TPC by the ABTS assay (r = 0.804), indicating a degree of association between phenolic content and antioxidant capacity.

The phenolic content and antioxidant properties of BP extracts can be significantly improved by PEF treatments, but the level of improvement is highly dependent on the applied field strength and treatment parameters.

These findings are in line with previous studies on citrus matrices. Luengo et al. (2013) reported up to an increase in polyphenol extraction from orange peel following PEF treatment at 7 kV/cm, and Peiró et al. (2019) using 30 µs pulses at 7 kV/cm in lemon peel residues.

The results obtained in the experimentation suggest a synergistic effect of PEF and hydroalcoholic solvents to improve the extraction of bioactives from different plant-based matrixes beyond citrus fruits. As noted by Hwang et al. (2021), PEF-induced membrane permeabilization coupled with the solvent properties of ethanol-water mixtures significantly enhances extraction efficiency of phenolic and antioxidant compounds. The intermediate polarity of ethanol facilitates solubilization of both polar and non-polar compounds, thereby maximizing recovery of structurally different phytochemicals. This synergistic effect is confirmed by Dent et al. (2013), who demonstrated superior extraction performance of ethanol–water mixtures for *Salvia officinalis*, and by Yan et al. (2017), who highlighted the influence of pulse duration on compound selectivity. According to these authors, short pulses favour extraction of low-molecular-weight compounds, while long pulses promote release of larger, more complex polyphenols.

The enhancement of extraction efficiency in this study was supported by significant increases in antioxidant capacity, as evidenced by ABTS and DPPH assays. Obtained results are consistent with Luengo et al. (2013), who observed significant increases in antioxidant activity following PEF treatment of orange peel extracts. Similarly, Niu et al. (2020) reported that PEF enhances antioxidant properties by facilitating bioactive compound release; however, they highlighted that excessive electric field intensities might cause compound aggregation, reducing bioactivity. This phenomenon is also supported by del Carmen Razola-Díaz et al. (2024), who found that intense PEF parameters, particularly when combined with probe ultrasound, induce phenolic aggregation and negatively affect antioxidant efficacy.

Sample	Electric field strength (kV/cm)	Pulse width τ (μs)	Treatment time (μs)	TPC (mg GAEg-1 dw)	DPPH (μmol TE g-1 dw)	ABTS (μmol TE g-1 dw)
CTR	-	-	-	17.33 ± 0.24^{f}	11.86 ± 0.15^{g}	54.38 ± 0.42^{d}
C	**2**	**15**	**900**	18.85 ± 0.13^{cd}	12.78 ± 0.11^{ef}	56.07 ± 0.28^{bc}
M	**5**	**5**	**1500**	18.81 ± 0.2^{cd}	13.39 ± 0.14^{bcd}	56.1 ± 0.76^{bc}
O	**5**	**10**	**900**	18.89 ± 0.16^{cd}	13.67 ± 0.16^{abc}	56.95 ± 0.28^{b}
L	**5**	**15**	**300**	18.85 ± 0.2^{cd}	12.94 ± 0.13^{def}	56.43 ± 0.51^{bc}
H	**8**	**10**	**1500**	20.13 ± 0.15^{a}	14.15 ± 0.13^{a}	60.24 ± 0.34^{a}
B	**8**	**5**	**900**	18.95 ± 0.2^{bcd}	12.88 ± 0.11^{def}	56.5 ± 0.33^{bc}
I	**5**	**5**	**300**	19.59 ± 0.16^{ab}	13.42 ± 0.12^{bcd}	56.95 ± 0.34^{b}
E	**2**	**10**	**300**	19.32 ± 0.13^{bc}	13.12 ± 0.18^{cdef}	56.28 ± 0.27^{bc}
N	**5**	**15**	**1500**	19.09 ± 0.16^{bc}	12.97 ± 0.18^{def}	56.12 ± 0.33^{bc}
D	**8**	**15**	**900**	18.65 ± 0.18^{cd}	13.3 ± 0.06^{cde}	57.28 ± 0.6^{b}
G	**2**	**10**	**1500**	17.79 ± 0.11^{ef}	12.59 ± 0.1^{f}	55.51 ± 0.38^{cd}
A	**2**	**5**	**900**	18.34 ± 0.17^{de}	12.66 ± 0.09^{f}	55.62 ± 0.35^{c}
F	**8**	**10**	**300**	19.58 ± 0.49^{ab}	13.88 ± 0.14^{ab}	56.86 ± 0.42^{b}
Sign.				**	**	**

Table 1 *Effect of pretreatment on TPC, DPPH and ABTS. Different letters show significant differences between each group as assessed by Tukey's post hoc test ($p < 0.05$).*

3.2 Process condition optimization

The BBD enabled a systematic evaluation of the interactions among the independent variables E, τ, and t to identify optimal conditions for maximizing TPC and antioxidant activity in BP extracts obtained from PEF-treated matrices.

The quadratic regression model (Equation 1) developed for TPC was statistically significant ($p < 0.0002$), with an F-value of 8.23, indicating a strong fit to the experimental data. The lack-of-fit test was non-significant ($p = 0.3185$), confirming the model's adequacy. Analysis of variance (ANOVA) identified the linear effect of E ($p < 0.0015$), the interaction between E and t ($p < 0.0018$), and the quadratic effect of t ($p < 0.0274$) as significant predictors of TPC.

$$TPC = 20.51 - 0.139 \cdot E - 0.00347 \cdot t + 0.000289 \cdot E \cdot t + 0.000000948 \cdot t^2 \quad \text{(Eq. 1)}$$

An adequate precision value of 10.094 indicated a good signal-to-noise ratio. The contour plot in Figure 1 shows that TPC increased synergistically at higher values of both E and t, defining the optimal processing region.

For antioxidant activity, the DPPH assay data were modelled using a reduced quadratic equation (Equation 2), which also showed a significant fit ($p < 0.0001$, F = 11.86) and a non-significant lack-of-fit ($p = 0.0645$). Significant terms included the linear ($p < 0.0001$) and quadratic ($p < 0.0366$) effects of E, and the quadratic effect of τ ($p < 0.0001$).

$$DPPH = 10.40 + 0.405 \cdot E + 0.398 \cdot \tau - 0.0278 \cdot E^2 - 0.0203 \cdot \tau^2 \quad \text{(Eq. 2)}$$

The model exhibited good predictive robustness with an adequate precision of 11.014. The results indicate parabolic relationships between both E and τ with DPPH activity, suggesting optimal parameter values within the studied range. The individual effect of E on DPPH activity (Figure 1) shows a clear increase at intermediate field strengths, followed by a decline.

Similarly, ABTS antioxidant activity was modelled with a highly significant quadratic equation (Equation 3) ($p < 0.0001$, F = 10.85), and a non-significant lack-of-fit ($p = 0.2307$). Significant predictors included the linear effect of E ($p < 0.001$), the interaction between E and t ($p < 0.0003$), and the quadratic effect of τ ($p < 0.0086$).

$$ABTS = 54.93 - 0.212 \cdot E + 0.567 \cdot \tau - 0.00259 \cdot t + 0.000578 \cdot E \cdot t - 0.0274 \cdot \tau^2 \quad \text{(Eq. 3)}$$

An adequate precision of 13.968 confirmed the robustness of the model. E had a significant influence on ABTS activity, modulated by t. The quadratic term for τ suggested the presence of an optimal value beyond which antioxidant activity declined. The contour plot in Figure 1 confirmed these trends, showing enhanced ABTS activity at moderate E and intermediate t levels.

Optimal conditions (sample H) were identified at E 8 kV/cm, t 1500 μs, and τ 10 μs.

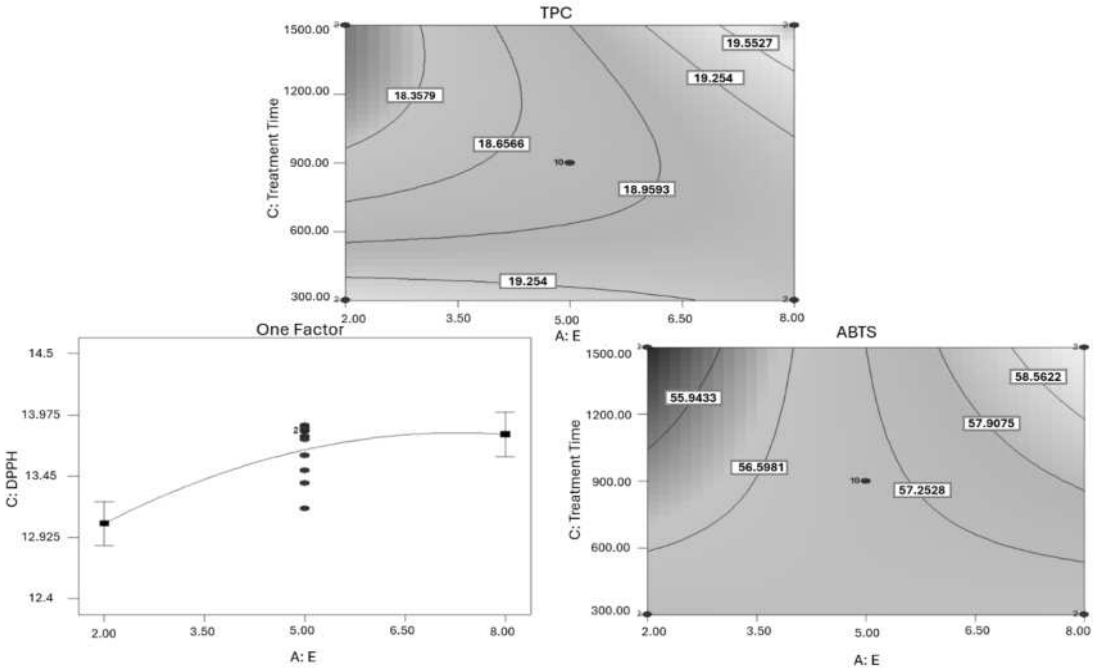


Figure 1 *Two-dimensional graphs showing the interaction between E and t (TPC and ABTS) and E (DPPH).*

3.3 *Listeria Innocua* inactivation

PEF-BP extract without ethanol, with a TPC content 12.18 ± 0.07 mg GAE/mL, exhibited significant bactericidal activity against *Listeria Innocua* with an initial bacterial load of approximately 8.10 ± 0.003 Log CFU/mL. A reduction of 2.29 Log units was observed following prolonged exposure up to 120 minutes, resulting in a bacterial count of 5.81 ± 0.04 Log CFU/mL. These findings demonstrate a time-dependent decrease in *L. Innocua* viability, characterised by a typical logarithmic inactivation pattern. Under the tested conditions, the inactivation efficacy is primarily governed by the duration of contact time between the extract and the microorganism (Figure 2).



Figure 2 *Inactivation of Listeria Innocua.*

The antimicrobial activity observed for BP extracts obtained via PEF pretreatment is consistent with the inhibitory effects. Citrus-derived extracts demonstrate notable antimicrobial activity in addition to their antioxidant properties as reported by Fisher and Phillips (2006) for bergamot essential oil against *L. monocytogenes*, both in vitro and within food matrices. PEF-BP extract demonstrated effective antimicrobial activity against *Listeria Innocua*. These results suggest that under the tested conditions, the duration of microbial exposure to the extract plays a critical role on inactivation efficacy. According to Ariza et al. (2021) and Bajpai et al. (2019), flavonoids such as hesperidin exhibit significant efficacy against Gram-negative bacteria, whereas naringin and its derivatives are particularly effective against Gram-positive strains, including *Listeria* spp. The underlying antimicrobial mechanisms involve disruption of cellular membranes, alterations in membrane permeability, and interference

with DNA synthesis.

4. Conclusions and Future Perspectives

The present study confirms the efficacy of PEF technology as a non-thermal pretreatment method for enhancing the extraction of polyphenols and the antioxidant capacity from bergamot pomace. The capacity of PEF to disrupt cell membranes through electroporation facilitates the release of phenolic compounds within the cell, resulting in a significant enhancement of the total polyphenol content and antioxidant capacity of the final extracts. The application of food-grade solvents further supports sustainable processing strategies consistent with circular economy principles. This methodology promotes the valorization of agro-industrial waste, it reduces environmental impact and supports the development of high-value functional ingredients from underutilized biomass. Additionally, the bergamot extracts obtained via PEF exhibited significant antimicrobial activity against *Listeria Innocua*. These encouraging results provide a basis for future research into the potential application of this extract as natural antimicrobial agent against other target microorganisms typical of food systems. Another important aspect is the use of this extract as a substitute to synthetic additives for food preservation or for the enrichment of edible films, with respect and care for the modern consumer.

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Integrated strategies to overcome lignocellulosic recalcitrance for the recovery, fractionation and characterization of functional fibers

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The structural recalcitrance of lignocellulosic biomass limits the recovery of functional fiber components. This PhD work explores different approaches for the fractionation and characterization of dietary fiber from plant residues, with a focus on the development of both selective (mostly hemicellulose-targeted) and non-selective extraction strategies. By adopting and/or combining sequential treatments, including enzymatic, physical, microbiological, and innovative solvent-based methods, the aim was to improve biomass accessibility and enable the recovery of functionally relevant fiber fractions under milder conditions than those conventionally employed.

Strategie integrate per facilitare il recupero, la separazione e la caratterizzazione di fibre funzionali da materiale lignocellulosici

La complessità strutturale delle biomasse lignocellulosiche rappresenta un limite importante al recupero di componenti funzionali della fibra. Questo lavoro di dottorato si concentra sull'esplorazione di diverse strategie per il frazionamento e la caratterizzazione della fibra alimentare proveniente da residui vegetali, con particolare attenzione allo sviluppo di metodi estrattivi sia selettivi (principalmente indirizzati all'emicellulosa) che non selettivi. Attraverso l'adozione e la combinazione di trattamenti sequenziali di tipo enzimatico, fisico, microbiologico e basato su solventi innovativi, l'obiettivo è stato migliorare l'accessibilità del materiale e ottenere frazioni di fibra integre, utilizzando condizioni più blande rispetto a quelle tradizionalmente impiegate.

Key words: lignocellulosic material, fractionation and characterization, hemicellulose, enzymatic and physical pretreatments, mild conditions

1. Introduction

Hemicellulose extraction from lignocellulosic biomasses and its valorization have been significant research topics over the last decade ((Moure et al., 2006)). Conventional methods often involve harsh conditions (Delidovich et al., 2014)), leading to the formation of non-food-grade degradation compounds and requiring multiple purification steps ((Otieno & Ahring, 2012)). This research aims to extract hemicellulose (and other fiber fractions) from food industry by-products using novel and milder techniques. These approaches involve the use of pretreatments to promote matrix disaggregation and lignin removal, enabling hemicellulose extraction under milder conditions. This PhD dissertation describes the main stages of the research, as well as key results concerning the adopted strategies, their impact on extraction efficiency, and the characterization of the extracted compounds.
The following key steps were performed:

1. Development of a protocol for fractionating and characterizing fibers
2. Systematic multistep extraction process for the total valorization of fiber fractions from fruit seeds
3. Hydrothermal extraction of fibers from hazelnut shells: effect of temperature, physical pre-treatments, lignin-degrading enzymes, and degradation compounds
4. Optimizing hydrothermal extraction of fibers from hazelnut shells: effect of temperature and different lignin-degrading enzymes
5. Optimizing hemicellulose hydrothermal extraction from lignocellulosic biomasses using an enzymatic approach: effect of temperature, lignin-degrading enzymes, and matrix
6. Extraction and fractionation of hemicellulose-rich fibers from hazelnut shells using Deep Eutectic Solvents (DESs)

2. Results and discussion

2.1. Development of a protocol for fractionating and characterizing fibers

The first study addressed the limitations of current enzymatic-gravimetric methods for fiber quantification, such as AOAC and Van Soest analyses, which quantify soluble and insoluble dietary fibers (DF) but lack detailed characterization of fiber fractions. An advanced protocol was developed that integrates the official AOAC 991.43 method with sequential fiber fractionation, exploiting the different resistances of fiber fractions to acid hydrolysis (TFA and H_2SO_4). Using hazelnut shells as a case study, each hydrolyzed fraction was quantified and characterized

through GC-MS monosaccharide analysis. The results obtained for soluble fiber, hemicellulose, cellulose, and lignin fractions were then compared with those from the standard Van Soest method. The key differences observed:

- Hemicellulose: higher values were obtained with the sequential acid hydrolysis method, indicating improved extraction and separation efficiency, and monosaccharide analysis revealed the presence of arabinolxylan as main component
- Cellulose: yields were comparable, but the developed method provided more detailed compositional information, highlighting the presence of a residual fraction of hemicellulose (xylose) strictly bound to cellulose.
- Lignin: slightly lower values were obtained, due to a more efficient separation from cellulose residues.

These results confirm the method's ability to provide a more comprehensive and accurate profile of dietary fiber., This comprehensive procedure, currently under validation, can be applied to a variety of food and nutraceutical products, emphasizing the importance of DF characterization for understanding their bio-functional properties.

2.2. Systematic multistep extraction process for the total valorization of fiber fractions from fruit seeds

A systematic multistep extraction protocol was developed to recover and characterize functional fiber fractions from various fruit seeds, including cherry, lemon, pumpkin, peach, apricot, mango, avocado, and litchi. The process was designed to sequentially isolate soluble fibers, residual pectins, and hemicelluloses, using a mild enzymatic treatment based on protease, an extraction with acidified hot water, and a final hydrothermal step at 175 °C. The extracted fractions were analyzed by GC-MS for monosaccharide composition, HPSEC-RID for molecular weight distribution, and ^{1}H NMR to assess the degree of methylation and acetylation.
The results showed considerable variability among seed types. Cherry, lemon, and pumpkin seeds proved most promising for fiber valorization, particularly in the hydrothermal step, yielding hemicellulose-rich fractions with purities up to 76% and potential recoveries up to 125 kg per ton of dry seed. These fractions were mainly composed of xylose and showed significant acetylation, suggesting applications as functional food ingredients. In contrast, mango, avocado, and litchi seeds, due to their high starch content, were less suitable for fiber extraction but may be considered for starch recovery.

2.3 Hydrothermal extraction of fibers from hazelnut shells: effect of temperature, physical pre-treatments, lignin-degrading enzymes, and degradation compounds

This study focused on extracting hemicellulose from hazelnut shells using milder conditions compared to conventional hydrothermal treatments, which typically require harsh conditions and generate non-food-grade degradation products. To enable milder extraction, several pretreatments were evaluated to promote matrix disaggregation and lignin removal.
Initially, the effect of temperature on hydrothermal extraction was assessed: no hemicellulose could be extracted at 125 °C, while yields improved at 150–175 °C, declining at 200 °C due to degradation. Thus, 125 °C was chosen for further testing of physical (pulsed electric fields and mechanochemical milling) and enzymatic (laccase from *Aspergillus sp*) pretreatments.
Physical treatments alone did not significantly enhance extraction yields but influenced the types of fibers extracted. In contrast, enzymatic pretreatment with laccase notably improved sugar recovery (+37%), mainly through enhanced pectin extraction. Laccase also promoted the release of specific sugars (e.g., mannose and galactose) in physically pretreated samples and generated new fiber fractions compared to controls.
In conclusion, while hemicellulose could not be efficiently extracted at low temperatures, enzymatic pretreatment improved pectin recovery, and physical treatments were excluded from further development due to limited impact.

2.4 Optimizing hydrothermal extraction of fibers from hazelnut shells: effect of temperature and different lignin-degrading enzymes

Due to the limited efficiency of physical pretreatments and the promising results observed with laccase, the study was expanded to include two additional lignolytic enzymes: laccase from *Trametes versicolor* (T) and manganese peroxidase (M). Enzyme dosages were optimized to enhance industrial feasibility. Hazelnut shells were treated with these enzymes and subjected to extractions at various temperatures. In addition to a one-pot extraction at 150 °C, a novel sequential temperature-based fractionation was performed at increasing temperatures (125, 140, 150, and 160 °C) on the laccase-pretreated matrix. Monosaccharide composition and total sugar yields were analyzed by GC-MS and ^{1}H NMR.
Results demonstrated distinct behaviors among the enzymes: Aspergillus laccase (L) enhanced sugar yields at 150 °C, while *Trametes* laccase (T) was effective already at 125 °C. The sequential extraction approach substantially increased total sugar yields compared to the one-pot method and allowed better fiber fractionation, resulting in higher hemicellulose purity in the final extract.

2.5. Optimizing hemicellulose hydrothermal extraction from lignocellulosic biomasses using an

enzymatic approach: effect of temperature, lignin-degrading enzymes, and matrix

Based on these findings and the commercial availability of laccase from *Aspergillus* sp. (L), further tests were conducted to evaluate the effect of different matrices on the extraction process. In addition to hazelnut shells, other matrices were selected based on their varying lignin content, including almond shells, hops leaves, and processing residues from pineapple, melon, orange, and apple. These agro-food by-products were selected specifically for their differing lignin-to-hemicellulose ratios.

The same enzymatic pre-treatment procedure with L was applied across all matrices. Results showed that, in some cases, extraction temperature and/or enzymatic pretreatment significantly increased total sugar yields (up to 25-30%). Additionally, a comparative test using microbial cultures capable of producing laccases (*Pseudomonas fluorescens*, *Lactobacillus rhamnosus*, and *Bacillus licheniformis*) was conducted on hops leaves to evaluate potential biological pretreatments. These microbial cultures were monitored over time (30 days), with samples analyzed both during fermentation and after the hydrothermal treatment. Notably, sugar concentration in solutions had already dropped sharply within the first two days of the fermentation period, indicating that the selected microorganisms rapidly metabolized the available carbohydrates, even the complex ones. This extensive consumption compromised the efficiency of the process, confirming the unsuitability of such microbial pretreatments for sugar recovery purposes.

2.6. Extraction and fractionation of hemicellulose-rich fibers from hazelnut shells using Deep Eutectic Solvents (DESs)

Hazelnut shells were also used as a model matrix in a study employing deep eutectic solvents (DES) to fractionate biomass and isolate hemicellulose. Two DES formulations were tested: an acidic DES (lactic acid + choline chloride, 2:1 molar ratio) and a neutral DES (ethylene glycol + choline chloride, 2:1 molar ratio). Extractions were carried out at 80 °C for 4 and 24 hours. A water extraction was included as a control.

Following extraction, samples were centrifuged, and both the supernatants and dried pellets were analyzed. The supernatants were analyzed by HPSEC-RID to assess molecular weight distribution, and the pellets (after a hydrolysis in sulfuric acid) by HPAEC-PAD for monosaccharide composition. The acidic DES proved more efficient in extracting xylan and yielded higher total extractions, while the neutral DES was relatively ineffective, performing only slightly better than water. Despite this, about 70% of the xylan in the matrix remained unextracted in the pellet (30% extraction yield).

To improve yields, the material was further processed using a planetary ball mill. Coarse material was milled for 0.5, 1, 2, and 4 hours, followed by extraction at 80 °C for 4 and 24 hours. Extraction yields increased in all cases. When applied to ball-milled material, the neutral DES extraction significantly improved the yield, increasing it from 5–10% to approximately 35%; however, other polysaccharides were also co-extracted. The acidic DES nearly doubled the xylan yield (exceeding 60% in the extract), although in this case as well, additional polysaccharides were recovered.

Remarkably, intensive milling enabled selective extraction of xylan with water alone (Figure a), achieving up to 70% recovery of the original xylan in 4-hour ball milled samples. The extracted xylan had an estimated molecular weight of ~20 kDa (Figure 1b), indicating the recovery of high molecular weight xylans. Most of the xylan was recovered in the water extract, with only minor amounts remaining in the pellet.

The water extracts were split into two portions: one dried as is, and the other precipitated with ethanol, to evaluate the mass balance and characterize both fractions. This work represents the final part of the PhD project, and analyses are ongoing. The aim is to further characterize the extracted compounds in terms of degree of polymerization and other structural features. Furthermore, since the pellet primarily contains lignin and cellulose, enzymatic removal of cellulose could provide access to a purified lignin fraction. This lignin could then be characterized (e.g., via 2D NMR) and potentially employed in various applications.

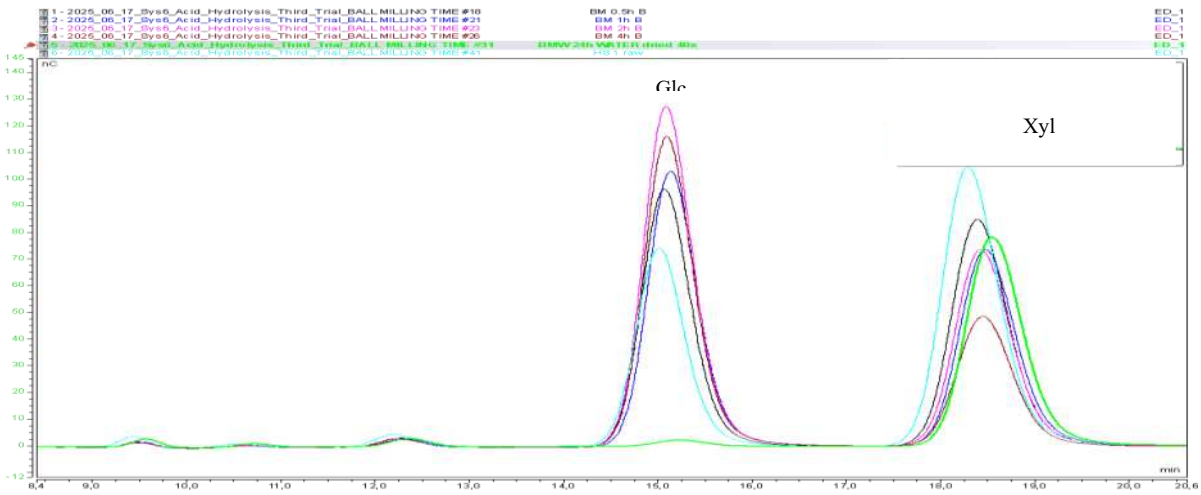


Figure 1a. Monosaccharide composition (HPAEC-PAD analysis) of the pellets obtained from the water extracts of samples ball milled for 0.5, 1, 2, and 4 hours. The chromatograms show a progressive decrease in the xylose peak with increasing milling time, indicating enhanced extraction. The green trace represents the water extract of the 4-hour ball milled sample, in which only the xylose peak is detected.

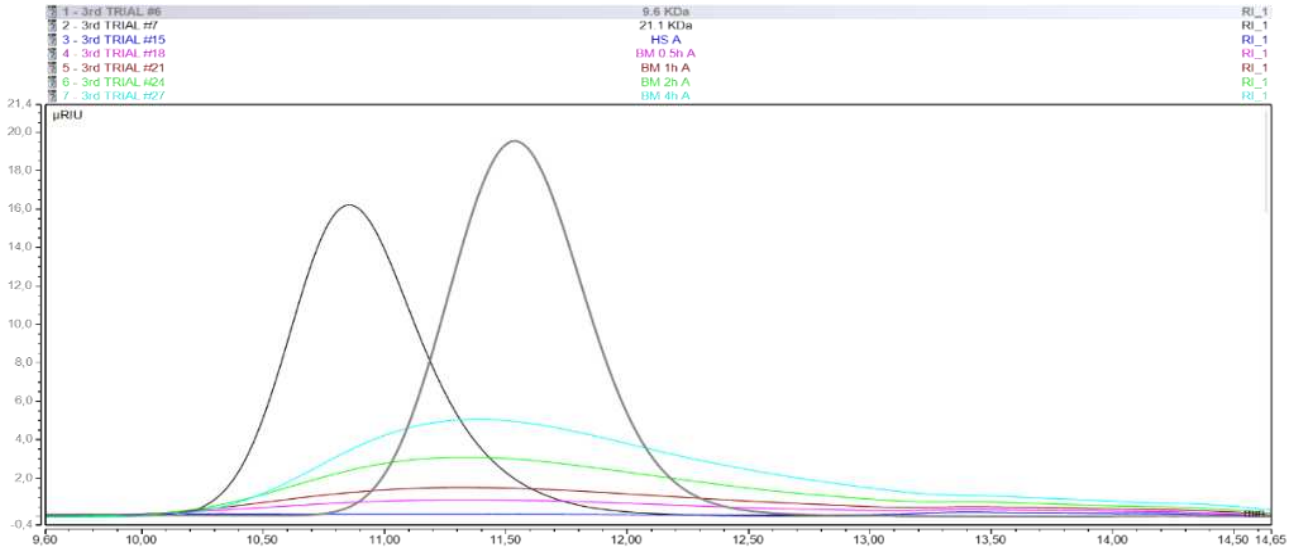


Figure 1b. Overlay of HPSEC chromatograms of water extracts obtained from coarse material (HS A in the legend) and from samples ball-milled for 0.5, 1, 2, and 4 hours, along with molecular weight standards of 21 kDa and 9.6 kDa, used for molecular weight estimation.

3. Conclusions and Future Perspectives

This PhD research contributed to the development of milder and more targeted methods for extracting and characterizing dietary fiber fractions from lignocellulosic biomass, with a focus on hemicellulose. Various strategies—including enzymatic, physical, microbial, and solvent-based pretreatments—were explored to improve extraction efficiency under gentler conditions than conventional harsh methods.

The fractionation and characterization protocol allowed detailed profiling of hemicellulose, cellulose, and lignin fractions. Enzymatic pretreatments with laccases from *Aspergillus* sp. led to moderate but consistent increases in sugar yields, although the effectiveness varied depending on the biomass matrix. Microbial pretreatments proved ineffective due to rapid consumption of sugars by microrganisms. Deep eutectic solvents (DESs) showed promise, particularly the acidic formulation, which was able to extract approximately 50% of xylan at the unusually low temperature of 80 °C, which is remarkable considering that typical xylan extraction usually requires temperatures above 150 °C. However, overall extraction yields with DES remained moderate and co-extraction of other polysaccharides was observed.

A major improvement was achieved by planetary ball milling as mechanical pretreatment. This approach substantially enhanced extraction yields using water alone, enabling selective recovery of high-molecular-weight xylan up to 70% of the initial content after 4 hours of milling, without the need for complex solvents or enzymatic steps. This represents a practical and scalable strategy for hemicellulose valorization.

Future research should focus on:

- Scaling up the most promising protocols, especially water extraction following planetary milling, to pilot scale, optimizing the milling time and temperature extraction.
- Combine laccase treatment with DES
- Deepening the structural characterization of extracted hemicelluloses and lignins using advanced analytical tools (e.g., NMR, MALDI-TOF).
- Investigating applications of the purified fractions in food, pharma, and biomaterial sectors.
- Evaluating environmental and economic sustainability through life-cycle assessment (LCA).

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Sustainable Recovery of High Value Added Products from Secondary Raw Materials of the Milling Industry

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This PhD research, conducted in collaboration with the industrial partner Casillo Next Gen Food Srl (Corato, BA), is based on the valorisation of secondary raw materials derived from industrial durum wheat milling. In the third year of the PhD, the research focus shifted towards a more in-depth investigation of the qualitative characteristics of debranning fractions from the pre-milling process of durum wheat kernels. Specifically, the content of wheat germ agglutinin (WGA), a biomarker of wheat germ, was analysed across various durum wheat kernel fractions. Additionally, the protein composition of wheat by-products was examined using the Osborne fractionation method.

Recupero Sostenibile di Prodotti ad Alto Valore Aggiunto da Materie Prime Secondarie dell'Industria Molitoria

La ricerca di dottorato, condotta in collaborazione con il partner industriale Casillo Next Gen Food Srl di Corato (BA), è basata sulla valorizzazione di materie prime secondarie derivanti dalla macinazione industriale del grano duro. Nel terzo anno di dottorato, l'attenzione si è spostata sull'approfondimento di alcuni aspetti qualitativi delle frazioni ottenute dalla decorticazione pre-macinazione di cariossidi di frumento duro. È stato determinato il contenuto di un biomarker del germe di grano (WGA) in diverse frazioni della cariosside di grano duro. Successivamente, sono state esaminate le proteine contenute nei sottoprodotti della lavorazione del grano duro, attraverso la procedura di frazionamento di Osborne.

Key words: Durum wheat; By-products; Wheat Germ Agglutinin; Osborne fractionation

1. Introduction

The sustainability of the food sector is among the main targets of the EU Green Deal (European Commission, 2020), promoting the reuse of waste to increase production revenue and circularity. In durum wheat milling, the seed coat/pericarp, not included in refined flour, represents a largely available biomass generally used in the feed sector. However, wheat bran and germ are rich of proteins, fibre and bioactive compounds (Prückler et al, 2014). These compounds are usually excluded during the debranning phase, conducted prior to milling. Dry fractionation techniques, such as pin milling and air classification, can be worthy strategies for the valorisation of wheat by-products.
In previous activities, pin milling was employed on defatted selected milling by-products of durum wheat, and air classification was used to separate the material into a fine and a coarse fraction based on differences in particle size and density. The obtained fractions showed enhanced nutritional characteristics and have been used as ingredients for the formulation of functional pasta (Cuomo et al, 2024). Wheat proteins, as explained by Osborne (1924), are divided into four classes, with different characteristics, and specific solubility in different solvents. A field of interest regarding the debranning process of durum wheat kernels involves the diverse concentration of the germ within the debranning fractions. This issue will be addressed through a specific Elisa analysis since there are still few data on this topic in the scientific literature.

2. Materials and methods

2.1. Materials

Three debranning fractions (DF1, DF2, DF3), with DF1 the more external one and DF3 the more internal toward the endosperm, were supplied by Casillo Next Gen Food Srl and analysed for fat, protein and ash content. Casillo Next Gen Food also supplied a mix of durum wheat by-products (mainly composed of debranning fractions 2 and 3) and the same mix of by-products after defatting treatment. The treatment specificities cannot be revealed for patent related issues, but they involve defatting of the material and a thermal treatment. Durum wheat germ was manually removed from whole kernels with a needle.

2.2 Chemical composition analysis

The moisture, lipid and ash content of the samples were determined according to ICC methods 109/1, 136, and 104/1, respectively (ICC, 1995). The protein content was inferred by a Leco nitrogen analyser, model FP 528 (Leco Corp. St. Joseph, MI, USA) according to the Dumas combustion method, AACC method 46–

30.01(AACC, 2010).

2.3 Wheat Germ Agglutinin

The content of Wheat Germ Agglutinin (WGA) was quantified using a commercially available ELISA kit (Wheat Germ Agglutinin ELISA Kit, Novatein Bio, Woburn, MA, USA; Catalog No. NB-E90036), following the manufacturer's instructions. The Novatein Bio WGA ELISA Kit is a competitive enzyme immunoassay designed for the quantitative detection of Wheat Germ Agglutinin (WGA) in vitro. + The assay relies on competition between sample-derived WGA and biotinylated WGA (tracer) for binding to anti-WGA antibodies immobilized on the plate. Following incubation with streptavidin-HRP and TMB substrate, the resulting colorimetric signal is inversely proportional to the WGA concentration in the sample. Quantification is achieved by comparison to a standard calibration curve. The preparation of the sample was carried out as follows: 2 grams of sample were weighed in a 50 ml vial, then 20 ml of RIPA buffer was added. The vial kept shaking at 200 rpm on a rocking table at room temperature for four hours. After that, ten cycles of ultrasonic bath (medium power) and stroke homogenizer were applied to the vial. In the end, an aliquot of the sample was centrifuged at 13000 g and the supernatant was diluted 1:10 since this dilution was found to fall always within the standard curve range of WGA provided by the kit (10000 µg/g – 2.4 µg/g) and then used for the analysis.

2.4 Osborne fractionation

The Osborne fractionation was conducted following the Osborne method (1924) with slight modifications. The procedure started with the albumin's extraction: approx. 2 grams of the sample were weighted in a 50 ml vial and 20 ml of distilled water were added. The sample was then mixed in a vortex mixer for one minute and placed on a horizontally shaking platform to be kept in movement for 30 minutes. The sample was then centrifuged at 3000 rpm for 10 minutes obtaining the albumin-rich supernatant. The extraction was repeated three times, and the supernatants were pooled constituting the albumins fraction. On the precipitate obtained after albumin extraction, the same procedure was applied to extract the globulins using NaCl 0,5 M. After globulins, the gliadins were extracted in the same way with a 70% ethanol solution. Finally, to extract glutenins, 20 ml of NaOH 0,05 M were added to the vial, which was placed into a horizontally shaking water bath at 60°C for 30 min. This step was also repeated three times, and the supernatants were pooled, constituting the glutenins fractions. The protein content of an aliquot of the extracts was analysed with the Kjeldahl method, AACC 46.11 A (AACC, 2010).

2.5 SDS-PAGE

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was conducted on liquid extracts of the Osborne fractionation in order to identify the proteins by comparison of their molecular weight with those present in the literature. The employed gels were NuPAGE Novex 12% Bis-tris gels (ThermoFisher Scientific). The extracts were first diluted with Milli-Q water to achieve a protein concentration of 1 mg/mL. The samples were then prepared putting in an Eppendorf tube 65 µl of the extract with 25 µl of 4X LDS loading buffer and 10 µl of DTT (1,4-Dithiothreitol) 1M, applying reducing conditions. The solution was mixed on a vortex mixer and then heated at 80 °C for 10 minutes at 350 rpm with a thermo-mixer. Afterwards, the gels were placed in an electrophoresis cell filled with a running buffer composed of 40 mL of 20X MES SDS and 760 mL of Milli-Q H_2O. Then, 15 µl of the prepared samples were inserted in the wells together with 3 µl of the molecular weight marker (Novex™ sharp pre-stained protein standard - LC5800 from Invitrogen). Electrophoresis was conducted at 150 V for approximately 1 hour. Following electrophoresis, the gels were stained using Coomassie blue (a mixture of 0.5 g of brilliant blue G in 25 mL Milli-Q water) within an equilibration buffer (20 mL of 86 % phosphoric acid, 150 g of ammonium sulphate, 820 mL of Milli-Q H_2O and 180 mL of 96 % ethanol) overnight. On the subsequent day, the gels were rinsed two times with Milli-Q H_2O for distaining and were finally scanned to obtain the final pictures.

3. Results and Discussion

3.1 Chemical analysis of three consecutive debranning fractions

Table 1 shows the content in protein, fat, and ash of the three debranning fractions DF1, DF2, DF3. It can be noted that the content of lipids increases from the external to the internal part of the durum wheat kernel, passing from 6.11% (DF1) to 8.62% and to 9.62% in DF2 and DF3 respectively. It is possible to recognize the same trend regarding the protein content. Indeed, DF1 shows by far the lowest content (13.26%), while it increases in DF2 and DF3, that have respectively the 18.75% and the 21.71% of proteins on dry matter.

***Table 1**: Content (g/100g d.m.) of fat, protein (N*6.25), ash and moisture (g/100g) of debranning fractions 1, 2 and 3 (DF1, DF2 and DF3) provided by Casillo Next Gen Food. Different letters in column indicate statistically significant differences (p < 0.05)*

Sample	Fat	Protein	Ash	Moisture
DF1	6.11 ± 0,46[c]	13.26 ± 0,07[c]	6.12 ± 0,07[a]	11.3 ± 0.27[b]
DF2	8.62 ± 0,02[b]	18.75 ± 0,09[b]	6.10 ± 0,05[a]	11.3 ± 0.13[b]
DF3	9.62 ± 0,06[a]	21.71 ± 0,07[a]	6.06 ± 0,01[a]	12.0 ± 0.13[a]

The protein content of DF2 and DF3 is very high because of the presence of the germ and the aleurone layer, that are known to be part of the kernel with the higher percentage of proteins, and lipids. As regards the ash content of the fractions, it doesn't seem to show considerable differences.

3.2 Content of Wheat Germ Agglutinin in debranning fractions and wheat germ

The biomarker Wheat Germ Agglutinin (WGA) was used to gain insight into the distribution of wheat germ within durum wheat debranning fractions. Furthermore, the analysis was conducted also on manually extracted wheat germ and on the mix of by-products selected by Casillo Next Gen Food Srl.

Table 2: *Content of WGA (µg/g) expressed as mean value and relative interval, in debranning fraction (DF1, DF2, DF3), in the mix of by-products supplied by Casillo Next Gen Food and in durum wheat germ. The whole wheat kernels were included in the analysis*.

Whole Wheat kernels	27 (16 – 38)
Wheat by-products:	
DF 1	120 (113 – 127)
DF2	152 (131 – 173)
DF3	214 (200 – 228)
Mix of by products	204 (162 – 245)
Durum wheat germ	655 (591 – 719)

Interestingly, the lipid concentration (Table 1) is in line with the results of the biomarker of the germ (WGA), that also increases from DF1 to DF3. The mix of wheat by-products selected by Casillo Next Gen Food has a content of WGA (range 162-245 µg/g) of around one third of the germ (range 591-719 µg/g), manually extracted. These results confirm the high presence of the germ in the mix of by-products. Although the Elisa method is generally used to analyse WGA, it exhibited high variability, as shown by the range of values obtained.

3.3 The effect of treatments on the composition of proteins as analysed through the Osborne Fractionation

Following the identification of wheat germ agglutinin (WGA) in both the wheat by-product mixture and durum wheat germ, further similarities between the two matrices were investigated through Osborne fractionation. The Osborne fractionation was therefore carried out on the mix of by-products supplied by Casillo Next Gen Food Srl before and after treatments (defatting and drying processes) and on durum wheat germ. Albumins and globulins, that display a lower presence in the endosperm where gluten proteins are abundant, are considered more valuable proteins respect to gluten proteins thanks to their more balanced amino acidic profile (Cuomo et al, 2024).

Table 3: *Relative percentages of total N contained in the four Osborne protein classes as found in the mix of by-products (MB), on the defatted mix of by-products (DMB) and in durum wheat germ (WG). The results are expressed on the nitrogen content of the sample. Different letters in columns indicate statistically significant differences* ($p < 0.05$)

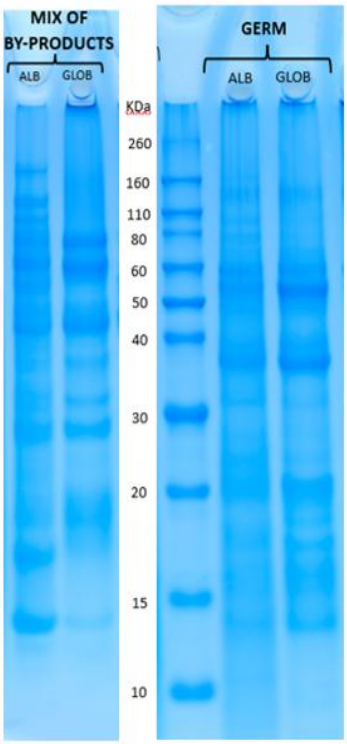
Sample	Albumins (%)	Globulins (%)	Gliadins (%)	Glutenins (%)	Total N (%)
MB	39.9 ± 1.4^{b}	15.1 ± 0.5^{a}	14.1 ± 0.4^{a}	22.2 ± 1.4^{b}	2.9
DMB	23.3 ± 0.35^{c}	12.3 ± 0.1^{b}	8.5 ± 0.6^{b}	48.0 ± 1.1^{a}	3.3
WG	58.3 ± 4.5^{a}	8.8 ± 0.9^{c}	0.62 ± 0.35^{c}	11.3 ± 0.3^{c}	5.3

Table 3 clearly indicates that treatments applied to MB and DMB influence the protein composition of durum wheat by-products. Specifically, prior processing, the percentage of water-soluble albumins was 39.9%. Following the treatments, this value decreased to 23.32%. It is therefore hypothesised that the treatments applied caused a partial denaturation of albumins, that lost their solubility and cannot be extracted anymore in distilled water, causing in turn a redistribution of the protein classes. A similar trend is observed with respect to glutenins. The decline in albumin content during treatments appears to be associated with a corresponding increase in glutenins post-treatment. This is plausible given that glutenin extraction involves significantly harsher conditions (0.05 N NaOH at 60 °C) compared to the relatively mild water-based extraction of albumins. Overall, the data indicate a loss of solubility of albumins resulting from treatments. Finally, the high albumin content of the wheat germ (58.3%) contributes to the elevated albumins levels found in the by-products (39.9%), prior treatments.

3.4 SDS-PAGE

Figure 1shows the SDS-PAGE profile of the albumins and globulins liquid extracts from the Osborne fractionation of the mix of by-products supplied by Casillo Next Gen Food Srl and from those of durum wheat germ.

Figure 1*: SDS-PAGE profile of the liquid extracts of albumins and globulins from the mix of by-products and from durum wheat germ. Alb: albumins, Glo: Globulins. The standard of know molecular weight proteins is at the centre (KDa) of the*



As shown in Figure 1, the albumin and globulin fractions from both the wheat by-products and the germ display multiple shared protein bands, indicating a notable degree of compositional overlap. This similarity may, in part, result from the solubility characteristics of these protein classes: albumins are water-soluble, and the presence of salts in the samples could facilitate the partial solubilization of globulins, leading to their co-extraction in the albumin fraction. On the other side, albumins are extractable in NaCl 0.5 N, therefore unextracted albumins can be extracted together with globulins. Further supporting this observation, previous studies have demonstrated that albumin and globulin fractions can contain common protein bands, (Zhang et al., 2020). According to De Brier et. al, (2015), proteins with molecular weights of 17, 22, 25, 27, 29, 32 and 40 kDa were present in albumin/globulin fractions while, in the study of Wu et. al, (2017), also band of 44, 51, 65, 71, 74, 78 and 90 KDa were found in albumin extract, even if carried out on another sample. These bands are present also in Fig. 1, even if blurred to some extent in the low molecular weight range.

Focusing on the albumin fractions, a notable similarity in the protein banding patterns can be observed between albumins of the mix of by-products and those of the germ. Both extracts show a predominant presence of protein

bands within the low to mid-molecular weight range (approximately 15–50 kDa), and in the higher range (from 50 to 110 KDa), with several bands appearing at corresponding positions, suggesting the presence of shared and structurally related proteins between the two matrices.

Conclusions

Although the results obtained with the different methods were not entirely consistent, taken together they provided evidence for the presence of wheat germ in the by-product mix. These results indicate a promising potential for the valorization of such secondary raw materials although further studies are recommended to characterize their composition more comprehensively.

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Oral presentation sessions
PhD students – 3rd year
Abstracts

PARALLEL SESSION 6
Food, Microbiota and Health

Ultra-Processed foods: from the nutritional characteristics to the impact on human health

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This PhD thesis aimed to explore the role of food processing, serving sizes, and ultra-processed foods (UPFs) in food-based dietary guidelines and human health. The research included: Investigating the role of serving sizes and consumption frequencies in shaping global dietary guidelines and examining the influence of UPFs; analysing the nutritional characteristics and contaminants in foods processed in home-like versus industrially produced foods; reviewing literature on the health impacts of UPFs; and conducting a randomized controlled trial to assess the effects of incorporating UPFs into a Mediterranean diet on health markers.

Cibi ultra-processati: dalle caratteristiche nutrizionali all'impatto sulla salute umana

Questa tesi di dottorato ha avuto l'obiettivo di esplorare il ruolo del processo alimentare, delle porzioni e degli alimenti ultra-processati (UPFs) nelle linee guida alimentari basate sugli alimenti e nella salute umana. La ricerca ha incluso: l'indagine sull'inclusione delle porzioni e delle frequenze di consumo nella definizione delle linee guida alimentari globali e l'esame della presenza in esse degli alimenti UPFs; l'analisi delle caratteristiche nutrizionali e dei contaminanti negli alimenti lavorati in ambienti domestici rispetto a quelli prodotti industrialmente; la revisione della letteratura sugli impatti sulla salute degli UPFs; e la realizzazione di uno studio controllato randomizzato per valutare gli effetti dell'inclusione degli UPFs in una dieta mediterranea sui marker di salute.

Key words: Ultra-processed foods, Mediterranean Diet, Nutrition, Health, Home-made, Industry-made, serving size, consumption frequencies, food based dietary guidelines.

1. Introduction

In accordance with the PhD thesis project previously described, this oral communication reports the main results of the following four activities directed to advancing our understanding of the role of food processing in food-based dietary guidelines, the importance of serving size in a healthy diet, and the impact of ultra-processed foods (UPFs) on human health. All activities are described as follows:

A1) The first-year activity focused on exploring the importance of serving sizes and consumption frequencies in shaping national food-based dietary guidelines globally. Additionally, the role of UPFs and food processing was examined to determine their influence on the development and implementation of these guidelines worldwide.

A2) The second-year activity focused on investigating the nutritional characteristics and contaminants derived from cooking in foods processed in different environments. Here, the nutritional profile and contaminant levels such as acrylamide and furosine in foods were analysed, comparing home-like food preparation to industrial food processing. This investigation aimed to assess how different processing methods affect the nutritional quality and safety of the food we consume.

A3) The third-year activity consisted in reviewing literature on ultra-processed foods and their impact on human health. This activity involved an extensive review of existing literature on the health implications of UPFs, focusing on evidence regarding their role in modulating human health. Key findings from intervention studies were synthesized to better understand the relationship between UPFs and various health outcomes.

A4) Final third year activity consisted in the design and implementation of a randomized controlled cross-over trial to assess the effects of incorporating 5 portions of UPFs daily into a Mediterranean diet (MD). Specifically, the MD was maintained, with UPF consumption kept at a level consistent with typical intake levels reported in the literature for Italians (approximately 20% of total energy intake). The trial aimed to evaluate the potential impact of a MD diet with 5 portions of UPF on various health markers after 12 weeks intervention, and compare these effects on cardiometabolic markers with those of a traditional MD.

2. Healthy diets: a matter of quality and quantity

The debate over whether healthy diets are primarily about quality or quantity remains central in nutritional science. While much attention has focused on food composition, growing evidence underscores the importance of how much and how often we eat. Portion size, eating frequency, and total food intake are critical factors in the development of non-communicable diseases (NCDs). Excess caloric intake is the most direct contributor to weight gain and obesity (Afshin et al., 2019). This is supported by robust epidemiological and experimental evidence (Sadler et al., 2021; Ibrahim et al., 2021; WHO, 2024). The rise in portion sizes, particularly in high-income societies, has closely paralleled the increase in obesity over recent decades (Embling et al., 2023; Papagiannaki &

Kerr, 2024). The Global Burden of Disease Study ranks high BMI among the leading global risk factors for early death and disability, an outcome driven largely by chronic caloric surplus (GBD, 2019). Ultimately, the health impact of a diet lies not in individual foods, but in broader dietary patterns and behaviors (Martini et al., 2023). In this context, scientific evidence has consistently demonstrated the beneficial effects of a Mediterranean dietary pattern, which emphasizes plant-based foods, moderate portions, and balanced consumption, supporting long-term health and prevention of NCDs (Dernini et al, 2015; Dinu et al, 2017).

3. Ultra processed foods in healthy eating patterns

The Mediterranean Diet (MD) is well recognized for its health and sustainability benefits due to its emphasis on plant-based, minimally processed foods (Dernini et al., 2015; Dinu et al., 2017). However, traditional MD components are increasingly being replaced by ultra-processed foods (UPFs), even in Mediterranean countries (Marino et al., 2021). Since 2009, nutrition science has shifted from focusing solely on nutrients to also considering the degree of food processing (Monteiro et al., 2010; Koios et al., 2022; Sadler et al., 2021). High UPF consumption is linked to obesity and other non-communicable diseases (Herforth et al., 2019). Among various classification systems, NOVA is the most discussed, categorizing foods based on processing rather than nutrient content (Monteiro et al., 2019). While adopted in many food-based dietary guidelines, NOVA has been criticized for ambiguities, possible misclassification, and lack of cultural sensitivity (Visioli et al., 2022). Some UPFs often contain additives and excess salt, sugar, or fat, and may negatively impact health through mechanisms like satiety disruption and gut microbiota alterations (Pagliai et al., 2021; Song et al., 2023). Yet only one clinical trial has explored UPFs' health effects, with limitations (Hall et al., 2019). From a sustainability perspective, UPFs may conflict with FAO and WHO principles of sustainable diets, which call for nutritional adequacy, cultural relevance, and low environmental impact (FAO & WHO, 2019). While the MD aligns well with these principles (Dernini et al., 2015), the health and environmental consequences of incorporating UPFs into the MD are still underexplored, indicating a need for further research.

4. Materials and Methods

4.1 Serving Size and Consumption Frequencies in Food-Based Dietary Guidelines: A Global Scoping Review

A global scoping review of the literature was conducted relative to the inclusion of information on serving sizes and frequency of consumption in national FBDGs. Data were sourced mainly from the FAO FBDGs repository (Feb 2023–Mar 2024) and supplemented by web searches. The study categorized dietary guideline data as quantitative, semi-quantitative, or non-quantitative and grouped foods into plant-based, animal-based, or "other" categories (i.e. water, alcoholic beverages, salt, fats, and sugars and sugary foods). Guidelines were included if they targeted the general population, were national, and available in English or Italian. Information was classified into qualitative, semi-quantitative, and quantitative types. Three authors independently collected and cross-verified the data for accuracy.

4.2 Food processing in worldwide dietary guidelines: status and future directions

This review used the FAO's FBDG online repository as the main data source, updated in Sept 2024, and supplemented by checks on official health websites. Only the most recent national guidelines aimed at the general healthy adult population, in English, French, Italian, or Spanish, were included. For each guideline, details like publication year, language, and availability of a search function were recorded, and countries were classified by income level. The analysis focused on the inclusion of food processing concepts, using specific keywords identified through digital or manual review. Mentions of food processing were classified by type (explicit or implicit) and context (nutritional, non-nutritional, or environmental). Data extraction was done independently by three reviewers and cross-checked for accuracy

4.3 Home-made vs industry-made: nutrient composition and content of potentially harmful compounds of different food products

Four widely consumed industrial food products: plum cake, fish sticks, tomato sauce, and cereal bars, were reproduced under standardized domestic conditions, closely following the ingredient lists and processing methods used in their commercial production. The home-made versions were then systematically analyzed and compared to their industrial counterparts to evaluate differences in nutritional composition, including energy, macronutrients, dietary fiber, and salt content. In addition, targeted chemical analyses were performed to quantify the presence of potentially harmful compounds formed during processing, such as acrylamide and Maillard Reaction products (e.g., furosine, CML, CEL, and HMF). This approach aimed to assess how industrial vs. home-prepared versions differ in both nutritional quality and exposure to processing-induced contaminants**.**

4.4 Ultra-Processed Foods and Health: What Do We Know from Intervention Studies? A Systematic Review

of Literature

This review is currently underway and follows the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. A systematic search was conducted on November 29, 2024, using three academic databases: PubMed®, Scopus, and Embase. The search included terms such as "UPF", "ultraprocessed", and "ultra-processed", combined with "trial or study", and was restricted to studies published between 2009 and 2025. The objective is to identify clinical trials and randomized controlled trials that mention ultra-processed food (UPF) terminology in their titles, abstracts, or keywords, ensuring a comprehensive and current overview of available evidence. The focus is on dietary intervention studies assessing the health impacts of UPF consumption compared to either a control diet (often the participant's habitual diet) or diets composed of minimally or non-ultra-processed foods. Eligible studies must involve human participants of any age, sex, BMI, or health status, and report outcomes related to health markers. Exclusion criteria include non-English publications, studies unrelated to health outcomes, and those not meeting the intervention model (i.e., not clinical dietary trials). Data extraction is being conducted independently by three reviewers and cross-checked for accuracy. The review has been initiated and is currently being registered in PROSPERO.

4.5 Consumption of Ultra-Processed Foods in the Context of the Mediterranean Diet: Effects on Human and Planetary Health. The PROMENADE Study

Fifty overweight adults (25 per center: RU1 - University of Milan, and RU2 - University of Florence) were recruited and screened for eligibility via medical history and clinical examination. Inclusion criteria included age >18, BMI 25.0–29.9 kg/m², and at least one cardiovascular risk factor per ESC guidelines. Exclusion criteria involved severe or unstable health conditions, pregnancy/lactation, recent antibiotic or supplement use, and restrictive diets. Following ethics approval, participants were enrolled in a 7-month randomized controlled crossover trial, which included two 3-month dietary interventions (HIGH UPF MD vs. LOW UPF MD) separated by a 1-month wash-out and preceded by a 2-week run-in phase. Block randomization (1:1) assigned participants to: HIGH UPF MD: Mediterranean Diet with 5 daily servings of ultra-processed foods (UPFs) per NOVA classification, LOW UPF MD: Mediterranean Diet with minimally processed substitutes, maintaining typical Italian UPF intake based on published data. Both diets were isocaloric, matched for macronutrient distribution (50–55% carbohydrates, 25–30% fat, 15–20% protein), and tailored to Italian dietary habits. Weekly menus and educational materials were provided. At each timepoint (T0, T1, T2, T3), participants underwent anthropometric and body composition measurements, fasting biological sample collection, and dietary assessments, including the MediLITE questionnaire to evaluate adherence to the Mediterranean Diet (Sofi et al., 2017).

5. Results and Discussion

5.1 Serving Size and Consumption Frequencies in Food-Based Dietary Guidelines: A Global Scoping Review

Totally 100 FBDG documents were identified across world regions, and 60 FBDGs were included in the final analysis, revealing major differences in how countries define and present dietary advice. Some provide detailed quantitative recommendations (N=35), others use semi-quantitative (N=16) or purely qualitative guidance (N=9). Serving sizes and consumption frequencies vary widely, influenced by cultural practices and local foods. Many FBDGs lack standard units or clarity, some use vague visuals or inconsistent measures. Even within countries, documents sometimes contradict themselves. Only a few, like Saint Kitts and Nevis, provide precise portion manuals, though these are often not part of the official guidelines. This lack of harmonization complicates consumer understanding, health policy, and nutrition labelling. To improve global nutrition communication, there is a clear need for standardized, regionally adapted benchmarks for serving sizes and food frequency recommendations.

5.2 Food processing in worldwide dietary guidelines: status and future directions

Of 100 FBDGs, 75 were analyzed, mostly from upper-middle and high-income countries. While 89% addressed food processing, only 25% mentioned "ultra-processed" or "highly processed" foods, and just 9% referenced the NOVA classification. Most focused on limiting processed meats (53%) and promoting fresh foods (52%), but few (8%) discussed environmental impacts. Regional and income disparities emerged, with low-income countries more likely to mention UPFs. Use of processing-related terms has grown over time, though "industrial" is declining. Terms like "fast food" and "junk food" were inconsistently applied and rarely linked to environmental concerns.

5.3 Home-made vs industry-made: nutrient composition and content of potentially harmful compounds of different food products

Nutritional analysis revealed identical compositions for all the four food pairs under study. Significant difference in fat content ($p < 0.005$) was found for fish sticks, but this was related to the cooking methods used (oven vs. pan), rather than the processing settings. Homemade foods are not immune to the formation of harmful compounds during cooking, as confirmed by Michalak et al., (2016). These findings challenge the perception that HM foods are inherently healthier or safer than industrial foods. While industrial processing uses additional ingredients and

methods, this does not necessarily result in poorer nutritional quality or higher levels of harmful compounds. It is possible to replicate industrial food at home with similar nutritional properties.

5.4 Ultra-Processed Foods and Health: What Do We Know from Intervention Studies? A Systematic Review of the Literature

A total of 11 articles emerged but considering that 4 are secondary analyses of related publications, there are 7 unique studies, a total of 777 participants were involved in the intervention studies included in this review. The main outcomes assessed in the studies included in this review were: energy intake (n=5), hunger (n=2), satiety (n=2), eating rate (n=2), satiety-related hormones (n=2), weight (n=8), BMI (n=3), body composition (n=7), lipid profile (n=4), glycemic profile (n=5), blood pressure (n=3), and liver function parameters (n=2). In general, it is not possible to draw a conclusion since data are discordant and heterogeneous. UPF consumption may promote higher calorie intake, weight gain, and unfavorable changes in body composition and possibly liver function. However, evidence is inconsistent, especially in studies with short durations, poor adherence, or indirect effects (e.g., school meals). Combined strategies (reduced UPF, increased fiber, physical activity, and Mediterranean diet) showed the most consistent health benefits.

5.5 Consumption of Ultra-Processed Foods in the Context of the Mediterranean Diet: Effects on Human and Planetary Health. The PROMENADE Study

Participants from the first intervention arms showed no significant inter-group differences for anthropometric parameters (body weight, BMI, waist and hips circumference, lean and fatty mass), and biochemical parameters such as glycaemia, hematocrit, TSH, and triglycerides. Reduction for both LDL and HDL-cholesterol was observed in the Low UPFs group (LDL 145 ± 31 mg/dL to 134 ± 22 mg/dL, p=0.02; HDL 57 ± 15 mg/dL to 51 ± 10 mg/dL, p=0.02), while LDL cholesterol did not significantly decrease in the High UPFs group (136 ± 27 mg/dL to 132 ± 29 mg/dL, p=0.52). Insulin sensitivity improved in both diets. Preliminary results suggested that including UPFs in an isocaloric MD did not compromise key cardiometabolic markers. However, a conclusion will be drawn once the full samples size is recruited and all volunteers complete both intervention arms. Finally, analysis of gut microbiota and inflammatory markers will be necessary to clarify UPFs' role within a MD.

6. Conclusions and Future Perspectives

Recent Global Burden of Disease findings show that rising non-communicable diseases (NCDs) are more linked to low intake of protective foods than to "unhealthy" foods. Thus, the concept of a healthy diet should move beyond labeling foods as simply "good" or "bad." Food-Based Dietary Guidelines emphasize not only what we eat but also how much and how often. Since 2009, food classification has evolved from focusing solely on nutrients to including the degree of processing. Food processing ensures food security and safety; industrial and homemade foods with the same ingredients often have similar nutritional profiles, challenging the idea that homemade foods are always healthier. However, confusion remains about the role of ultra-processed foods (UPFs). The Mediterranean Diet (MD), known for health benefits, is typically plant-based and minimally processed. Yet recent findings show that selected UPFs can be included in the MD without harming health. A 12-week intervention found no negative health effects from UPFs within the MD. These results highlight the importance of overall dietary patterns rather than considering single foods and their processing levels. Future research should explore the complex role of UPFs in healthy diets to inform better food policy and public health strategies.

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In Vitro Evaluation of Probiotic, Enzymatic, and Botanical Supplements on Gut Microbiota and Digestion

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This PhD research evaluated nutraceutical supplements for gastrointestinal (GI) health using in vitro models. The three main studies focused on (1) the spore-forming probiotic *Heyndrickxia coagulans* LMG S-24828, (2) fungal enzyme blends to improve digestion, and (3) the impact of botanical extracts on the gut microbiota. Results showed that the probiotic is genetically stable, safe, and able to germinate in GI conditions. Enzyme blends improved the digestion of proteins, fats, carbohydrates, and lactose. Botanical extracts selectively influenced certain bacteria without disrupting the microbial balance. These findings support the development of effective and microbiota-compatible supplements.

Valutazione in vitro di integratori probiotici, enzimatici e botanici su microbiota intestinale e digestione

Questa ricerca ha valutato integratori per la salute gastrointestinale utilizzando modelli in vitro. I tre principali studi si sono concentrati su (1) il probiotico sporigeno *Heyndrickxia coagulans* LMG S-24828, (2) miscele enzimatiche fungine per migliorare la digestione, e (3) l'impatto di estratti botanici sul microbiota intestinale. I risultati hanno mostrato che il probiotico è geneticamente stabile, sicuro e in grado di germinare nelle condizioni gastrointestinali. Le miscele enzimatiche hanno migliorato la digestione dei principali macronutrienti. Gli estratti hanno influenzato selettivamente alcuni batteri senza alterare l'equilibrio microbico. Questi dati supportano lo sviluppo di integratori efficaci e compatibili con il microbiota.

Keywords: Probiotics; Spore-forming bacteria; Fungal enzyme blends; In vitro digestion; Gut microbiota; Botanical supplements; Microbiome compatibility.

1. Introduction

In recent years, the relationship between diet, digestive function and gut microbiota has become a major focus in human health and nutrition research. There is growing scientific and commercial interest in functional foods and supplements designed to support digestion, modulate the gut ecosystem and provide health benefits without disturbing microbial balance. Developing effective and microbiome-compatible products requires reliable experimental models and thorough evaluation of how these supplements behave under physiologically relevant conditions. This PhD project evaluated three types of interventions related to gut health and microbiome. These were spore-forming probiotics, fungal enzyme supplements for digestive support and commercial botanical extracts. All studies used in vitro systems, particularly the INFOGEST protocol, which simulates human gastrointestinal conditions and allows reproducible digestion experiments. Additional fecal microbiota incubations and microbial culture assays were performed to assess microbial viability, function and community response. The main goal was to characterize how specific products, *Heyndrickxia coagulans* LMG S 24828, two fungal enzyme blends and three botanical extracts, interact with gut-related processes. The focus was on safety, functional performance during digestion and effects on selected probiotic or commensal strains. The findings contribute to the development of scientifically supported strategies for improving digestive efficiency and maintaining microbial balance through "microbiome-friendly" supplements.

2. Characterization of *Heyndrickxia coagulans* LMG S-24828 as a Spore-Forming Probiotic

The first study focused on the characterization of *Heyndrickxia coagulans* LMG S-24828, a spore-forming bacterial strain marketed as Weizy®. Unlike traditional probiotics such as lactic acid bacteria and bifidobacteria, spore-forming strains offer greater stability and resistance to stress, making them suitable for industrial applications and gastrointestinal delivery. Originally isolated from human feces, the strain underwent genomic and phenotypic evaluation. Whole genome sequencing confirmed its taxonomic identity and absence of plasmids, supporting genetic stability. No transferable antibiotic resistance genes were detected. The presence of acquired (potentially transmissible) antibiotic resistances was also investigated using the ISO 10932 IDF 223:210 protocol recommended by the EFSA. None of the minimum inhibitory concentrations (MICs) determined for strain LMG S-24828 exceeded the EFSA breakpoints for the nine antibiotics recommended in the EFSA guidelines, including vancomycin. Functional assays showed that the strain does not produce D-lactate, does not deconjugate bile salts, and lacks hemolytic activity, meeting current safety standards for probiotic use.

Phenotypic tests also revealed metabolic versatility, including the ability to hydrolyze complex carbohydrates and adhere to intestinal epithelial cells. For example, LMG S-24828 was tested for its ability to utilize mucin by growing it in glucose-free MRS medium supplemented with porcine gastric mucin (~~fig.~~ Figure 1). After 48 hours, the strain showed a significant growth increase ($+0.9 \pm 0.3$ $\log_{10}$ CFU/mL), comparable to growth in the presence of glucose. In contrast, *Lacticaseibacillus rhamnosus* GG, used as a negative control, showed no growth on mucin. Genomic analysis identified four genes encoding glycosyl hydrolases (GH31 and GH42), supporting the strain's ability to degrade mucin, which may help sustain its metabolism during gastrointestinal transit.

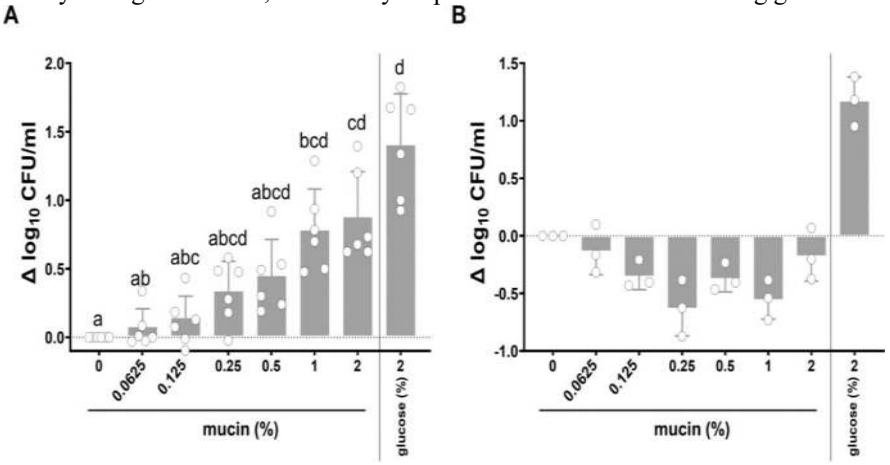


Figure 1 *Modification of viable counts of* Heyndrickxia coagulans *LMG S-24828 (A) and* Lacticaseibacillus rhamnosus *GG (B) after 48 h incubation in MRS broth without glucose, supplemented with porcine gastric mucin. MRS with 2% glucose was used as a positive control. Histograms represent the mean ± standard deviation of data collected from six (A) or three (B) independent experiments. Each dot in the graph indicates the result of a different experiment. Different lowercase letters indicate significant differences at $p < 0.05$ according to the Kruskal–Wallis nonparametric ANOVA with Dunn's multiple comparisons test*

The survival of *H. coagulans* LMG S-24828, as a freeze-dried preparation, during gastrointestinal transit was evaluated using the INFOGEST static in vitro digestion model (Figure 2a). The initial preparation contained only viable endospores, as confirmed by identical viable counts before and after pasteurization. The strain showed strong resistance to digestion, with negligible loss in viability (from 11.18 to 11.15 $\log_{10}$ CFU/g). Additionally, the proportion of pasteurization-sensitive cells increased from 3% to 17%, indicating partial spore germination during the simulation. Spore germination was demonstrated in vitro by incubating the freeze-dried preparation in human fecal samples under anaerobic conditions at 37 °C (Figure 2b), with over 85% of cells germinating within 15 minutes. In a pilot human trial, five healthy adults ingested 6 billion spores daily for three days (Figure 2c). Viable cells were detected in all fecal samples (mean 6.3 ± 0.2 $\log_{10}$ CFU/g), and pasteurization-sensitive cells (10 to 78% of total CFUs) confirmed in vivo germination. Although viable recovery from feces has been reported for a few strains (e.g., *B. animalis* BB12, *L. rhamnosus* GG), data on spore-forming probiotics remain limited. This study provides evidence that *H. coagulans* LMG S-24828 can survive gastrointestinal transit and germinate efficiently, potentially aided by its adhesion to intestinal cells.

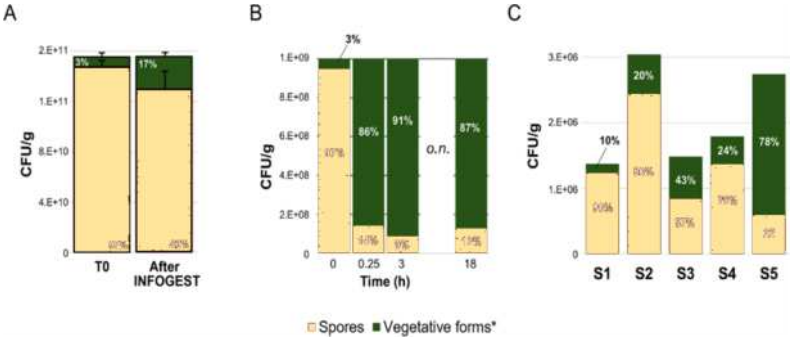


Figure 2 *Survival and germination of* Heyndrickxia coagulans *LMG S-24828 spores in the gastrointestinal tract. A Spores and vegetative forms before and after the INFOGEST simulated gastrointestinal transit. B Spores and vegetative forms determined during incubation at 37 °C in a human fecal sample; o.n., overnight. C Spores and vegetative forms recovered from the feces of five healthy volunteers after 3 days of LMG S-24828 intake. The asterisk symbol (*) indicates vegetative forms have been determined as pasteurization-sensitive units*

These properties indicate that LMG S-24828 has significant potential for use in probiotic formulations, warranting further investigation in clinical trials to fully elucidate its health benefits.

3. Evaluation of Fungal Enzyme Blends for Enhanced Digestion of Convenience Meals

The second study assessed the digestive performance of two fungal enzyme blends, Poolzyme® MULTI and Poolzyme® DAIRY, using a standardized in vitro digestion model. These products are designed to complement human enzymatic activity and are marketed for individuals with functional digestive complaints, such as bloating or lactose intolerance. Both formulations contain protease, lipase and lactase; Poolzyme® MULTI additionally

includes amylase and cellulase and is intended by the manufacturer to be used for a wide range of meals while Poolzyme® DAIRY is specifically optimized for dairy-rich meals. The study employed the INFOGEST protocol to simulate oral, gastric, and intestinal phases of digestion. Two common food matrices were selected to represent realistic dietary challenges: a fast-food hamburger with fries and a frozen cheese pizza. The selection of the two test meals was driven by the need to evaluate enzymatic activity on a food matrix containing a diverse combination of macronutrients. The hamburger with fries represents a meal rich in proteins, fats, and complex carbohydrates, whereas the frozen white pizza was chosen for its lactose and casein protein content, making it ideal for testing the specificity of the Poolzyme ® DAIRY formulation. These convenience meals are commonly consumed in Western countries, including Italy (Scarsi et al., 2024), and their nutritional profile may influence digestion, particularly in individuals with functional dyspepsia (Amerikanou et al., 2023). Each meal was tested under three conditions: digestion with no added enzymes (control), digestion with the enzyme blend at the recommended dose, and digestion with a higher dose. The resulting samples were analyzed either via enzymatic kits (Lactose/Galactose and primary amino nitrogen for the degree of protein hydrolysis) or UHPLC-HR-MS after appropriate extraction phases. Quantitative measurements at the end of the intestinal phase showed that enzyme blends enhanced nutrient release. Protein hydrolysis increased significantly, as indicated by higher levels of total and branched-chain amino acids as well as primary amino nitrogen levels increasing (Figure 3). In the cheese-rich matrix, lactose levels decreased with Poolzyme® DAIRY treatment, confirming effective lactase activity (Figure4). Enzyme addition also improved glucose and free fatty acid release in both meals, indicating enhanced carbohydrate and lipid digestion. These effects were dose-dependent, particularly for lactose degradation and amino acid release. Treated samples showed more complete substrate breakdown than controls, suggesting additive or synergistic effects with endogenous digestive enzymes.

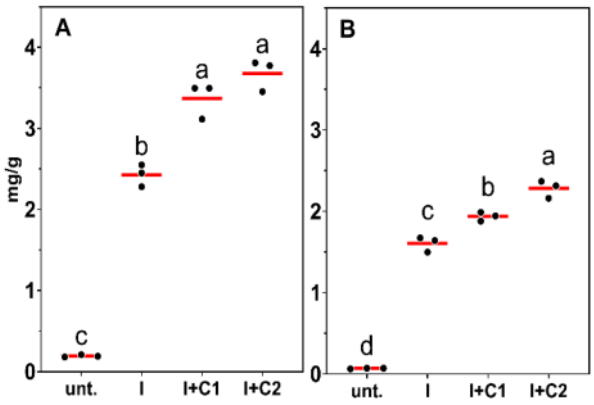


Figure 3 *Overall protein hydrolysis assessed using Primary Amino Nitrogen (PANOPA) Assay, expressed as mg of free terminal nitrogen groups per gram of test meal. The data are shown for the untreated (unt.) meal, INFOGEST (I) with no addition of enzyme blend, and INFOGEST with enzyme blend-treated samples at the low dosage (I+C1) and at the high dosage (I+C2). Panel A presents data for the pizza test meal with the Poolzyme® DAIRY enzyme blend, while panel B presents data for the hamburger meal with the Poolzyme® MULTI enzyme blend. For all conditions, three independent replicates were performed, and the median is shown as a red bar. Statistical significance was assessed using one-way ANOVA followed by Tukey's multiple comparisons test. Post-hoc Compact Lettering Display (CLD) was assigned with a significance threshold of $p < 0.05$. Significant differences between samples are indicated by different lowercase letters*

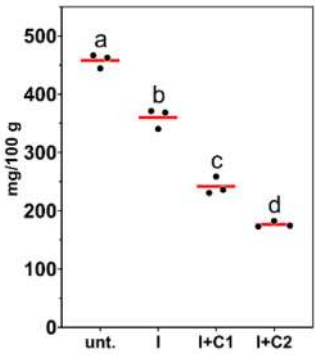
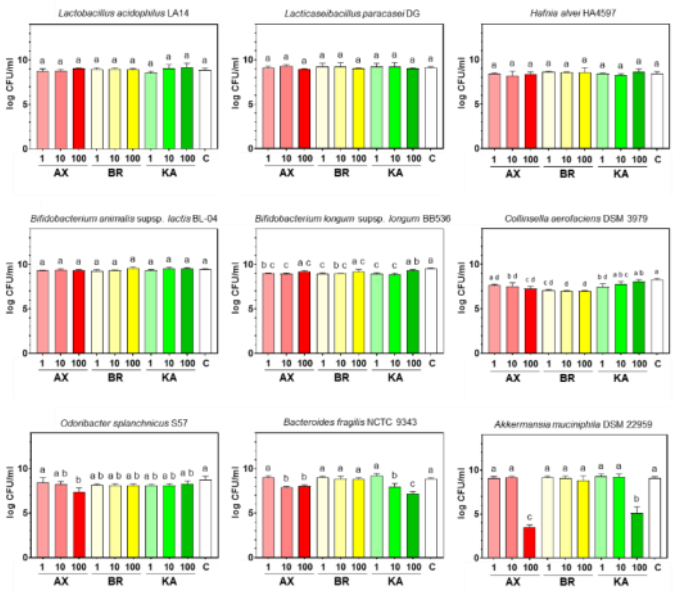


Figure 4 *Lactose quantification in "white" pizza samples. Data are reported as mg of lactose per 100 g of initial food. Un, undigested food (no INFOGEST); I, food after in vitro digestion without Poolzyme® DAIRY; I+C1, food after in vitro digestion in the presence of the lowest amount of Poolzyme® DAIRY (one capsule corresponding to 300 mg of Poolzyme® DAIRY); I+C2, food after in vitro digestion in the presence of the highest amount of Poolzyme®two capsules corresponding to 600 mg of Poolzyme® DAIRY). Each condition has been tested in three independent experiments using aliquots of the same meal. Statistical significance was assessed using one-way ANOVA followed by Tukey's multiple comparisons test. Post-hoc Compact Lettering Display (CLD) was assigned with a significance threshold of $p < 0.05$. Significant differences between samples are indicated by different lower-case letters*

This study strengthens the scientific foundation supporting microbial enzyme supplementation for digestive enhancement. Both Poolzyme® MULTI and Poolzyme® DAIRY significantly improved macronutrient digestion under simulated gastrointestinal conditions. Notably, these enhancements were achieved without modifying the digestive model parameters, emphasizing the intrinsic efficacy of the enzyme blends. Future research should explore enzyme supplementation in diverse dietary contexts, including plant-based diets with high fiber or antinutritional factor content, to extend the applicability of this approach beyond the tested meal types. Controlled clinical trials remain essential to translate in vitro findings into tangible gastrointestinal health benefits.

4. Impact of Botanical Supplements on Gut Microbiota and Selected Bacterial Strains

The third study investigated the effects of three commercial botanical supplements: Kalita (bergamot secondary



juice extract), Bromeyal (pineapple stem extract), and Axtragyl (Astragalus root extract) on the human gut microbiota using in vitro models. These products, used in dietary supplements, contain bioactive compounds such as polyphenols and proteolytic enzymes that may influence microbial populations. Antimicrobial activity was first assessed in monocultures of nine commensal or probiotic strains, including *Lacticaseibacillus paracasei* DG, *Hafnia alvei* HA4597, *Bifidobacterium longum BB536*, *Akkermansia muciniphila* DSM22959, *Collinsella aerofaciens* DSM3979 and *Odoribacter splanchnicus* S57 (Figure 5). Only minimal, strain-specific inhibition was observed at a high, non-physiological concentration of 100 mg/mL. The most affected bacteria were *Akkermansia muciniphila*, *Bacteroides fragilis*, and *Collinsella aerofaciens*.

Figure 5 *Impact of botanical extracts on the growth of selected human gut bacterial strains at varying concentrations (1, 10, and 100 mg/mL). The botanical extracts evaluated include Axtragyl® (AX), Bromeyal® (BR), and Kalita® (KA). Bacterial growth was quantified by enumerating colony-forming units (CFU) on agar plates, expressed as the decimal logarithm (log_{10} CFU). The control (C) represents cultures incubated in media devoid of botanical additives. Each bar denotes the mean ± standard deviation derived from three independent experiments. Distinct letters above the bars signify statistically significant differences ($P < 0.05$), as determined by one-way ANOVA followed by Tukey's post hoc test*

To evaluate broader microbiota responses, each botanical extract, both in native and digested form (using the INFOGEST protocol), was incubated with human fecal slurries under anaerobic conditions for 48 hours. 16S rRNA gene profiling revealed no significant changes in alpha-diversity (within-sample richness; fig. 6) or beta-diversity (between-sample composition), indicating that the overall structure and diversity of the microbial community remained stable.

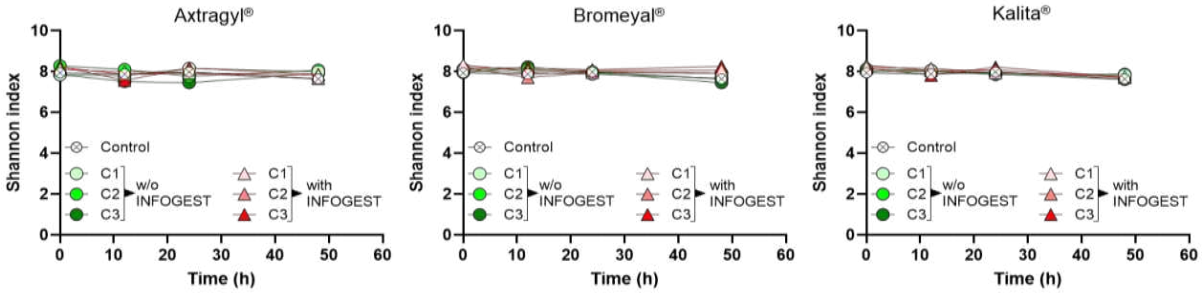


Figure 6 *α-diversity analysis using the Shannon index of fecal samples incubated with three botanical ex-tracts (Axtragyl®, Bromeyal®, and Kalita®), with or without INFOGEST pre-treatment. Extracts were added at concentrations of 50 (C1), 500 (C2), and 5000 (C3)*

Despite the absence of broad community shifts, selective modulation of specific taxa was observed (Figure 7). Kalita and Axtragyl were associated with an increase in *A. muciniphila*, a species linked to gut barrier health. Bromeyal consistently reduced the abundance of *C. aerofaciens* in both community incubations and monoculture assays. Digested Axtragyl also led to a minor reduction in *Collinsella* spp. Importantly, keystone species such as *Faecalibacterium prausnitzii* remained unaffected.

Overall, these findings suggest that Kalita, Bromeyal, and Axtragyl are microbiota-friendly botanical ingredients. While they do not disturb overall microbial diversity or essential taxa, they can exert targeted effects on specific gut bacteria. This selective modulation may contribute to their physiological activity and supports their use in functional foods and dietary supplements.

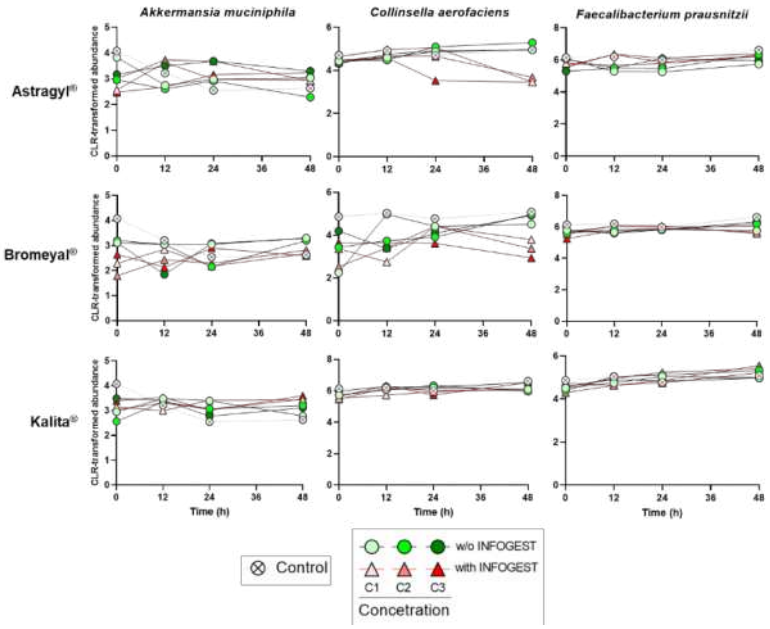


Figure 7. *Impact of botanical preparations on the relative abundance of selected bacterial species in the fecal suspension. Extracts were applied at 50 (C1), 500 (C2), and 5000 (C3) mg per 100 g of fecal material. Abundances are shown as centered log-ratio (CLR) transformed read counts. Each bo-tanical was tested both in its native form (w/o INFOGEST) and after simulated in vitro digestion using the INFOGEST protocol (w/ INFOGEST).*

5. Conclusions and Future Perspectives

This PhD research employed standardized and mechanistically informed in vitro models to evaluate microbiome-related products under conditions simulating human digestion and gut environments. The studies addressed three key areas: the viability and functional characteristics of a spore-forming probiotic strain, the digestive efficacy of fungal enzyme blends, and the selective microbiota modulation induced by commercial botanical supplements. The first study confirmed that *Heyndrickxia coagulans* LMG S-24828 is genetically stable, safe, and able to germinate during gastrointestinal transit. These features make it a promising candidate for probiotic formulations requiring resilience to processing and digestive conditions. The second study demonstrated that fungal enzyme blends improve the breakdown of proteins, fats, carbohydrates, and lactose in convenience meals. The observed effects were dose-dependent and complementary to endogenous digestive enzymes, supporting their potential use for individuals with impaired digestion or reduced nutrient absorption. The third study revealed that the botanical supplements tested do not alter overall gut microbiota diversity or structure but selectively influence specific bacterial taxa. This suggests their potential for targeted microbiota modulation without causing dysbiosis, an important factor in designing microbiome-compatible supplements. Together, these findings support the development of functional supplements that are both effective and compatible with the gut microbial ecosystem. The use of the INFOGEST digestion model and fecal incubation assays were essential in assessing digestive performance and microbial safety. Future work should focus on translating these in vitro results into clinical trials and on investigating complex microbial ecosystems using multi-donor fecal models. This research provides practical methodologies and reference data for designing evidence-based microbiome-supportive dietary interventions.

6. Selected References

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Genomic characterization of Lactic Acid Bacteria strains for the development of a novel psychobiotic product

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This PhD thesis explored the indigenous microbial communities of typical Italian fermented products to design novel microbial consortia with psychobiotic properties to realize a plant-based psychobiotic yogurt able to transfer neurotransmitter-producing strains to the human gut, opening new avenues for microbiome modulation for the benefit of mental health and linking health promotion to the use of a sustainable product.

Caratterizzazione genomica di ceppi di batteri lattici per lo sviluppo di un nuovo prodotto psicobiotico

Questa tesi di dottorato ha esplorato le comunità microbiche autoctone di prodotti fermentati tipici italiani per progettare nuovi consorzi microbici con proprietà psicobiotiche e per realizzare uno yogurt psicobiotico a base vegetale in grado di trasferire ceppi produttori di neurotrasmettitori all'intestino umano, aprendo nuove strade per la modulazione del microbioma a beneficio della salute mentale e collegando la promozione della salute all'uso di un prodotto sostenibile.

Key words: Neurotransmitters, psychobiotic, comparative genomic analysis, fermented foods.

1. Introduction

In accordance with the PhD thesis project previously decribed (Calvanese, 2023), this oral communication reports the main results of the following four activities directed to:

(A1) comparative genomic analysis of genomes available in public databases, to identify the LAB species more promising for psychobiotic activity, supporting the isolation;
(A2) the genome analysis of selected LAB strains isolated from traditional Italian FFs for the presence of microbial genes/pathways related to the production of neurotransmitters and neuroactive molecules (Berding et *al*., 2023).
(A3) in vitro-test for neurotransmitter production of most promising strains in accordance with genomic analysis;
(A4) Growth performance in plant-based matrices and development of plant-based yogurt-like with benefit on mental health.

2. Gut-brain axis and Psychobiotics

Improving human health through modulation of microbial interactions during all phases of life is an evolving concept that is increasingly important for consumers, food manufacturers, health-care professionals and regulators. In the field of mental health, it has recently been shown that lactic acid bacteria (LAB) produce neuroactive molecules, capable of modulating mood and cognitive functions in humans (psychobiotics). Fermented foods (FF) can be considered as a still unexplored microbial reservoir of beneficial microbes, which can positively influence human mental health, releasing molecules with the potential to modulate microbiome-gut-brain axis pathways. Indeed, it has recently been suggested that a diet rich in fermented foods may have a positive impact on stress (Berding et al., 2023). The growing volume of genomic information may facilitate systematic efforts to determine the metabolic pathways that can lead to the achievement of desired psychobiotic metabolites. Therefore, Table 1 reports the main neuroactive molecules produced by bacteria in the human intestine, including their precursors and their regulatory functions (*Casertano et al.,* 2022).

Table 1 Representative list of neurotransmitters produced from bacteria within the human gut, precursors, and their regulatory functions.

Neuroactive compound	Precursors	Genus	Regulatory function
Gamma-aminobutyric acid (GABA)	Glutamate	*Lactobacillus, Bifidobacterium, Bacteroides*	Stress responsiveness, anxiety
Acetylcholine	Choline	*Lactobacillus, Bacillus*	Encoding of new memories
Dopamine	Tyrosine	*Lactococcus, Lactobacillus, Streptococcus*	Motivational decision-making
Serotonin	Tryptophan	*Lactococcus, Lactobacillus, Streptococcus*	Modulation of intestinal secretion and motility; Brain development

3. Materials and Methods

For comparative genomic analysis, genes related to the biosynthesis of neuroactive molecules and their precursor are selected from the available scientific literature (Casertano et *al.*, 2022) and genomes (n=3998) belonging to 33 LAB species were selected and downloaded from the NCBI public database. For each of these genomes, the genes were predicted using the PROKKA software and were mapped against previously selected genes of interest using DIAMOND software. Finally, the results were statistically processed with the R Studio software.
LAB isolation was carried out from i) 20 samples of traditional Italian cheeses; ii) 80 samples of fermented table olives from all over the Mediterranean basin; iii) 10 sourdough samples. Seventy-four LAB strains were isolated, identified by MALDI-TOF Biotyper and their genomes were sequenced and analyzed according to the same pipeline used in the comparative genomic analysis. Strains with the most promising profiles from the functional analysis, were inoculated and grown in MRS medium broth at 37°C. A fresh overnight culture of the strains were individually subcultured (1%, v/v) in MRS medium at their optimal temperature, supplemented with an amino-acid precursor of the neurotransmitter. Cells were removed by centrifugation (4000 rpm, 4°C, 10 min) and the supernatant was used for metabolomic analysis. The most promising strains from the metabolomic analysis were used as starters to produce a vegetal yogurt, following the biotechnological protocol of *Pontonio et al.,* 2020. The base of the yogurt-like was prepared by mixing flour and water in a ratio of 1:4. The flours were mixed together in a ratio of 2:1:1 (rice: lentils: peas). The subsequent gelatinization phase was carried out at 80° C for 15 minutes. The formulation with the best technological performance was selected as final product with benefit on mental health.

4. Results and Discussion

4.1 Comparative genomic analysis for screening potentially psychobiotic species.

From comparative genomic analysis we can observe a presence of species-specific patterns as shown in Figure 1. We want to highlight:

- the highest occurrence of genes related to the production of GABA (γ-Aminobutyric acid) in *Levilactobacillus brevis*, *Lactiplantibacillus plantarum* and *Lactiplantibacillus paraplantarum* species;
- the highest occurrence of genes related to metabolism of tryptophan (leading to the production of some neuroactive metabolites such as serotonin) in the genera *Lacticaseibacillus*, *Lentilactobacillus* and *Lactiplantibacillus;*
- genes related to conjugate linoleic acid production, propionate and taurine production were present in most of the species included in the dataset;
- the occurrence related to the production of catecholamines, histamine and imidazole propionate production was low in all species included in the dataset.

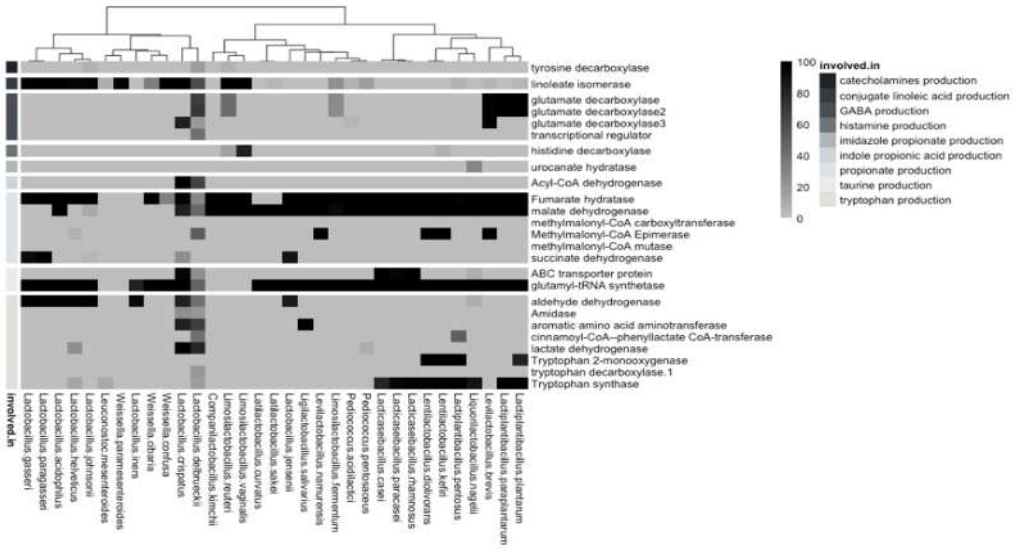


Figure 1 *Prevalence (%) of genes related to the production of neuroactive molecules in LAB genomes downloaded from public databases*

4.2 Developing a collection of Lactic Acid Bacteria from typical Italian FFs

In accordance with the comparative genomic analysis, the strains with the highest potential for psychobiotic activities fall into the species of *Lactiplantibacillus plantarum* (B1, B95, HA2, TO02, TO05, TO06, TO11, TO15, TO22), *Lactiplantibacillus pentosus* (E2), *Lactobacillus delbrueckii* (H2), *Levilactobacillus brevis* (TO10), *Lentilactobacillus diolivorans* (A1, B92), and *Lentilactobacillus kefiri* (KL) as shown in Figure 2. These selected strains will be tested in vitro.

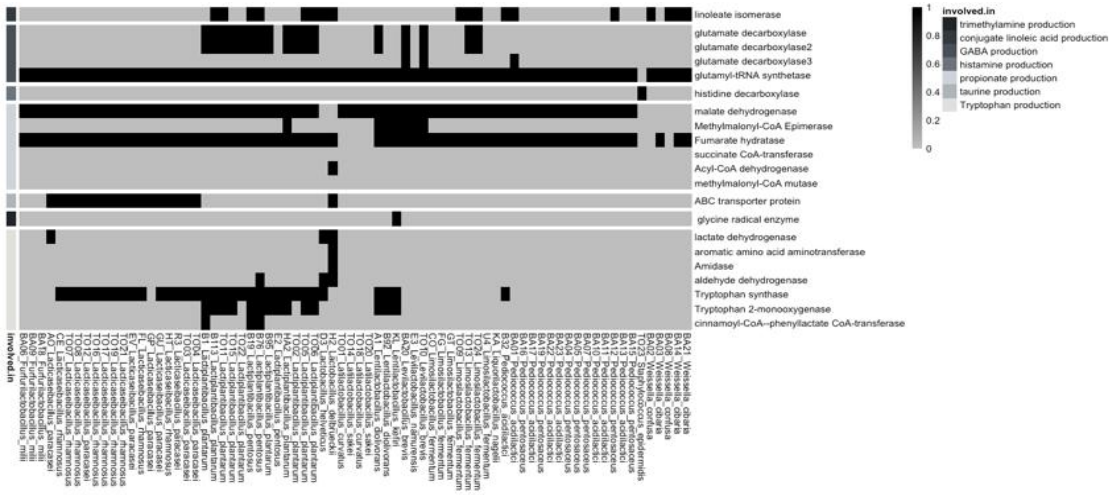


Figure 2 *Prevalence of genes related to the production of the main neuroactive molecules in strains isolated from typical Italian FFs.*

4.3 Metabolomic analysis for neurotransmitter production of most promising strains

Metabolomic analysis showed that only a few strains reported the production of catecholamines. *L. plantarum* B1 was the strain with the highest concentration of catecholamines (adrenaline, noradrenaline and dopamine). *L. plantarum* TO05 showed a higher concentration of adrenaline and noradrenaline, but no dopamine was detected. In contrast, only dopamine was detected in *L. plantarum* TO06.
As for acetylcholine, it was detected in all strains in a range between 1.89 mg/L (*L. fermentum* TO24) and 10.47 (*L. plantarum* B1).
GABA production was detected in 5 strains (*L. plantarum* E2, *L. fermentum* TO24, *L. diolivorans* B92, *L. brevis* TO10, *L. brevis* BR126). In particular, *L. brevis* TO10 reported the highest concentration (0.82 mg/L), while *L. brevis* BR126 reported the lowest concentration (0.22 mg/L). Serotonin production was not detected in any strain, except *L. brevis* BR128, with a concentration of 0.12 mg/L (Figure 3).

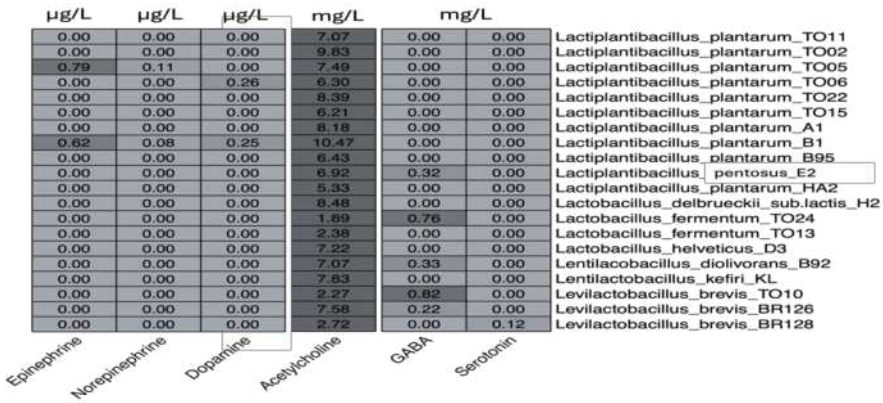


Figure 3 *In vitro tests on the most promising strains for neurotransmitters production by HPLC analysis*

4.4 Metabolomic analysis of the selected microbial consortia in a plant-based matrices

From the results obtained from genomic analysis, in vitro tests, and fermentation trials, the strains 144 - B92 - BG153 - LA10B were tested in a plant-based matrix. These strains were inoculated in two different time/temperature conditions: i) 16 hours/30°C ii) 24 hours/30°C. Furthermore, respecting the two conditions mentioned above and independently from each other, the single strains, combinations of two, three and four strains were inoculated to highlight any differences. Detection of GABA and Acetylcholine by HPLC analysis on the inoculated strain combinations in a plant-based matrices are shown in Figure 4 and Figure 5 respectively.

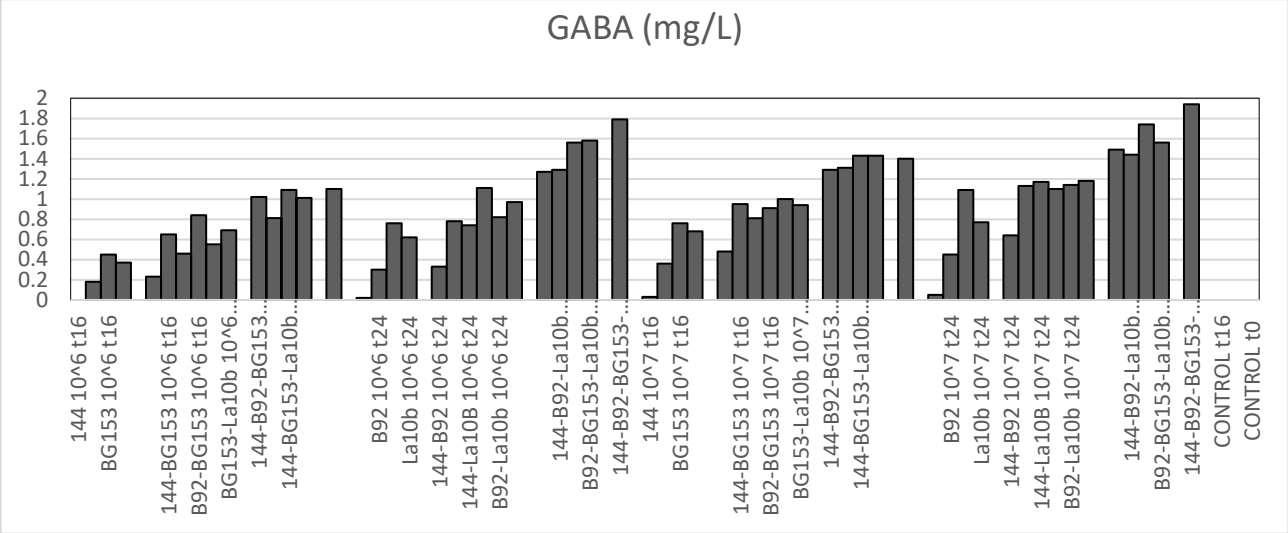


Figure 4 *Detection of GABA on the inoculated strain combinations in a plant-based matrices to detect neurotransmitters production by HPLC analysis.*

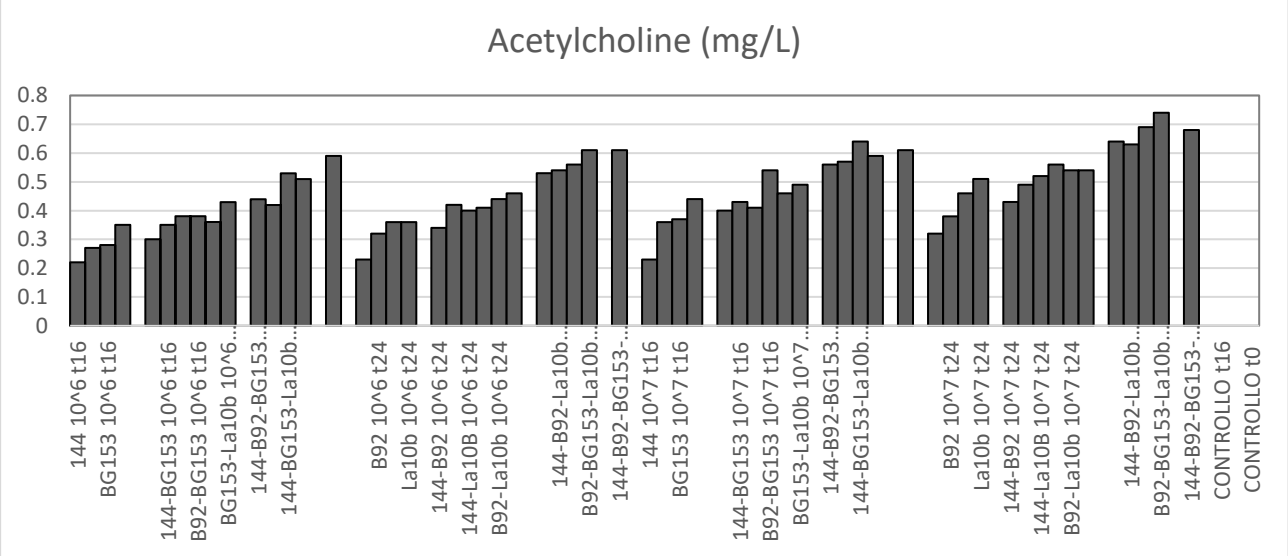


Figure 5 *Detection of Acetylcholine on the inoculated strain combinations in a plant-based matrices to detect neurotransmitters production by HPLC analysis.*

The combination of strains 144-BG153-LA1OB inoculated at concentrations of 10^7 CFU/ml for 24h (T24), showed the highest concentrations of acetylcholine and GABA in a plant-based matrix.

5. Conclusions and Future Perspectives

In recent years, the trillions of microorganisms residing in the gut, known as the microbiota, have been identified as a crucial regulator of gut.brain axis, the bidirectional communication between the digestive system and the nervous system. Gut microbes can synthesize key brain neurotransmitters like GABA, acetylcholine, serotonin, and dopamine, and can also convert dietary precursors (glutamate, choline, tryptophan and tyrosine) into these neurochemicals. It is well known that validating probiotics is complex due to a lack of standardization in traditional *in vitro* and *in vivo* test batteries, which often don't accurately reflect real-world efficacy or safety, and strains can use multiple mechanisms for health benefits, making their function hard to predict. Advances in sequencing technology, coupled with sophisticated bioinformatics tools, provide a solid foundation for exploring the gut microbiota. Combining these techniques, both at the community and strain levels, with 'omic approaches, has greatly advanced our understanding of microbial mechanisms. The purpose of this study was to demonstrate how metagenomic and genomic approaches can facilitate the selection and design of psychobiotics for clinical use, deepening the understanding of how microbes can influence the nervous system and host behavior.

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Study of the Effects of Plant-Derived MicroRNAs on the Human Gut Microbiota

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PhD project addresses the potential of plant-derived miRNAs as mediators of cross-kingdom communication by characterizing their stability during digestion, predicting their bacterial gene targets, and evaluating their functional effects on *Lacticaseibacillus rhamnosus* GG (LGG) physiology and gut microbial metabolism.

Studio degli effetti dei microRNA di origine vegetale sul microbiota intestinale umano

Il progetto di dottorato affronta il potenziale dei microRNA di origine vegetale come mediatori della comunicazione intraregno, attraverso la caratterizzazione della loro stabilità durante la digestione, la predizione dei geni target batterici, e la valutazione dei loro effetti funzionali sulla fisiologia di *Lacticaseibacillus rhamnosus* GG (LGG) e sul metabolismo microbico intestinale.

Key words: MicroRNAs; *Lacticaseibacillus rhamnosus* GG; Gut Microbiota;

1. Introduction

MicroRNAs (miRNAs) derived from plants are small RNA molecules that do not code for proteins but play a role in regulating gene expression within plants. Recently, it has been suggested that these miRNAs might also cross species boundaries to affect biological processes in mammals (Li et al., 2019). Although the stability of plant miRNAs in the gastrointestinal tract remains controversial, emerging evidence supports their relative resistance to digestive enzymes and acidic conditions, enabling them to reach the gut microbiota and host tissues intact (Anzola et al., 2018). The human gut microbiota, a complex community of microorganisms, plays a vital role in health, including digestion, immunity, and metabolism (Teng et al., 2018). Among health-supporting microbes, *Lacticaseibacillus rhamnosus* GG (LGG) is a well-documented probiotic known for its adhesion capacity and biofilm formation, which are crucial for its colonization and interaction with the host (Lebeer et al., 2008). Microbial communities in the human gut influence health and metabolism through interactions shaped by diet, environment, and genetic regulation. While bacterial gene expression is studied in response to host signals, evidence suggests food-derived miRNAs may regulate gut microbes through cross-kingdom interactions. However, their impact on bacterial physiology remains unexplored.

2. Experimental Procedure

Following the PhD thesis project, this oral communication reports the main results of the following four activities directed to:

A1) Characterization of miRNA-enriched extract, including miRNA read counts, and secondary structure of miRNA;
A2) Comparative analysis of the digestive stability of plant-derived miRNAs;
A3) Computational prediction of plant miRNA targets in LGG related to cell wall biosynthesis, along with experimental validation through biofilm quantification;
A4) Evaluation of short-chain fatty acid (SCFA) production in response to two concentrations of miRNA-enriched extract using the simulator of the human intestinal microbial ecosystem (SHIME®) model

3. Materials and Methods

Small RNA was isolated from a spinach-derived extract obtained from Mirnagreen and subjected to sequencing on the NextSeq500 platform. Raw data were processed using sRNAbench within the sRNAtoolbox suite, employing standard parameters and Bowtie alignment against the PmiREN and miRBase hairpin databases for sequence annotation. The secondary structures of the plant miRNAs were predicted using RNAstructure software with input parameters reflecting physiological conditions: 37°C, 10 mM Na^+, and 1.0 mM Mg^{2+} (Xu et al., 2024). Spinach-derived extract was reconstituted in nuclease-free water (0.25 g in 10 mL) and subjected to a simulated digestion protocol (Brodkorb et al., 2019) mimicking the human digestive system (oral, gastric, and intestinal phases). Following each digestion phase, small RNA was isolated using a method based on Mathioni et al. (2017) with a slight modification. Quantification of target miRNAs (ath-miR159a, ath-miR166a, and ath-miR168a) was performed using quantitative real-time PCR with TaqMan® MicroRNA Assays (Applied Biosystems). To investigate the potential cross-kingdom interaction between spinach-derived plant miRNAs and LGG, a computational approach was employed, with target genes (*GlmU*, *GlmS, pgmL*, *pgi*, *SpaA*, *SpaC* and EPS gene

cluster) retrieved from NCBI due to their roles in cell wall biosynthesis and adhesion, hypothesized to be influenced by plant miRNAs. Plant miRNA sequences were screened against LGG nucleotide sequences using psRNATarget to evaluate miRNA–mRNA hybridization sites. To validate the computer simulation, biofilm formation was quantified using a modified crystal violet staining assay (Deng et al., 2022). LGG was adapted through two sequential 24 h subcultures in minimal media supplemented with 5%, 10%, and 15% miRNA-enriched spinach extract. Following adaptation, the cultures were maintained under the same conditions and transferred into fresh MRS medium for a 24 h incubation at 37°C. A negative control was included (synthetic broth without LGG). After incubation, plates were washed with 0.9% (w/v) NaCl, treated with methanol (200 µL, 10 min), and stained with 0.2% (w/v) crystal violet (15 min). Excess dye was removed via NaCl washes, and bound dye was solubilized using 95% ethanol. Optical density (OD at 595 nm) was measured with a Tecan microplate reader. The SHIME® model was employed to assess the impact of two distinct concentrations (0.6 and 6 g) of a miRNA-enriched extract on the gut ecosystem. SCFA levels were quantified in both the proximal colon (PC) and distal colon (DC) reactors at multiple time points: T0 (baseline, before treatment), T1 (third day of treatment), T2 (fifth day of treatment), and during the washout period at W1 and W2.

4. Results and Discussion

4.1 miRNA read counts and secondary structure of miRNA

According to sequencing results, the miRNA-enriched extract contained a diverse miRNA population. htu-miR159a was the most abundant, exceeding 30,000 reads, alongside other highly expressed miRNAs like ttu-miR159a and gma-miR393a. This indicates a heterogeneous profile, with a few miRNAs dominating (Figure 1a). The overall thermodynamic stability of the identified miRNAs was further detailed by the distribution of their predicted ΔG values (Figure 1b). Most miRNAs exhibited negative ΔG values, confirming the formation of stable hairpin secondary structures. The distribution peaked around 0 to +1 kcal/mol; however, a substantial number of miRNAs revealed more favorable (more negative) ΔG values, extending to approximately −4 kcal/mol. Notably, while high abundance often correlated with some degree of stability, such as htu-miR159a (ΔG=−0.4 kcal/mol), several low-abundance miRNAs like ath-miR168b-5p (ΔG=−3.2 kcal/mol) demonstrated high structural stability. These data highlight the potential for selecting specific miRNAs for further investigation based on their abundance and inherent structural stability, which might potentially impact their bioavailability and functional impact.

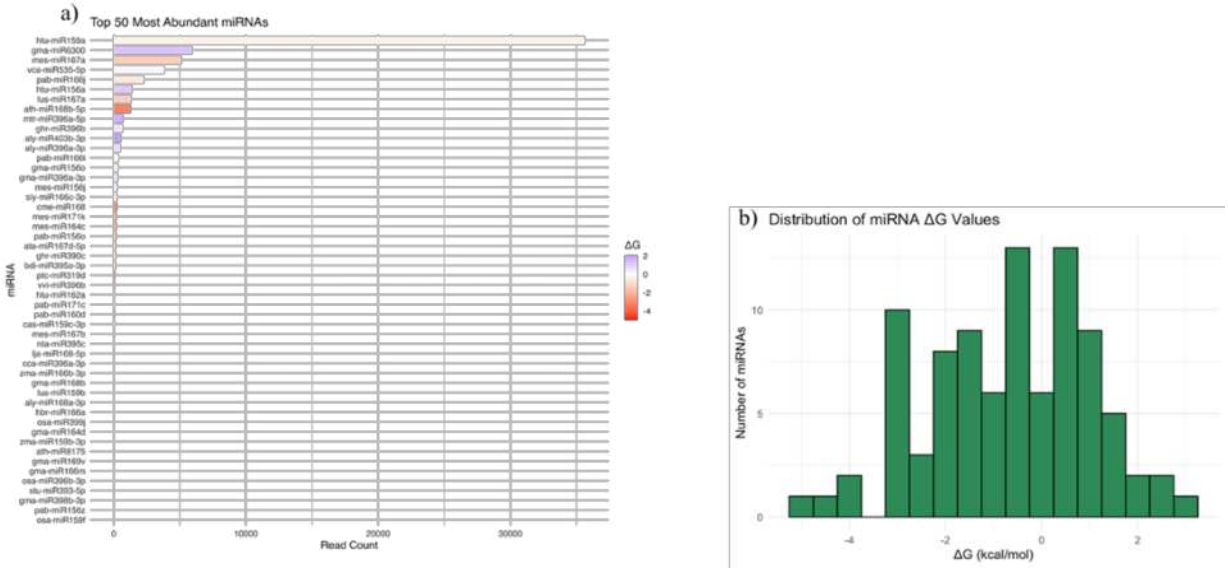


Figure 1. *Abundance and Thermodynamic Stability of Plant-Derived Mature miRNAs present in miRNA-enriched extract. (a) Bar plot showing the top 50 most abundant plant-derived mature miRNAs ranked by read count. Each bar represents an individual miRNA, with the color gradient reflecting the corresponding Gibbs free energy (ΔG) values of their predicted secondary structures, as calculated by RNA structure. (b) Histogram illustrates the overall distribution of ΔG values across all detected plant-derived mature miRNAs. The spread of ΔG values reflects the variability in predicted secondary structure stabilities among the datasets.*

4.2 Digestive stability of plant-derived miRNA

For all three miRNAs, the spinach-derived extract significantly enhanced their stability against enzymatic degradation compared to their synthetic oligos (Figure 2a–c; all $p < 0.05$). Particularly, synthetic miRNAs were promptly degraded, retaining less than 5% of their original levels by 120 min. In contrast, ath-miR168a from the spinach extract retained over 80% of its initial concentration after just 2 min of oral digestion, markedly outperforming the synthetic version. These results correspond with the predicted secondary structures of the miRNAs, which indicate a more stable conformation for ath-miR168a compared to ath-miR159 and ath-miR166. This variation in stability suggests that miRNA survival during digestion may be influenced by sequence

characteristics, secondary structure, and GC content. MiRNAs with less stable secondary structures may be more vulnerable to enzymatic degradation, consistent with findings by (Yang et al. 2018), who reported similar susceptibility in other plant miRNAs.

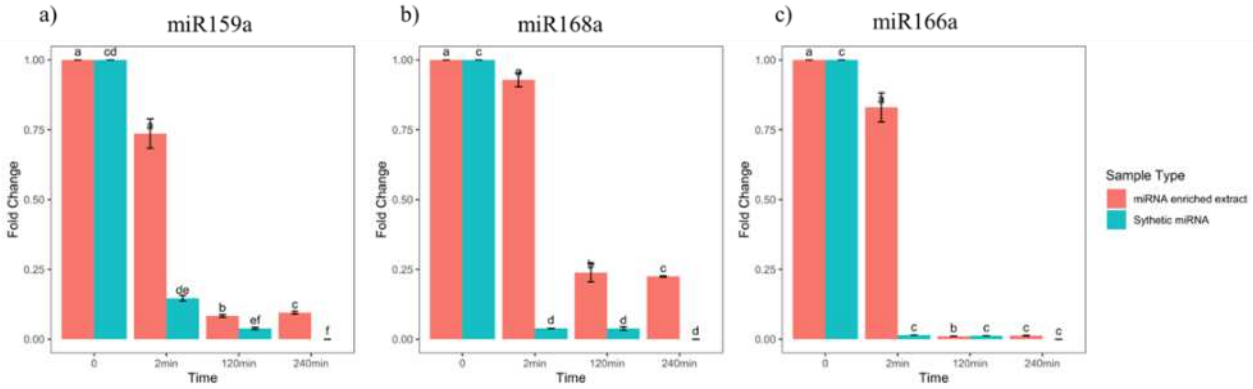


Figure 2. *Digestive stability of spinach extract of three plant microRNAs (miRNAs), ath-miR159a, ath-miR166a, and ath-miR168a. The figure is divided into three panels: a) ath-miR159a, b) ath-miR166a, and c) ath-miR168a, each depicting the fold change of the respective miRNA across different stages of digestion. The x-axis represents the time points (T0, T1, T2, and T3) corresponding to each digestion stage (oral, gastric, intestinal). The y-axis represents the fold change, indicating the relative abundance of the miRNA compared to the control (T0, the initial extract). Data are presented as the means of three replicates ± standard deviations. Bars with different superscript letters differ significantly (p< 0.05).*

4.3 Plant miRNA Target Prediction and Biofilm Modulation in LGG

A total of 26 mature plant-derived miRNAs were predicted to interact with LGG genes, potentially influencing key bacterial functions through either mRNA cleavage or translation repression. Notably, htu-miR159a expressed the highest abundance (35,641 reads) and targeted genes within the EPS (exopolysaccharide) gene cluster via translational inhibition, with a moderate expectation score (5.0) and relatively low unpaired energy (UPE = 12.605), suggesting accessible and biologically relevant binding. Similarly, several miR156 family members (htu-miR156a, gma-miR156o, mes-miR156j) were highly expressed (1400–352 reads) and predicted to target *welI*, a key EPS biosynthesis gene, primarily through cleavage. miRNAs such as pab-miR160d and htu-miR160a were predicted to repress *GlmU*, an essential enzyme involved in UDP-N-acetylglucosamine biosynthesis, via translation inhibition. These miRNAs had 62 read counts and relatively stronger binding energies (ΔG = -3.2 to -1.5 kcal/mol), implying a potential impact on LGG's cell wall integrity. Additionally, miRNAs like bna-miR168b and cca-miR396c were predicted to cleave *pgmL* and *pgi*. Taking together, the *in-silico* results suggest that highly abundant dietary plant miRNAs, primarily from the miR156, miR159, and miR160 families, may exert cross-kingdom regulatory effects on LGG, influencing genes associated with biofilm formation and cell wall biosynthesis.

To validate the computer simulation, the biofilm formation of LGG was assessed under different conditions to examine the influence of spinach-derived plant miRNAs. LGG grown in minimal media (MM) alone indicated low levels of biofilm formation with an optical density measured at OD 595 nm of 0.486 ± 0.006, while growth in nutrient-rich MRS medium resulted in higher biofilm levels (0.798 ± 0.079). Supplementing MM with miRNA-enriched spinach extract at 5% and 10% significantly increased biofilm formation to 0.918 ± 0.053 and 0.908 ± 0.024, respectively, whereas 15% supplementation yielded a slightly lower but still elevated biofilm level (0.765 ± 0.047) compared to MM alone. It is noteworthy that, after two subcultures in MM with spinach miRNAs, transferring LGG to fresh MRS medium triggered a substantial increase in biofilm formation, with OD 595 nm values of 1.308 ± 0.226, 1.318 ± 0.161, and 1.509 ± 0.098 for the 5%, 10%, and 15% treatment groups, respectively. These outcomes support the idea that prior exposure to plant miRNAs enhances LGG's ability to form biofilm in nutrient-rich environments, indicating a lasting effect and supporting the idea of cross-kingdom regulation by dietary miRNAs. Teng et al. (2018) demonstrated that LGG selectively take up ginger-derived miRNAs, which regulate genes such as the transcription repressor *LexA* and pilus protein *SpaC*, as well as *ycnE* affecting metabolism and host immune responses. This supports our findings that spinach-derived miRNAs (miR156, miR159, miR160) may have the potential to modulate LGG genes involved in biofilm formation and cell wall biosynthesis, indicating cross-kingdom regulation.

Mature miRNA	Read count	delta G	Expectation	UPE	Inhibition	Target
cca-miR396c	3	0.7	5.0	15.249	Cleavage	pgi
bna-miR168b	2	-4.1	5.0	16.972	Cleavage	pgmL
htu-miR160a	4	-1.5	4.0	22.236	Translation	GlmU
pab-miR160d	62	-3.2	4.0	22.236	Translation	GlmU
csi-miR1515b-5p	2	-0.3	5.0	13.868	Translation	GlmU
htu-miR156a	1400	1.1	5.0	14.038	Cleavage	welI
gma-miR156o	352	0.5	5.0	15.7	Cleavage	welI
nta-miR395c	57	-1.1	5.0	19.649	Cleavage	welF
osa-miR399j	12	-1.9	4.5	23.114	Cleavage	EPS gene cluster
gma-miR172g	11	2.4	5.0	19.025	Translation	wze
mes-miR156j	280	0.5	4.5	15.631	Translation	EPS gene cluster
htu-miR159a	35641	-0.4	5.0	12.605	Translation	EPS gene cluster
mes-miR164c	226	-2.3	5.1	14.226	Translation	EPS gene cluster
htu-miR160a	4	-1.5	5.0	N/A	Translation	spaC
pab-miR160d	62	-3.2	5.0	N/A	Translation	spaC
gma-miR168b	32	-3.2	5.0	N/A	Cleavage	spaA
htu-miR160a	4	-1.5	4.0	N/A	Translation	glmU
pab-miR160d	62	-3.2	4.0	N/A	Translation	glmU
csi-miR1515b-5p	2	-0.3	5.0	N/A	Translation	glmU
cca-miR396c	3	0.7	5.0	N/A	Cleavage	pgi
pab-miR156k	2	0.7	4.0	N/A	Cleavage	welI
htu-miR156a	1400	1.1	5.0	N/A	Cleavage	welI
ssp-miR156	2	1.1	5.0	N/A	Cleavage	welI
gma-miR156o	352	0.5	5.0	N/A	Cleavage	welI
pde-miR159	4	-1.9	4.0	N/A	Cleavage	WelH
csi-miR1515b-5p	2	-0.3	4.5	N/A	Cleavage	wzy
hci-miR156a	2	1.1	4.5	N/A	Cleavage	welF
nta-miR395c	57	-1.1	5.0	N/A	Cleavage	welF
ppt-miR319d-3p	2	-3.1	5.0	N/A	Cleavage	welG

Table 2 *Predicted interactions between plant-derived mature miRNAs and genes in LGG, based on in silico target prediction analyses. Each miRNA includes the read count, predicted binding free energy (ΔG, kcal/mol), target accessibility (UPE: Unpaired Energy), and target prediction confidence (Expectation score). The table also categorizes each interaction by predicted mode of inhibition, either mRNA cleavage or translation repression, and lists the LGG gene targeted.*

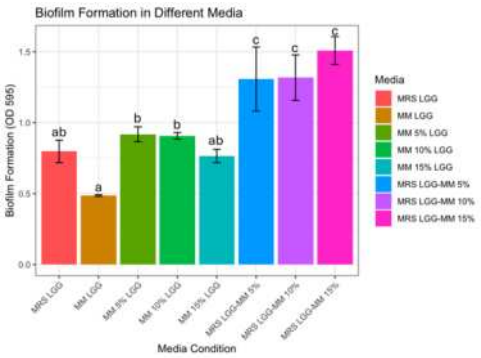


Figure 2 *Biofilm formation of LGG across diverse media conditions, the x-axis indicates the media type, and the y-axis represents the quantified biofilm (OD 595 nm). Bars are presented as the means of three replicates ± standard deviations. Values with different superscript letters differ significantly (p< 0.05).*

4.4 Impact of miRNA-Enriched Spinach Extract on Short-Chain Fatty Acid Production in a Human Gut Model

SCFA concentrations were quantified in a SHIME® model to assess the influence of two doses (0.6 g and 6 g) of a miRNA-enriched spinach extract on gut microbial fermentation. Changes in SCFA levels may act as indicators of shifts in the gut ecosystem in terms of activity and composition of the microbiome (Sanna et al. 2019). According to acetic acid quantification, both extract concentrations significantly increased acetic acid levels during the treatment phase (T1 and T2) in both PC and DC reactors. The 6 g dose consistently yielded the highest concentrations, peaking at 71.3 ± 1.43 mM in PC and 91.0 ± 0.87 mM in DC by T2 ($p<0.05$). Following washout (W1, W2), acetic acid decreased, returning to baseline. The effects of propionic acid production were dose-dependent in both bioreactors. For instance, a 6 g extract dose significantly increased propionic acid at T2, reaching 169.0 ± 3.67 mM in the PC reactor and 63.4 ± 2.19 mM in the DC reactor ($p < 0.05$), while a 0.6 g dose led to a modest increase to 94.4 ± 0.51 mM, observed only in the PC reactor. Taken together, these results indicate that the miRNA-enriched spinach extract positively modulated gut microbial fermentation in the SHIME® model, leading to increased production of short-chain fatty acids. This aligns with findings from (Liu et al. 2022), which similarly demonstrated that plant-derived miRNAs can enhance SCFA concentration, reinforcing the concept of dietary plant miRNAs to influence gut microbial metabolism and consequently, host intestinal health.

Acetic acid (mM)

	PC		DC	
	0.6 gram	6 gram	0.6 gram	6 gram
T0	47.3 ± 0.69^{d}	46.9 ± 0.66^{a}	80.1 ± 0.57^{a}	79.5 ± 0.24^{a}
T1	50.8 ± 0.62^{a}	66.3 ± 1.86^{b}	87.0 ± 0.71^{a}	85.2 ± 0.71^{b}
T2	53.4 ± 0.28^{b}	71.3 ± 1.43^{c}	90.3 ± 0.35^{b}	91.0 ± 0.87^{c}
W1	51.2 ± 0.19^{c}	55.3 ± 0.59^{c}	90.9 ± 0.44^{b}	89.1 ± 0.65^{d}
W2	47.0 ± 0.68^{c}	54.1 ± 0.90^{d}	87.6 ± 0.81^{c}	79.5 ± 0.44^{d}

Propionic acid (mM)

	PC		DC	
	0.6 gram	6 gram	0.6 gram	6 gram
T0	86.7 ± 2.16^{a}	88.0 ± 1.32^{a}	24.7 ± 0.26^{a}	24.7 ± 0.46^{a}
T1	93.8 ± 0.51^{a}	163.0 ± 2.49^{a}	22.5 ± 0.39^{ab}	28.3 ± 0.87^{b}
T2	94.4 ± 2.41^{b}	169.0 ± 3.67^{b}	22.3 ± 0.93^{b}	63.4 ± 2.19^{b}
W1	88.5 ± 1.66^{b}	90.5 ± 1.37^{b}	23.2 ± 0.34^{b}	28.1 ± 0.37^{c}
W2	81.2 ± 0.68^{c}	85.6 ± 1.00^{b}	21.8 ± 0.72^{b}	21.5 ± 0.20^{d}

Butyric acid (mM)

	PC		DC	
	0.6 gram	6 gram	0.6 gram	6 gram
T0	24.3 ± 2.53^{a}	28.2 ± 1.47^{a}	35.9 ± 0.72^{a}	36.7 ± 0.42^{a}
T1	26.9 ± 1.16^{a}	25.9 ± 1.89^{ab}	37.5 ± 0.46^{a}	50.0 ± 1.03^{a}
T2	28.5 ± 1.45^{ab}	21.1 ± 1.34^{bc}	40.2 ± 0.74^{ab}	48.0 ± 0.92^{ab}
W1	31.5 ± 0.62^{bc}	31.0 ± 0.54^{c}	41.1 ± 1.53^{bc}	45.0 ± 0.31^{b}
W2	32.5 ± 0.93^{c}	34.3 ± 0.64^{d}	40.7 ± 1.31^{c}	36.8 ± 7.97^{b}

Table 2 *SCFA Concentrations (mM) in PC and DC Reactors Across Treatment and Washout Periods. The SHIME® model was employed to assess the impact of two distinct concentrations of a miRNA-enriched extract on the gut ecosystem. SCFA levels were quantified across multiple time points: before treatment (T0), three days (T1) and five days (T2) into the treatment, as well as during the washout phase at W1 (two days post-treatment) and W2 (five days post-treatment). Data are presented as the means of three replicates ± standard deviations. Values with different superscript letters differ significantly (p< 0.05).*

5. Conclusions and Future Perspectives

This study highlights the emerging epigenetic role of plant-derived miRNAs as potential mediators of cross-kingdom communication between diet and the gut microbiome. The concept that small RNAs from edible plants can withstand gastrointestinal conditions and influence bacterial physiology opens new avenues for understanding host–microbe–diet interactions. By demonstrating that dietary miRNAs may modulate microbial responses such as biofilm formation or core metabolic networks, this work contributes to the growing body of evidence supporting RNA-based inter-kingdom signaling. The results suggest a transformative approach in personalized nutrition, highlighting that the influence of dietary elements extends beyond traditional nutrients to encompass gene-regulating molecules. Further investigations are needed to explore the mechanisms of miRNA uptake and function across microbial species, as well as their potential to modulate microbiome composition and function *in vivo*. Integrating advanced multi-omics, including single-cell RNA sequencing, spatial transcriptomics, and metabolomics, can help resolve cell-type-specific responses and community-level adaptations.

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Influence of food matrix on metabolic fate of polyphenols: role of phenol-nutrient interactions

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This PhD thesis explored how the co-occurrence of polyphenols and other nutrients in food and by-product matrices having different nutritional compositions modulate their bioaccessibility and bioavailability *in vitro*. Sustainable processing methods, such as fermentation, and the valorization of agro-industrial by-products have been proposed to formulate innovative food products. These food matrices were tested to better understand their functional properties, with the aim of enhancing their nutritional quality and potential health benefits.

Influenza della matrice alimentare sul destino metabolico dei polifenoli: il ruolo delle interazioni polifenoli-nutrienti

Questa tesi di dottorato ha esplorato come la presenza contemporanea di polifenoli e altri nutrienti nelle matrici alimentari e nei sottoprodotti con differenti composizioni nutrizionali moduli la loro bioaccessibilità e biodisponibilità in vitro. Sono stati proposti metodi di trasformazione sostenibili, come la fermentazione, e la valorizzazione dei sottoprodotti agroindustriali per formulare prodotti alimentari innovativi. Queste matrici alimentari sono state testate per comprendere meglio le loro proprietà funzionali, con l'obiettivo di migliorarne la qualità nutrizionale e i potenziali benefici per la salute.

Key words: food matrix, by-products, polyphenols, interactions, bioaccessibility, bioavailability.

1. Introduction

In accordance with the PhD thesis project previously described (Chiacchio, 2023), this oral communication reports the main results of the following four activities directed to:

A1) Investigate the impact of fermentation of chickpea flour by lactic acid bacteria isolated from various ecosystems on the enhancement of bioactive compound content compared to its unfermented counterpart.

A2) Assess the *in vitro* functional potential of the by-product obtained from the production of a plant-based beverage derived from tiger nuts in comparison to the beverage.

A3) Evaluate the effects of incorporating fermented chickpea flour at different levels into a wheat-based product, in comparison to their conventional and unfermented counterparts, with a focus on functional properties.

2. Food matrix matters: polyphenols-nutrient interactions modulate their bioaccessibility and bioavailability

Polyphenols are plant-derived bioactive compounds with well-documented health benefits, including anti-inflammatory, anti-cardiovascular, and anti-diabetic effects (Xue et al., 2024). These benefits are largely attributed to their antioxidant capacity, either through direct scavenging of reactive oxygen species (ROS) or by enhancing endogenous antioxidant enzyme systems (Bié et al., 2023). However, their efficacy depends on bioavailability, i.e., the fraction that reaches systemic circulation, which is influenced by their bioaccessibility, or release from the food matrix during digestion (Xue et al., 2024). The food matrix encompasses both the nutrient composition and the physical organization of food components, where complex interactions occur among nutrients and non-nutrients (Weaver & Givens, 2025). Polyphenols commonly interact with carbohydrates, proteins, lipids, and dietary fibers, and these interactions can modify digestion, absorption, and functionality (Sęczyk et al., 2021). Such binding may reduce availability through chemical modifications or, conversely, protect polyphenols from degradation, ensuring delivery to absorption or fermentation sites (Ribas-Agustí et al., 2018). For example, polyphenols can influence fat digestion by interfering with emulsification and inhibiting lipase, or they may be stabilized in lipid micelles, enhancing bioavailability and supporting cardiovascular and metabolic health (Cianciosi et al., 2022). In plant-based foods, polyphenols are often bound to dietary fibers, which limit their release in the upper gut but help deliver them to the colon, where they may act as local antioxidants within fiber-polyphenol complexes (Rocchetti et al., 2022). During digestion and processing, they may also form reversible or irreversible complexes with proteins (Wang et al., 2022). These interactions, affected by molecular characteristics, pH, temperature, and food composition, can alter protein functionality and polyphenol activity by masking reactive sites or changing solubility and emulsification (Cianciosi et al., 2022). Aggregation can hinder or enhance protein digestibility and influence polyphenol bioavailability, depending on the nature of the interaction. Some complexes may dissociate during digestion, forming new polyphenol-peptide complexes with potential bioactivity (Zhang et al., 2021).

3. Sustainable food processing strategies to enhance the bioaccessibility of dietary polyphenols

Chemical structure and matrix interactions are important food-related factors that hamper the bioaccessibility and bioavailability of phenolic compounds and can be overcome by applying appropriate processing techniques (Ribas-Agustí et al., 2018). Food processing induces structural and physicochemical changes that can affect the amount and metabolism of phenolic compounds (Shahidi and Pan, 2022). Such changes include chemical modifications into more absorbable forms, cleavage of bonds linking phenolic compounds to macromolecules in the food matrix, disruption of microstructural barriers (e.g., cell walls) that hinder their release, and the formation of complexes that protect phenolics during digestion until absorption (Ribas-Agustí et al., 2018). Conversely, invasive technologies (e.g., thermal treatments) may promote oxidation and degradation of phenolic compounds, thereby reducing their content in processed foods. Fermentation has emerged as a sustainable and non-invasive technique with the potential to enhance the nutritional profile of food products. For instance, lactic acid bacteria produce various enzymes capable of breaking down complex macromolecules, thereby releasing bound phenolic compounds, increasing their total amount and bioaccessibility, and/or generating novel bioactive metabolites (Zhao et al., 2021).

4. Experimental Procedure

In this PhD thesis a three-step experimental design was set up that included: i) fermentation of chickpea-based products as a strategy to improve polyphenols, bioactive peptides, and reduce anti-nutrients; ii) study of bioaccessibility and bioavailability of polyphenols from tiger nut beverage and its by-products through *in vitro* digestion and Caco-2 cells; and iii) design and formulation of innovative crackers with partial replacement of fermented or non-fermented chickpea flour and study of bioaccessibility and bioavailability of polyphenols through *in vitro* digestion and Caco-2 cells. Applying this experimental design it was possible to evaluate: a) the effect of an eco-friendly process like fermentation on the nutritional profile of a matrix rich in polyphenols, fibers, and proteins like chickpea (i); b) the different digestive behaviours and functional properties in terms of antioxidant activity, inhibition of digestive enzymes along with bioaccessibility and bioavailability in Caco-2 cells of polyphenols derived from the same matrix (tiger nut) but in different states (liquid beverage and solid by-product) and different nutritional composition (ii); and c) the effect of inclusion of fermented chickpea flour, as wheat flour replacer in crackers, on the bioaccessibility of polyphenols and their bioavailability on Caco2 cells (iii).

5. Materials and Methods

A1. Fourteen chickpea-based purées (20% w/v) were produced at laboratory scale and fermented using 14 lactic acid bacteria (LAB) strains isolated from different ecosystems. Following fermentation, the samples were freeze-dried to obtain flours, which were analyzed for polyphenol content, bioactive peptides (BAPs), free amino groups (FAG), total antioxidant activity (TAC), and phytic acid content using both spectrophotometric and chromatographic techniques. A detailed description of these methods is reported in Chiacchio et al., 2025.
A2. A tiger nut-based beverage was produced at laboratory scale, and both the beverage and its corresponding by-product were subjected to *in vitro* digestion. The samples collected at each digestion step were analyzed polyphenol release and TAC. The non-bioaccessible fraction (the digestive residue) of each sample was also evaluated for its antioxidant potential. Additionally, the intestinal bioaccessible fractions were assessed for their inhibitory activity on digestive enzymes (α-amylase, α-glucosidase, and lipase) and were used to evaluate the bioavailability of polyphenols and antioxidant activity in a Caco-2 cell model. Spectrophotometric and chromatographic techniques were used. A detailed description of these methods is reported in Llorens, Chiacchio et al., 2024.
A3. Six fermented chickpea flours were obtained by fermenting chickpea purées with six different LAB strains at laboratory scale. Based on the total antioxidant capacity, the most promising flour was selected for incorporation into a cracker formulation. A control cracker (100% wheat flour) and innovative crackers containing 10% or 25% of fermented or non-fermented chickpea flour were produced. Polyphenol content and TAC were measured in the final products and their intestinal bioaccessible fractions. These fractions were also tested for their inhibitory activity against digestive enzymes (α-amylase, α-glucosidase, and dipeptidyl peptidase-IV) and were applied to Caco-2 cells to assess polyphenol bioavailability.

6. Results and Discussion

6.1 Lactic acid fermentation improves nutritional and functional properties of chickpea flour

The flours fermented by *Enterococcus hirae* and *Enterococcus faecium* had higher FAG and BAPs, including inhibitors of Dipeptidyl peptidase-IV and Angiotensin-converting enzyme. Flours fermented by *Leuconostoc mesenteroides* OM94, *Lactiplantibacillus plantarum* 299v, *Lactiplantibacillus plantarum* E75, *Lactiplantibacillus plantarum* LPPB, and *Lacticaseibacillus casei* LBC491 contained higher amounts of polyphenols, had soluble TAC that was 10-fold and direct TAC 3-fold higher along with lower phytic acid content

than the control flour. Pyrogallol was detected in fermented products for the first time, and protocatechuic 4-*O*-glucoside increased three times in chickpea flour fermented by *Leuconostoc mesenteroides* OM94 compared to the control. Interestingly, 2 clusters were identified, and the variables that most contribute to the separation were total polyphenols, protocatechuic 4-*O*-glucoside, phytic acid, FAG, TAC, sinapoyl D-glucoside,and total BAPs. Specifically, the ochre and blue clusters indicate strains with putative proteolytic and saccharolytic patterns. Indeed, the total BAPs and FAG better characterized the ochre cluster, whereas polyphenols and TAC drove the separation of the blue cluster (Figure 1).

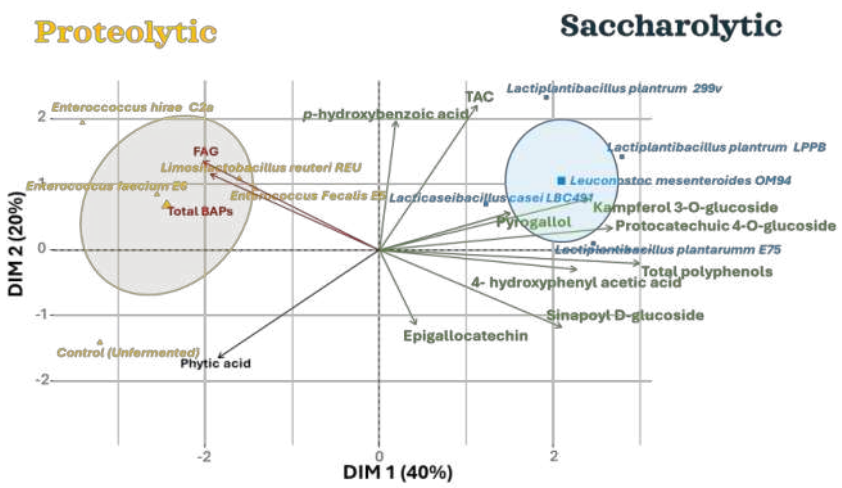


Figure 1 *K-means clustering via Principal Component Analysis based on total bioactive peptides (Total BAPs), free amino-groups (FAG), total antioxidant capacity (TAC), polyphenols, and phytic acid of chickpea flours fermented with 14 LAB strains and control (unfermented). Vectors representing polyphenols and TAC are shown in green, those representing BAPs and FAG are shown in red while phytic acid is shown as a black vector.*

6.2 Potential bioaccessibility and bioavailability of polyphenols and functional properties of tiger nut beverage and its by-products over *in vitro* digestion

The results revealed that the total polyphenol content in tiger nut beverage (TNB) was 3-fold higher than that in tiger nut by-product (TNBP), with ferulic acid glucoside, epicatechin derivative, and caffeic acid glucoside accounting for approximately 60% of the total polyphenols. The average bioaccessibility of polyphenols from TNBP was 56% compared to 24% from TNB; caffeic and ferulic acid hexosides were the most bioaccessible polyphenols, with 124% and 118% for TNBP and TNB bioaccessible fractions, respectively (Fig. 2A). Consistently, TAC increased 8 and 5 times in the bioaccessible fractions of TNBP and TNB, respectively, upon digestion. Regarding the fractions reaching the colon, TNBP showed a TAC 2- and 4-fold higher than TNBP (raw) and TNBP bioaccessible fraction, respectively (data not shown). TNBP bioaccessible fraction reduced α-glucosidase activity by 80%, whereas TNB inhibited lipase by 77% (Fig. 2C). Five polyphenols were absorbed through the Caco-2 cell monolayer and showed mean bioavailabilities of 69% and 90% for TNBP and TNB, respectively (Fig. 2B). Caffeic and ferulic acid hexosides from the by-product had absorption peaks at 2h and 4 h, respectively, and a maximum TAC after 3h whereas polyphenols from TNB peaked after 2h coinciding with the highest TAC (data not shown).

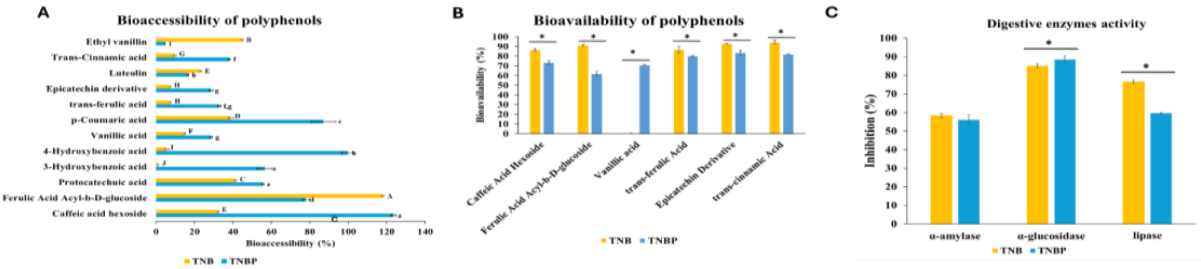


Figure 2 *Main results from the evaluation of functionality in vitro of tiger nut beverage (TNB, ochre) and tiger nut by-product (TNBP, blue):* ***A)*** *Polyphenol bioaccessibility in intestinal digesta. Different uppercase (TNB) and lowercase (TNBP) letters indicate significant differences (One-way ANOVA and Tukey's post hoc, $p < 0.05$);* ***B)*** *Polyphenols bioavailability. *Indicate differences between the samples for each phenolic compound (t-test, $p < 0.05$);* ***C)*** *Enzymatic inhibitory activity of intestinal digesta from TNB and TNBP. *Indicate significant differences between samples for the inhibition of each enzyme (t-test, $p < 0.05$).*

6.3 Development of fermented chickpea flour-based crackers and functionality over *in vitro* digestion and cellular model

Fermentation enhanced the antioxidant activity of chickpea flours by up to 4-fold, with the highest value observed for the strain *L. diolivorans* 13-4AB (data not shown). Prior to digestion, the selected fermented flour (FF) exhibited a higher polyphenol content than its unfermented counterpart (CF), and the cracker containing the highest percentage of fermented flour (C25FF) showed the highest TAC (Fig. 3A and Fig. 3B). Microbial fermentation

led to the formation of pyrogallol, which was exclusively found in FF and crackers formulated with this ingredient. Both FF and CF along with their respective crackers contained significant levels of protocatechuic acid 4-*O*-glucoside and sinapic acid glucoside. The former was more abundant in FF and derived crackers, whereas the latter predominated in the unfermented ones. Polyphenol bioaccessibility was 1.3 times higher in FF compared to the unfermented, and 1.2 times higher in the cracker with the highest inclusion level of fermented flour compared to the other formulations. Glucosides of sinapic acid and protocatechuic acid and pyrogallol, accounted for approximately 90% of the bioaccessible polyphenols and were also the most bioavailable. The TAC of the bioaccessible fractions from the crackers with high inclusion of non-fermented flour (C25CF) and C25FF, and with low inclusion of fermented flour (C10FF), was 2.8-, 1.5-, and 1.4-fold higher, respectively, than that measured in the undigested products. In contrast, the TAC decreased in the other bioaccessible fractions. In conclusion, crackers enriched with the highest levels of either fermented or non-fermented chickpea flour, C25FF and C25CF, showed the greatest polyphenol bioavailability up to 60% (Fig. 3D).

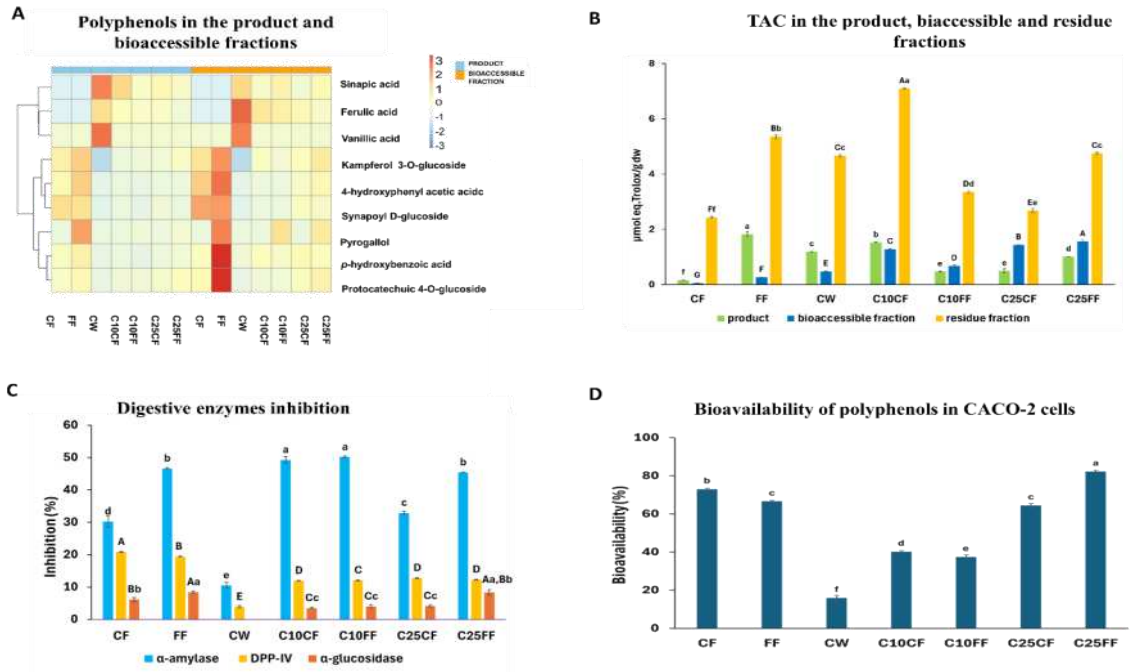


Figure 3 *Main results from the evaluation of functionality in vitro of chickpea flour (CF), fermented chickpea flours (FF), 100% wheat-based cracker (CW), 10% chickpea flour-enriched cracker, 10% fermented chickpea flour-enriched cracker, 25% chickpea flour-enriched cracker, 25% fermented chickpea flour-enriched cracker*: **A)** *Heatmap showing the abundances of polyphenols detected in the samples. The colored bars across the top of the graph depict the product (blue) and the bioaccessible fraction (orange)*; **B)** *Total antioxidant activity of product (green), bioaccessible fraction (blue), and residue fraction (ochre). Different lowercase letters indicate differences between the products, different uppercase letters indicate differences between the bioaccessible fraction, and upper-lowercase letters indicate differences between residue fractions (One-way ANOVA and Tukey's post hoc, $p < 0.05$);* **C)** *Inhibitory activity of α-amylase (blue bar), DPP-IV (ochre), and α-glucosidase (orange) by bioaccessible fractions. Different lowercase letters indicate differences between the bioaccessible fractions for α-amylase, different uppercase letters indicate differences between the bioaccessible fraction for DPP-IV, and upper-lowercase letters indicate differences between the bioaccessible fractions (One-way ANOVA and Tukey's post hoc, $p < 0.05$)*; **D)** *Bioavailability of polyphenols in CACO-2 cells. Different letters indicate differences between the samples (One-way ANOVA and Tukey's post hoc, $p < 0.05$).*

7. Conclusions and Future Perspectives

The work presented in this thesis highlights how polyphenol bioactivity is significantly influenced by their interaction with the food matrix components. Experimental results have shown that matrices with different nutritional compositions, along with their modification through fermentation, modulate the metabolic fate of polyphenols *in vitro*. Specifically, lactic acid fermentation of chickpea flour led to the release of polyphenols and short peptides with biological activities as potential inhibitors of DPP-IV, while reducing phytic acid and producing novel metabolites. These findings suggest that fermentation is a valuable approach for modifying the food matrix and producing functional food ingredients. A comparison between the tiger nut beverage and its derived by-product revealed that, although the beverage contained higher levels of total polyphenols, the by-product exhibited greater polyphenol bioaccessibility and antioxidant capacity following digestion. These results underscore the key role of the matrix state (liquid vs. solid) in modulating the bioavailability of polyphenols. In solid matrices, polyphenols are generally protected from enzymatic degradation, thanks to structural components such as fibers and proteins that limit their exposure. Conversely, in liquid matrices, polyphenols are often present

in free form, making them more susceptible to degradation by digestive enzymes and other gastrointestinal conditions Similarly, the inclusion of fermented chickpea flour in wheat-based crackers led to improved polyphenol content, antioxidant capacity, and bioavailability compared with unfermented and wheat-based crackers. This suggests that the nutritional improvements conferred by the fermentation of chickpea flour persist when the fermented ingredient is incorporated into more complex food matrices. Despite the promising outcomes of these studies, certain limitations must be acknowledged. Static in vitro digestion models do not fully mimic human gastrointestinal conditions, so future studies should validate these products *in vivo*. Moreover, the application of an untargeted metabolomics approach is essential to comprehensively identify bioactive compounds and their metabolic transformations during processing and digestion, allowing for the identification of novel biomarkers. Further research is needed to evaluate the feasibility of scaling up production and to ensure the functional stability of these foods during industrial processing and storage. In conclusion, this thesis provides valuable insights into the role of the food matrix in modulating polyphenol metabolism *in vitro* and supports the development of sustainable, plant-based functional foods through fermentation and by-product valorization strategies.

8. Nomenclature

TAC, total antioxidant capacity; CACO-2, human colorectal adenocarcinoma cell; LAB, lactic acid bacteria; FAG, free amino groups; DW, dry weight; BAPs, bioactive peptides; SD, standard deviation; TNB, tiger nut beverage; TNBP, tiger nut by-product; CF, chickpea flour; FF, fermented chickpea flour; CW, 100% wheat-based cracker; C10CF, 10% chickpea flour-enriched cracker; C10FF, 10% fermented chickpea flour-enriched cracker; C25CF, 25% chickpea flour-enriched cracker; C25FF, 25% fermented chickpea flour-enriched cracker.

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Methodologies to guide consumers towards a healthy and sustainable Mediterranean Diet

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The purpose of the current PhD thesis project is to assess and implement methodologies to support young people and families in embracing a healthy and sustainable diet and lifestyle, like the Mediterranean one. Accordingly, a variety of educational resources and web-based apps have been devised and tested within intervention studies to evaluate their effectiveness in improving nutritional knowledge and eating habits. This oral presentation underlines the results achieved.

Metodologie per accompagnare i consumatori verso una Dieta Mediterranea sana e sostenibile

Lo scopo del presente progetto di tesi di dottorato è quello di valutare e implementare metodologie per sostenere i giovani e le famiglie nell'adottare una dieta e uno stile di vita sani e sostenibili, in linea con il modello Mediterraneo. A tal fine, sono state ideate e testate diverse risorse educative e web-app in differenti studi di intervento per valutarne l'efficacia nel migliorare le conoscenze nutrizionali e le abitudini alimentari. Questa presentazione orale evidenzia i risultati ottenuti.

Key words: Mediterranean Diet, adherence, young people, family, health, sustainability.

1. Introduction

In accordance with the PhD thesis project previously described, this oral communication reports the main results of the following four research activities:

A1) Realisation of a systematic review to explore the demographic and socio-economic determinants affecting the adherence to the Mediterranean Diet (MD) in the adult population;
A2) Assessment of various intervention approaches to promote MD in different family members (adults, adolescents and children);
A3) Examining the impact of nutrition and sensory education and healthy snack exposure as interventions strategies to improve the nutritional knowledge, food-related perceptions and dietary habits of adolescents;
A4) Supporting healthy eating habits and improving nutritional knowledge in families with children through the development of the NUBI web-app;

2. Research studies

2.1 Investigating the demographic and socio-economic determinants affecting the adherence to the Mediterranean Diet in the adult population: a systematic review with metanalysis

Purpose and methods. Within the framework of the European PRIMA project MoreMedDiet (MoreMedDiet, 2022), the aim of the systematic review with metanalysis was to investigate the MD adherence in adult populations from European countries and the Mediterranean basin, in relation to their demographic and socio-economic determinants. A comprehensive literature review was performed considering PubMed, Embase, and Scopus electronic databases and filtering the publication years between Jan-2000 and Dec-2024. Descriptors together with keywords – as adherence, MD and adult population – where used for the search. Original peer-reviewed studies were included, with no language limitations. A systematic approach was employed, whereby 2 independent couples of researchers conducted the publication screening, starting from title and abstract and proceeding to full-text appraisal. Adherence to MD, healthy adults, European and Mediterranean populations and observational studies were considered as eligibility criteria for title and abstract. The presence of at least 1 socioeconomic and at least 1 demographic variable in correlation with MD adherence (the latter derived from a validated index or questionnaire) were also accounted when evaluating full-texts. No sample size restrictions were included among the selection criteria. To determine the validity of included studies, a standardized form for assessing the risk of bias in cross sectional studies was considered (JBI Critical Appraisal Checklist for Analytical Cross Sectional Studies, 2017). Data extraction is planned to be conducted continuously by an independent couple of researchers, who will report the data (e.g., study period and country, population characteristics, outcomes) in a pre-set excel file. The review protocol was uploaded to PROSPERO database (Reg. No. CRD42024562247).

Results. Searching electronic databases resulted in the collection of an initial pool consisting of 4083 records. Out of them 913 were excluded as not being original peer-reviewed studies. From the remaining 3170 publications, 1950 were discarded as they were not compliant with the eligibility criteria of title and abstract. Particularly, 136 were excluded since they did not address adherence to the MD, 1056 as the population did not consist of healthy adults or was only elderly (over 60-65 years), 595 as the study was not conducted in geographic Europe or in countries from the Mediterranean basin. Finally, 219 were removed, not being observational studies. Thus far, 990 documents were removed at the full-text screening stage, currently in progress.

2.2 Assessing three intervention approaches to promote the Mediterranean Diet among family members across age groups: the SWITCHtoHEALTHY project

Purpose and methods. As part of the 3-year European PRIMA project SWITCHtoHEALTHY (S2H, 2022; Calderón-Pérez et al., 2024), the purpose was to measure the effectiveness of different intervention strategies for encouraging positive changes in families' diets, aligned with the MD. A citizen-centered circular approach was thus applied, beginning at the community level and then returning innovative approaches. Accordingly, an observational cross-sectional study was conducted in 4 Mediterranean countries to assess dietary and lifestyle habits, the adherence to the MD, and barriers together with drivers related to this dietary model. Following, 3 intervention strategies were implemented and evaluated in a 3-month parallel, randomized, single-blinded, and controlled multicenter intervention study involving families with children and adolescents (3-17 years) from Spain and Türkiye (n=160 per country). Thus, healthy snacks for children, digital educational tools (App) and hands-on educational material were tested singularly and in combination among them, resulting in 7 intervention groups and a control group without any type of intervention. Data were collected over 3 visits: pre-assement phase at month 0, start visit after 10 months and final visit after 13 months from the beginning. The primary outcome was the adherence to the MD. Possible changes were also explored about dietary behavior and attitudes, nutritional knowledge, economy, and the environment, besides anthropometric information and vital signs. Concerning the strategies implementation, each country developed 8 healthy plant-based snacks for children and of these 4 were selected for the intervention phase. A web-app for the parents was additionally implemented to provide healthy weekly meal proposals for children, by providing menus with lunch-box options or complementing school food service with balanced proposals for all other meals of the day. Educational content for parents was also provided within the same tool, structured in 17 topics (e.g., nutrients, the 5 meals of the day, the healthy plate, grocery shopping). The app for children was finally integrated with a similar app to provide weekly and personalized meal plans for adults and a life-simulation game for the family. Regarding educational material, the following resources were implemented: 4 nutritional educational sheets with activities and games, 10 recipes, 10 take home messages, a short video and a handbook with activities for adolescents at school. The University of Parma (UNIPR) team focused on developing the web-app for children and part of the educational material for adolescents.
Results. The observational cross-sectional study (Yesildemir et al., 2025) was conducted collecting anonymized data from 812 adults (41 ± 11 years, 66% females): 202 from Italy, 200 from Spain, 201 from Türkiye and 209 from Lebanon. Part of the survey also allowed the collection of food habits information of 500 children, provided by their parents. Focusing on the MD diet adherence, this was medium in all countries both for adults (MEDAS questionnaire, García-Conesa et al, 2020) and children (KIDMED questionnaire, Serra-Majem et al., 2004). When observing the MD lifestyle in adults, MEDLIFE score (Sotos-Prieto et al, 2015) showed statistically significant differences between groups. Anyway, a substantial percentage of participants were ranked in the first quartile, showing very poor adherence to the MD lifestyle. Data collected through KIDMED, MEDAS and MEDLIFE questionnaire are summarized in Table 1.

Table 1 *Mediterranean Diet adherence and lifestyle among countries assessed by MEDAS score, KIDMED score and MEDLIFE score.*

	Türkiye (n=201)	Italy (n=202)	Lebanon (n=209)	Spain (n=200)	Total (n=812)	p
MEDAS SCORE (adults) – 0 to 14 points*						
Median (min-max)	8 (2 – 12)	8 (2 – 12)	8 (3 – 12)	8 (1 – 14)	8 (1 – 14)	0.353
KIDMED SCORE (children) – from 0 to 12 points**						
Mean ± standard deviation	6.29 ± 2.23	5.93 ± 2.44	5.93 ± 2.36	6.46 ± 2.47	6.18 ± 2.38	0.119
MEDLIFE SCORE (adults) - from 0 to 28 points*#						
Median (min-max)	16 (6 – 26)[a]	15 (6 – 27)[a]	18 (3 – 27)[b]	16 (4 – 23)[a]	16 (3 – 27)	**<0.001**

** Kruskal Wallis test was used for overall comparison. ** One-way ANOVA analysis of variance test was used.[#]The Bonferroni test was used for pairwise comparisons.*

Additionally, when evaluating the drivers of adherence to the MD the highest-rated factors were the belief that (a) the MD contains healthier and more nutritious foods, (b) healthier fats, (c) more fruits and vegetables, and (d) less

red meat and (e) processed food, as well as (f) more homemade food. Regarding the barriers the determinants receiving the highest score were the perception that (a) MD contains high-priced foods and (b) limited options in restaurants, (c) the difficulty and time required for preparing MD meals, (d) the belief that MD is not suitable for vegans and (f) that contains allergen foods.

Concerning the multicenter intervention study, a total of 860 families were initially recruited and of these 250 (130 from Spain, 120 from Türkiye) were randomized at T0 in: a control group (n=16 Spain; n=15 Türkiye), a group with the App (n=16 Spain; n=14 Türkiye), a group with the hands-on educational material (n=16 Spain; n=15 Türkiye), a group with the healthy snacks (n=17 Spain; n=16 Türkiye), a group with both the App and the hands-on educational material (n=18 Spain; n=15 Türkiye), a group with both the App and the snacks (n=15 Spain; n=15 Türkiye), a group with both the snacks and the hands-on educational material (n=15 Spain; n=15 Türkiye), and a group with all the 3 tools (n=17 Spain; n=15 Türkiye). The intervention phase (T1-T2, from September to December 2024) was completed by 68 and 83 families in Spain and Turkey respectively. Currently, the data analysis is proceeding to verify possible changes in diet quality and health, nutritional knowledge, behaviors and economic and environmental impacts.

2.3 Nutrition and sensory education and healthy snack exposure as interventions strategies to improve the nutritional knowledge, food-related perceptions and dietary habits of adolescents: Italian results from the PROmedLIFE project

Purpose and methods. PROmedLIFE project (PROMD, 2022), within the European PRIMA program, had the main objective of empowering the adherence to the MD by supporting the adoption of a healthy eating lifestyle. Moreover, it aimed to decrease the environmental and economic burdens related to food production and processing. Consistent with this, a multi-actor approach was applied in a 4-week intervention study, targeting high school students from Italy, Greece and Tunisia. The intervention was based on nutrition and sensory education together with snack exposure. The adolescents were thus grouped into a control group (C), a nutrition education group (N), a sensory education group (S) and a group with both sensory and nutritional intervention (NS). All groups were exposed daily to healthy snacks implemented specifically for the project. Nutritional and sensory education were provided as frontal lessons with supporting materials and activities or games to play. A web-app for students' families was also developed as part of the nutrition intervention to provide educational materials. Thus, MD adherence, nutritional knowledge, sociodemographic and lifestyle factors, picky eating, and food neophobia were assessed at the start of the intervention (T0), at its conclusion (T1) and successively to a follow-up period (T2, after 12 weeks). The following tolls were used for data collection: the KIDMED questionnaire (Serra-Majem et al., 2004) for MD adherence, a Nutritional Knowledge (NK) questionnaire realized for the study, a sociodemographic and lifestyle questionnaire derived from HBSC study (Inchley et al., 2018), the Picky Eating (PE) questionnaire (Menghi et al., 2020), the Food Neophobia Scale (FNS) (Pliner & Hobden, 1992) and a Food Choice (FC) task.
Results. The results from the Italian study are presented as follows. Enrolled from a school in Trento city, 197 students participated in the study (14 ± 1 years, 64% males): 56 in the C group, 50 in the N group, 54 in the S group and 37 in the NS group. Data analysis revealed that both N and NS group had a significant improvement in NK after the intervention and the follow-up compared to baseline ($p < 0.001$). Moreover, these groups showed statistically significant differences in comparison with the other group at both times: C-N, C-NS, S-N and S-NS groups at T1 ($p<0.001$); C-N groups ($p = 0.001$) and C-NS groups ($p = 0.005$) at T2. Regarding MD adherence no statistically significant changes were found, besides a significant overall time effect ($p = 0.022$). PE and FNS scores revealed no statistically significant differences. Nutrition education could be effective in improving knowledge on the MD and at least encourage a change towards it. Indeed, the nutritional education intervention also appeared to have an impact on participants' ability to identify Mediterranean foods (assessed by the FC task). Detailed results about FC outcomes and other findings regarding snack exposure are currently being prepared for submission and will be presented in a forthcoming publication.

2.4 The NUBI web-app: a digital tool to improve nutritional knowledge and healthy eating habits in families with children

Purpose and methods. NUBI Cremona project, within ONFOODS project funded by the European Union – NextGenerationEU (ONFOODS, 2025), is a randomized-controlled pilot study intended to assess the effectiveness of a web-app in improving the adherence to a healthy diet, as the MD, and nutritional knowledge in families with children in Cremona city (Italy). Specifically, the NUBI web-app provided parents with healthy and balanced weekly menu proposals, based on what primary and kindergarten children consumed at the school canteen. A rule-based algortim was developed and allowed the creation of healthy and balanced combinations. Parents, furthermore, were supported by educational content structured in tips and topics related to a sustainable and healthy diet, provided within the web-app. Therefore, a 4-week intervention study was conducted, succeeded by a follow-

up phase of 8 weeks. Two randomization groups were considered: an intervention group (I) receiving menus and educational content via the web-app and a control group (C) without any kind of intervention. For both groups, data collection was carried out via the same tool, proposing 4 self-administered questionnaires: a sociodemographic questionnaire, a food frequency questionnaire (FFQ), a food parenting practices (FPP) questionnaire derived from a validated tool (Vaughn et al., 2017) and a nutritional knowledge (NK) questionnaire. Questionnaires were to be filled out at the beginning of the study (T0), at the end of the intervention (T1) and at follow-up (T2). Recruitment of families was done through distribution of flyers and informative meetings, in collaboration with schools.

Results. The NUBI study involved 4 primary schools and 2 kindergartens in Cremona city, by which it was possible to enroll the families. For organizational reasons, the study took place in 2 slots: the first started in min-January and ended in mid-April (n=68), the second took place from mid-February to mid-May (n=63). A total of 131 families were recruited by signing the informed consent and of these only 1 family stated that they no longer wished to participate in the study. Thus 62 families were assigned to the C group, 68 to the I group. Each family was given credentials to access the NUBI web-app and was asked to choose one parent to fill out all the questionnaires at the 3 survey times. Thirty-three parents completed both the NK and FPP questionnaires at T0 and T1. The remaining ones completed only part of the questionnaires or did not fill them in at all. Out of these 33 parents (39 ± 5 years; 82% female) 12 belong to the C group and 21 to the I group. The data collected via the socio-demographic questionnaire revealed that 58% (n=19) of parents have a university-level education. Of the respondents, 91% (n=30) stated that both parents are employed, nearly half in office jobs (42%, n=14 answering parents and 39%, n=13 the other parents). Most of the sample (89%, n=29) is married or with a partner and more than half of the families are in a middle-income class (70%, n=23). Table 3 displays the scores from the NK questionnaire for both groups, at the beginning and after the 4-week intervention. Overall, nutritional knowledge remained medium to high with a slight improvement in group I. Nonetheless, there were no statistically significant differences between the times in each group (Wilcoxon signed-rank test) and between the groups at each time (Mann-Whitney U test).

Table 3 *Descriptive analysis of nutritional knowledge (NK) scores for the intervention group and the control group at baseline (T0) and at the end of the intervention (T1).*

	Time	Intervention group (n=21)	Control group (n=12)
Nutritional Knowledge (NK) score 0-15 points	**T0**	10.00 (10.00-12.00)	11.50 (9.25-12.75)
	T1	11.00 (9.00-12-50)	11.00 (10.00-13.00)

Data are expressed as median and interquartile range (due to non-normal distribution, Shapiro-Wilk test).

The results concerning FPP questionnaire at T0 and T1 in both groups are given in Figure 1. Among the collected information it emerged that at both T0 and T1 more than half of the parents in group I (67%, n=14 at T0; 62%, n=13 at T1) believed that they often justify why they are given rules about food and its consumption (item 6). In both groups, with slightly higher frequencies in the intervention, the use of TV during weekend meals is always denied by the majority (items 8.1, 8.2, 8.3). For instance, 50% (n=6) of parents in C group reported never allowing TV during snack time at both T0 and T1. In group I, this was stated by 57% (n=12) at T0 and 52% (n=11) at T1. Most of the samples, both in the case of the I group and the C group, always provide and make fruit and/or vegetables easily accessible at home for the children (items 9 and 10). Specifically, regarding item 9 “Do you have fruits and vegetables that your child likes available at home?” 75% (n=9) and 83% (n=10) of parents from C group answered “always”, respectively at T0 and T1; 57% (n=12) of parents from I group answered “always” at both times.

Analysis related to the FFQ and follow-up data will further investigate whether the intervention may have led to changes over time in the different otucomes explored.

3. General Conclusions and Future Perspectives

To summarize, embracing a healthy and sustainable diet requires considerable effort at the individual and community levels since many determinants could affect people’s food choices and daily dietary habits. Exploring and investigating the context, focusing on both general population and individual family members, is mandatory to provide valuable insights into food habits, nutritional knowledge, sociodemographic and economic factors, as well as other potential barriers and drivers to the adoption of healthy eating patterns, as the MD. Consistently, the results of the cross-sectional study as part of the SWITCHtoHEALTHY project and partly summarized in this report made it possible to assess the Mediterranean context with a focus on families with and without children. The systematic review with meta-analysis within the European MoreMedDiet project will provide further insight into the context of socioeconomic and demographic determinants that can most strongly affect MD adherence in adult population.

Nevertheless, further research is necessary to identify limitations and strengths of the already developed methodologies to achieve more effective approaches. Possibly to comprehensively evaluate the stretegies

summarized in this work, a longer intervention period may be required. Indeed, as pointed out in the PROmedLIFE study, which highlighted a short-term improvement in nutritional knowledge among high school students receiving nutrition education, translating such awareness into consistent and concrete habits requires more time and sustained efforts. Equally important is the adoption of a multi-target approach to foster intra-family support and engagement with several community actors. The NUBI study, part of whose preliminary analysis was presented, and the derived App for children implemented in the framework of SWITCHtoHEALTHY project are an example of a web-app-based methodology that bridges the school canteen scenario with the family environment to support parents in managing their children's diet.

For future perspectives, overall, the described methodologies could be further implemented to educate, for example, on proper food parenting practices and empower families in developing competencies and skills to increase not solely nutritional knowledge but more generally a comprehensive improvement in food literacy.

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Oral presentation sessions
PhD students – 3rd year
Abstracts

PARALLEL SESSION 7
Smart and Sustainable Food Processing

Innovative Technologies and Processes for Sustainable Frozen Foods

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This Ph.D. project examines the effects of innovative freezing technologies and eco-friendly freezing and frozen storage (FFS) systems on the quality of food products. This study aims to explore: i) the effect of freezing and storage in a frozen state in solar-powered freezers (SPF) on the quality of frozen carrots compared with products stored in conventional freezers (CF); ii) the impact of freezing and storage in a frozen state of fresh hop cones as a potential innovative preservation technology; iii) the effect of Pulsed Electric Field (PEF) as a pre-treatment for preserving the quality of chicken meat during freezing and frozen storage.

Tecnologie e processi innovativi per alimenti congelati sostenibili

Questo progetto di dottorato esamina gli effetti delle tecnologie di congelamento innovative e dei sistemi di congelamento e conservazione eco-sostenibili (FFS) sulla qualità dei prodotti alimentari. Lo studio si propone di analizzare: i) l'effetto del congelamento e della conservazione in stato congelato in congelatori alimentati a energia solare (SPF) sulla qualità delle carote surgelate, in confronto ai prodotti conservati in congelatori convenzionali (CF); ii) l'impatto del congelamento e della conservazione in stato congelato delle infiorescenze fresche di luppolo come potenziale tecnologia innovativa di conservazione; iii) l'effetto del trattamento preliminare con Campi Elettrici Pulsati (PEF) per preservare la qualità della carne di pollo durante il congelamento e la stoccaggio congelata.

Keywords: Quality, freezing, Solar-powered freezing

1. Introduction

In accordance with the PhD thesis project previously described (Ozel, 2024), this work reports the main results of the activities:
(A1) Monitor the temperature of SPF and CF during freezing and frozen storage, evaluate and compare the effects of freezing and frozen storage in SPF and CF systems on the physicochemical properties of the carrots.
(A2) Evaluate the effects of freezing and storing fresh hop cones in a frozen state as a potential novel method for preservation in conventional freezers.
(A3) Investigate the effects of PEF as a pre-treatment for preserving the quality of chicken meat during the freezing process and frozen storage.

1. Evaluation of the effects of SPF and CF on the quality parameters of carrots

Freezing is a common food preservation technology; however, it often faces criticism for its high energy consumption, emissions, and reliance on non-renewable resources. Sustainable methods, such as utilizing solar energy to power freezing systems, can help minimize the environmental impact. SPF uses solar panels to convert sunlight into electricity by using photovoltaic (PV) technology. The panels, made up of solar cells, capture sunlight and generate an electric current. This current, regulated by a charge controller, is stored in a battery bank as direct current (DC) electricity for later use. When the freezer needs power, an inverter converts the stored electricity (DC) from the batteries into the alternating current (AC) required to run the freezer (Ahmed et al. 2019). Nevertheless, solar-powered systems are susceptible to temperature fluctuations and need further development. To test SPF performance and compare with the CF, carrot slices were frozen and stored at –20 and –40°C up to 180 days. FFS temperatures were chosen to be below and above the product's T'g (glass transition temperature of the product frozen at maximally concentrated solution), where molecular mobility is limited and physicochemical degradation is minimized during storage. The physicochemical characteristics of carrot slices were analyzed, and a comparative study was performed between SPF and CF.

2. Freezing and frozen storage of fresh hop cones

Hop cones, the female flowers of the *Humulus lupulus* plant, are primarily known for their use in beer production, where they contribute bitterness, aroma, and preservative properties. They contain a complex mixture of bioactive compounds, including alpha acids (e.g., humulone) and beta acids (e.g., lupulone). They are rich in alpha and beta acids, polyphenols, flavonoids, and essential oils (Abram et al., 2015).

Fresh hop cones are typically dried after harvest to ensure stability during storage and transport, enhance shelf life, and minimize microbial spoilage. Nonetheless, the drying process, particularly at elevated temperatures, may lead to a reduction in volatile aromatic compounds and degradation of important bioactive components like polyphenols and bitter acids (Raut et al., 2020; Rybka et al.,2018). In this section of the project investigates the impact of FFS of fresh hop cones as potential innovative preservation technology. Fresh hop cones were frozen at –20, -30, and –40°C (below, above, and close to its T'g) up to 270 days in conventional freezers.

3. PEF as a pre-treatment prior to Freezing and Frozen storage

Controlling freezing rate, ice crystal size, and achieving uniform freezing are essential for minimizing structural damage and preserving the quality of frozen foods. Pulsed Electric Field (PEF**)** technology has emerged as a promising pre-treatment to enhance freezing efficiency and improve the preservation of sensory and nutritional properties. PEF induces electroporation in cell membranes, thus facilitating better heat and mass transfer, accelerating freezing, and promoting the formation of smaller, more uniform ice crystals. This contributes to better texture, color, and nutrient retention in various food products. PEF pre-treatment shows potential as a sustainable and effective tool for enhancing freezing processes and improving the quality of frozen foods. However, successfully implementing PEF at an industrial scale requires addressing several challenges, including scalability, high equipment costs, and the need to optimize treatment parameters to ensure consistent and effective results (Rondineli et al, 2024). In this section, the project aims to investigate the impact of PEF as a pre-treatment in preserving the quality of chicken meat during cold storage, freezing process and frozen storage.

4. Materials and Methods

The temperature of the cells was monitored using a real-time system provided by the collaborating company, while that of the samples by a Mini-T1 Temperature datalogger (XS Instruments, Carpi (MO) – Italy) with an external pointed steel probe (Ø 4mm L=80 mm, accuracy of ±0.5°C). Fresh carrots (origin: Italy) were bought in a local market. Upon arrival, they were peeled and sliced (D: 2 ± 0.5 cm, thickness 0.5 cm). Thermal analysis was carried out on 7-10 mg carrot samples by differential scanning calorimetry (DSC) using a PE 8500 (Perkin Elmer, Boston, MA) equipped with a cooling system Carrot slices were subjected to blanching in water (90 °C for 3 min), cooled in melting ice for 5 min to minimize overprocessing, then drained. The following analyses were carried out: residual activity of peroxidase (POD) and pectin methylesterase (PME) according to Neri et al. (2011); moisture content, color (spectrophotocolorimeter, CIElab), mechanical properties (Instron Universal Testing Machine, recovery work %), and phenolic extraction and total phenolic content (TPC) according to Santarelli et al. (2021). Fresh, not blanched samples were used as a control (NT). BL and NT carrot slices were packed in a single layer into freezing bags and subjected to freezing and frozen storage for up to 180 days at -20 and -40°C in SPF and CF. At each selected storage time, samples were thawed and equilibrated at 4°C for 16-17 h and subjected to analyses. Data collected during the frozen storage were processed by multifactorial ANOVA to evidence the single and combined effects of the freezing method (FM), frozen storage temperature (T), and time (ST) under frozen conditions.

Fresh hop cones (cv. Cascade) immediately after harvesting were packed in a single layer into freezing bags and subjected to FFS in conventionally powered freezers at three different temperatures (-20°C, -30°C, and -40°C) for up to 270 days. For the characterization of chemical and techno-functional properties at each storage time and temperature, fresh hop cones were subjected to freeze-dried, then phenolic extraction was performed using methanol extraction solvent and ultrasonic bath for 30 min. The antioxidant capacity (AOC) was evaluated using the FRAP and trolox equivalent antioxidant capacity (TEAC) by ABTS•+ assay, while the total phenolic content (TPC) was determined through the Folin–Ciocalteu method. Additionally, chromatographic analysis was used to evaluate both the individual and total content of bitter acids and polyphenols using high-performance liquid chromatography (HPLC) equipped with a diode array detector (DAD).

PEF treatment was applied to fresh, boneless, and skinless chicken breast fillets using electric field strengths between 0.5–1 kV/cm and a pulse width of 20 microseconds. Non-pretreated chicken fillets were used as a control. One group of samples was subjected to blast freezing and stored at –20 °C for up to 60 days, while another group of samples was kept under cold storage conditions at 6°C for 7 days within their shelf life. To assess the impact of PEF treatment, electrical and thermal conductivity, thermal properties (freezable and bound water content) using Differential Scanning Calorimetry (DSC), texture profile analysis (TPA), color (cieLab), water holding capacity, drip loss, and cooking loss analysis were conducted immediately after PEF treatment and following storage periods. Based on preliminary studies, an electric field strength of 0.8 kV/cm was selected for the PEF pre-treatment.

5. Results and Discussion

5.1. Evaluation of the effects of SPF and CF on the quality parameters of carrots

Based on the thermal properties of carrots (Tgmid = -38.18 ± 0.6 °C), FFS storage temperatures were selected accordingly for the carrot slices. The temperatures recorded in SPF units were -20 ± 1.7 and -40 ± 2.8 °C, while those in CF units were -20 ± 1.5 and -40 ± 0.4 °C. From all the data collected during frozen storage (data not shown), it is possible to observe that in the SPF system, there are high temperature fluctuations (±1.7-2.8°C) due to the use of photovoltaic generators that produce energy not constantly. This is currently requiring an improvement of the energy generator system which is ongoing to limit temperature fluctuations. BL samples showed residual POD and PME activity of less than 10%, meeting industrial standards. Overall, BL samples had higher moisture content and TPC values than NT samples, along with significantly ($p<0.05$) higher L* values and recovery work (%), indicating greater brightness and elasticity, respectively.
MANOVA results (*data not shown*) highlighted that moisture content was significantly influenced ($p<0.01$) by FM, T, and ST and combined effect FM×ST and FM×T×ST. In particular, samples frozen in CF showed higher moisture content compared to those frozen in SPF. In general, during storage time moisture content of both samples stored in SPF and CF systems decreased and all samples showed higher moisture content at lower temperature (-40°C) for both freezing methods. On the contrary higher drip-loss was observed in samples stored in SPF and during frozen storage an increase of the dry matter occurred in all samples.
All individual and combined factors significantly influenced TPC ($p<0.05$). Higher TPC values were observed in CF frozen samples compared to SPF samples. Overall, no significant TPC variation was observed in BL samples after 30 days of storage, irrespective of the freezing method and temperature. These results highlight the efficiency of the blanching pre-treatment.
L* was significantly ($p<0.05$) influenced by individual FM, ST and all combined factors except for FM×T. SPF samples showed a higher L* value than CF samples. a* and b* parameters were significantly ($p<0.01$) influenced by individual ST factor and combined FM×ST and T×ST factors; additionally, a* parameter was significantly ($p<0.001$) influenced by the FM factor. a* and b* values showed an increase during frozen storage, which was associated with the yellowness and redness of carrot slices.
Recovery work was significantly ($p<0.01$) influenced by ST, FM×ST, T×ST, and FM×T×ST. During storage time, a decrease in recovery work, indicating an elasticity loss, was observed at all the investigated temperatures due to mechanical damage induced by ice crystal formation and growth. NT samples showed a lower elastic property than BL ones, possibly due to PME activity. The storage both in SPF and CF at -40°C (< T'g of carrots) reduced the differences in the carrots' quality attributes.

5.2. Freezing and frozen storage of fresh hop cones

Frozen hop storage at –40 °C initially preserved AOA better than at –30 °C and –20 °C, but this effect decreased over time; after 30 days, a similar trend was observed across all samples. Non-linear trends in antioxidant activity and bitter acid content were observed during storage, possibly due to the release of free and bound polyphenols during extraction, possibly due to mechanical damage induced by freezing on the plant structure, chemical and enzymatic degradation of phenolic compounds, and polymerization of phenolic compounds, resulting in the formation of new products with potentially higher reducing capacity. The complex effects of enzyme degradation and polymerization of phenolic compounds triggered by mechanical damage and freezing-induced ice separation from the maximally concentrated solution.
Figures 4.1.1illustrate the changes in xanthohumol, isoxanthohumol, and caffeic acid content in hop cones during frozen storage at different temperatures. Throughout the storage period, phenolic compounds exhibited fluctuating trends, likely influenced by a combination of chemical and enzymatic degradation and polymerization reactions, which may lead to the formation of new compounds with varying properties.
Overall, an increasing trend was observed for xanthohumol, caffeic acid, and catechin levels, while isoxanthohumol showed a consistent decrease. Xanthohumol, the main prenylated flavonoid in hops, is known to isomerize into isoxanthohumol during processes involving heat or pH changes, such as brewing (Stevens et al., 2004; Bamforth, 2017). However, under frozen storage conditions, especially at low temperatures, a reverse isomerization from isoxanthohumol back to xanthohumol may occur. Residual enzymatic activity or chemical catalysts may also facilitate this behavior even at low temperatures (Friedman, 1997). Additionally, the gradual release of caffeic acid may result from enzymatic hydrolysis or structural breakdown due to freezing-induced mechanical stress.

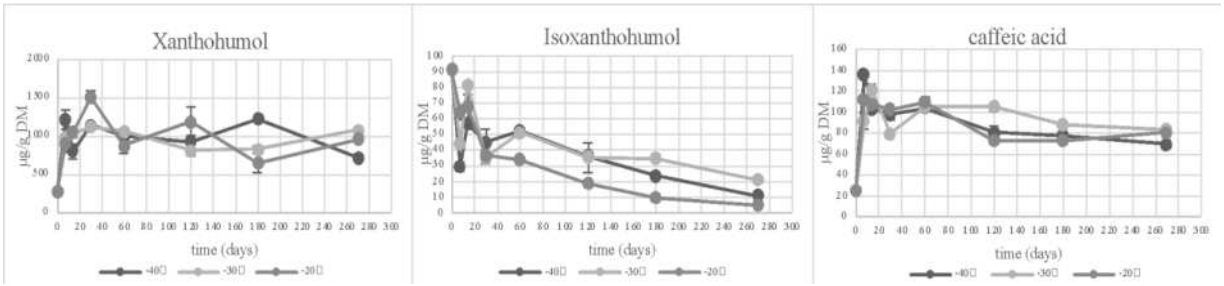


Figure 4.1. *Variation of xanthoumol, isoxanthohumol and caffeic acid of hop cones frozen and stored at different temperatures.*

5.3. PEF as a pre-treatment prior to Freezing and Frozen storage

Preliminary results indicate that PEF-treated samples exhibited significantly lower electrical conductivity ($p < 0.05$), higher lightness (L*) and redness (a*) values ($p < 0.05$), and reduced cooking loss compared to the control group. TPA revealed that PEF-treated samples had significantly lower hardness, gumminess, and chewiness ($p < 0.05$). No significant differences were observed in dry matter content, drip loss, or pH. Further analysis of the samples during frozen storage is currently ongoing.

6. Conclusions and Future Perspectives

Solar-powered freezers (SPF) offer an environmentally sustainable alternative to conventional freezing methods, though improvements are needed to reduce temperature fluctuations. Freezing and frozen storage show potential as preservation strategies for fresh hop cones, depending on the targeted compounds and their intended applications. Pulsed electric field (PEF) treatment is also a promising and eco-friendly innovation for enhancing freezing processes, but it requires further optimization and adaptation for large-scale industrial use.

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Integrating automation in food processing operations

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This PhD thesis focused on the integration of digital design for shape morphing of food and automation of food processing operations. Specifically, two food processes were investigated: the dynamic shape of food by digitally designed parametric surface grooves and the automation of the mixing ingredients.

Integrazione dell'automazione nelle operazioni di lavorazione degli alimenti

Questa tesi di dottorato è dedicata all'integrazione di tecnologie di digitalizzazione per la creazione di alimenti capaci di modificare la propria forma dinamicamente durante i processi di cottura (*morphing food*) e per l'automazione della fase di miscelazione di ingredienti al fine di migliorare la qualità di emulsioni alimentari.

Key words: automation; morphing food; modelling; controlled movement.

1. Introduction

According to the PhD project previously described (Di Palma 2023), this communication presents the results of three main experimental activities aimed at studying the following processes:

A1) Dynamic shape change of pasta triggered by digitally designed surface grooves.
A2) Computer-controlled system to create superficial grooves and precisely controlled shape-morphing behavior of cereal-based snacks during baking.
A3) Mixing oil/water emulsion using a Delta Robot.

2. State-of-the-art: Robotic Applications

Robotic systems have demonstrated significant potential for enhancing precision in food manufacturing by offering flexibility, operational efficiency, and reduced material waste (Table 1). Despite the widespread adoption of robotics in several industrial sectors, its use in the food industry remains largely confined to secondary operations such as picking, placing, sorting/grading, meat processing, packaging, and palletizing (Derossi et al., 2023).
Although the integration of robots in the food industry is of great interest, the full automation of *primary unit operations* remains a challenge.

Table 1 *Potential benefits and capabilities of robotic technology in food manufacturing (from EU, 2021)*

Industrial robot capabilities		Benefits
DECREASE	PRODUCTION COST	Reduce costs associated with manual labor and utility expenses
	MATERIAL WASTE	Increased efficiency allows for reduction of production material waste and less scrap from rejects
	FLOOR SPACE	Compact systems with mounting versatility
	PRODUCTION TIME	Higher speed and efficiency, fast re-configurability
IMPROVE	PRODUCT QUALITY	More efficient process control, high repeatability and accurate task execution
	PRODUCT UNIFORMITY	Errors caused by human error and fatigue eliminated
	WORKING ENVIRONMENT	Existing labor upgraded, removes humans from unfavorable conditions and tedious tasks
INCREASE	PRODUCTION RATES	Ability to produce 24/7 without disruptions
	FLEXIBILITY	Reconfigurable and easy to apply to a variety of tasks
	SAFETY COMPLIANCE	Works in hazardous environments, made of hygienic materials
	COMPETITIVE ADVANTAGE	Faster response to market demands, allows for product customization and personalization
	EFFICIENCY	Optimized processes, increased yield (reduces production material waste, scrap from rejects)

3. Experimental Procedure

The experimental activities were designed to study how a digitalized approach and automation can contribute to the development of innovative food products and the quality of oil-in-water emulsions. The research activities

were organized into two main work packages:

(i) Morphing food through the precise control of designed surface grooves and predictive modelling.
(ii) Advances in controlled mixing of emulsion by a Delta-Robot.

The first research line consisted of three steps:

1. Study of shape morphing of pasta samples triggered by surface grooves. Particularly, the effects of the thickness and the depth of the surface grooves of pasta samples and snacks were studied.
2. The mathematical description of the morphing dynamics under different groove configurations and baking conditions, with the purpose of describing the process and enhancing shape control.
3. The use of an automatic system to generate groove-patterning on the surface of snacks enabling the control of the dynamic change of the shape during baking in oven.

A 3D-printed plastic mold was employed to generate the grooves on the top surface of samples under various experimental conditions. These included variations in the thickness of the samples, groove depths, and applied pressure, as illustrated in Figure 1a. For both pasta and snack samples, the shape changes over time were assessed by measuring the bending angle during cooking. To describe the dehydration/rehydration kinetics and the morphing behavior, different empirical models - i.e. Peleg, Weibull, Four-Parameter Logistic, and Logarithmic regression models, were utilized. Moreover, to provide a physical description of the phenomenon and understand the relationship between sample geometry, material properties, and curvature behavior, a finite element model (FEM) was developed using COMSOL Multiphysics. This approach allowed simulating the thermomechanical behavior of the grooved samples during heating, offering predictive insights into how design parameters influence the final shape.

The second work package focused on the use of a 3D Delta Robot for the mixing of an oil/water emulsion (Figure 1b). To design unconventional mixing paths, Full Control G-code designer was used to digitally design the robot's trajectories. A Central Composite Design (CCD) was applied to explore the combined effects of the process variables - mixing speed, duration, and mixing path - on some key aspects of the emulsions including the viscosity and the size distribution of oil-droplet. These parameters were selected as indicators of emulsion stability and homogeneity and were used to compare the performances of the studied patterns.

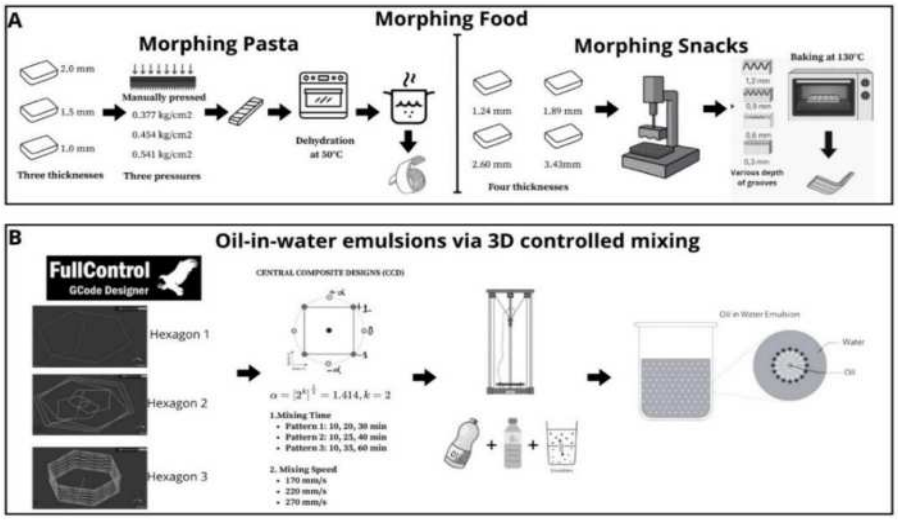


Figure 1 *A) Experimental conditions used to create surface grooves that induce morphing in two types of food: pasta and snacks. B) Experimental conditions for producing an oil-in-water emulsion through 3D-controlled mixing movements.*

4. Results and Discussion

4.1 Morphing Pasta: effect of the process variables on the rehydration rates and bending angles

Figure 2 shows the estimated rehydration rates, *k*, as a function of the thickness of the sheeted dough and the depth of the grooves. Results show a progressive increase in the rehydration rate of pasta samples as a function of the pressure applied to create the grooves, especially for samples of 1 mm and 1.5 mm thick. Particularly, values between 0.36 and 0.44 g H_2O/ g* min^{-1} for samples of 1.5 mm, and between 0.52 and 0.62 g H_2O/ g* min^{-1} for samples of 1.0 mm were estimated. Contrarily, the rehydration rates were almost constant at 0.25 ± 0.019 g H_2O/ g* min^{-1} for samples 2.0 mm thick. These results suggest that the rehydration rate of the pasta samples was mainly dominated by the thickness of the remaining layer of dough below the grooves, while it seems less dependent on

the depth grooves although significant effects have been revealed for the thinner samples.

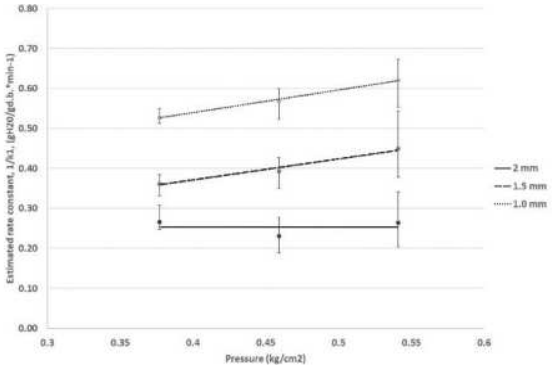


Figure 2 Estimated *rehydration rates, 1/k1, of pasta samples cooked in water at 95 ◦C.*

To better elucidate this behaviour, the estimated *k* values were compared as a function of both the depth of the grooves and the height of the remaining layers below the grooves. First, the significant differences of the rate constants well sustain the main effect of the original thickness of the sheeted dough. By analyzing the depth of the grooves (D) and the height of the remaining layers below such grooves, (L), significant differences were observed even when the same pressure was applied. For instance, for a pressure of 0.377 kg/cm^2, the depths of the grooves were 1,096.99 ± 57.01, 680.02 ± 77.95 and 336.68 ± 56.46 µm while the height of the remaining layers were 1,245.66 ± 86.66, 801.60 ±74.40 and 636.98 ± 56.54 µm, respectively for sheeted dough of 2.0, 1.5 and 1.0 mm. Furthermore, by applying a pressure of 0.377 kg/cm^2, D/L values of 0.88, 0.84 and 0.52 were obtained for the samples which are in agreement with the increased estimated rehydration rates of 0.26, 0.36 and 0.52 g H_2O/g* min^{-1} (Figure 2). These data confirm the primary effect of the height of the remaining layer of dough below the grooves on the rehydration rate of the samples during conventional cooking in hot water.

Figure 3 shows the bending angle of pasta samples as a function of cooking time in hot water. The value greater than 360° indicates the formation of more than one loop. For samples of 1.0 mm and 1.5 mm thick 5 min of cooking was enough to obtain a bending angle greater than 360°. On the other hand, about 8-11 min were necessary for samples of 2.0 mm thick to obtain similar bending, confirming the great effect of the initial thickness of the samples. The Four Parameter Logistic model satisfactorily described the shape change of the samples over cooking time showing correlation coefficients between 0.986 and 0.992. Especially, for samples 2.0 mm thick, the bending occurred slowly and almost independently from the applied pressure. For thinner samples, the rate of bending notably increased with values from 3.846°min^{-1}, for samples of 1.5 mm thick and P = 0.377 kg/cm^2, to value of 7.083◦min^{-1} for samples of 1 mm thick and P = 0.541 kg/cm^2. In addition, by increasing the applied pressure, a progressive increase of the rate of bending was observed even though such differences were significant ($p < 0.05$) only for the samples with thicknesses of 1.5 mm and 1 mm.
Moreover, the data clearly confirms that the height of the remaining dough layer below the grooves plays a primary role in determining the rehydration rate during conventional cooking in boiling water (Di Palma et al., 2025a).

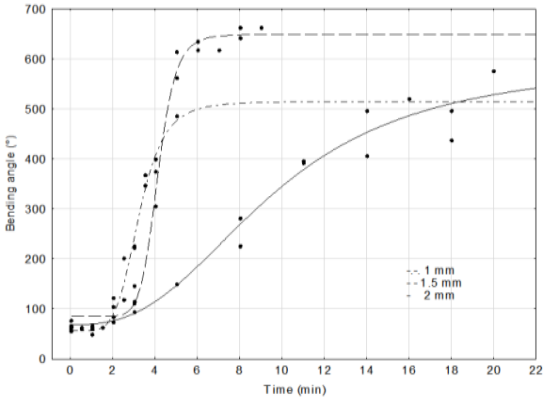


Figure 3 *Evolution of the bending angle of pasta samples with designed grooves over cooking time.*

4.2 Semi-automated system for surface groove formation and shape morphing of snacks during baking in the oven

A computer-controlled system was used to develop highly precise and constant depth of the surface grooves,

aiming to enhance the overall control on the shape morphing of cereal snacks while baking in oven. The system consists of a texture analyser mod. TA-XT Plus (Stable Micro Systems, Godalming, Surrey, UK) equipped with a cylindrical probe and the 3D printed plastic mold to control the displacement of the mold (%) from the surface of the samples. The relation between the displacement of the probe and the depth of the grooves was mathematically modelled as defined by Di Palma et al. (2025b) (Figure 4a). For the thicker samples (3.43 mm), displacement in the range of 25% - 45% generated depth grooves respectively of 747.50±51.87 µm and 1722.14±65.81 µm. Sheeted dough with a thickness of 2.60 mm showed groove depths of 594.21±51.16 µm and 1645.00±72.42 µm using the same displacements. In addition, the thinner samples (1.24 mm) displacements of 25% and 40% generated groove depths of 459.78±44.42 µm and 709.21±38.47 µm, respectively.
Figure 4b reports the fits of the experimental data obtained by using logarithmic regression. In all cases, the model precisely described the changes of groove depths as a function of displacement percentage, showing correlation coefficients, *r*, always greater than 0.94. Then, the estimated model's parameters were utilized to compute displacements necessary to obtain the desired groove depths and a second series of validation experiments were performed. When analysing samples of 3.43 mm thick, the following estimated and experimental values were respectively obtained: 1236.32 µm and 1175.83±53.15 µm for applied displacement of 32%, 932.50 µm and 862.33±69.66 µm for applied displacement of 27%, and values of 643.88 µm and 635.87±6.01 µm for applied displacement of 23%. In the case of the thinner samples (1.24 mm), depth grooves of 610.91±43.96 µm and 301.70±39.36 µm were obtained, precisely matching the estimated values of 666.07 µm and 311.14 µm when displacements of 31% and 25% were applied. A slightly higher deviation was obtained for displacements of 36% with measured and estimated data of 874.44±75.14 µm and 912.80 µm

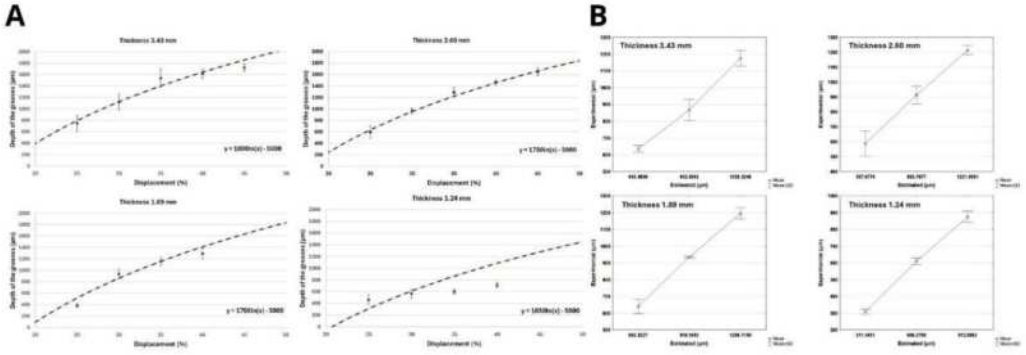


Figure 4 *A) Measured groove depths in dough sheets of different thicknesses plotted against the applied displacement percentages using the semi-automated system. Error bars denote experimental variability, and dotted lines represent the fitted model. B) Comparison of experimental and estimated groove depths in dough sheets of varying thickness obtained by the semi-automated system.*

Figure 5 shows the quantitative analysis of the bending angle of samples with thickness of 1.89 and 1.24 mm.

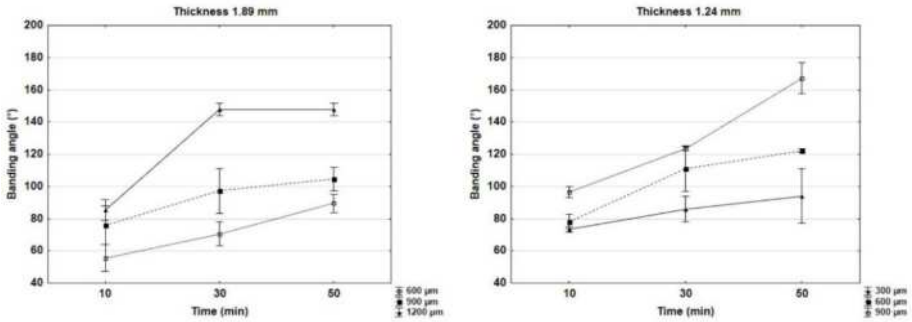


Figure 5 *Bending behavior of 1.89 mm and 1.24 mm thick samples under varying experimental conditions.*

4.3 Optimizing oil-in-water emulsions via 3D controlled mixing: assessing the impact of process parameters
By combining motion engineering with material science, the physical-chemical process for producing oil-in-water emulsions was optimized through the use of three-dimensional mixing movements, going beyond conventional planar systems. In this experiment, three different mixing patterns were digitally designed and tested. Various process conditions were explored, allowing for the evaluation of the individual and combined effects of mixing time and speed on the particle size distribution across the three patterns (Figure 6). The analysis focused on the size distribution of oil droplets with particular attention to the peak of dQ3(x), as a representative index of

emulsification performance.
To statistically evaluate the influence of process variables, a General Regression Model (GRM) was applied. This approach enabled the identification of significant linear and interaction effects of the independent variables on the responses. The regression coefficients, r^2, ranging from 0.976 to 0.998, demonstrated the ability of the models to describe the effect of the time and speed of the mixing on the particle size of the oil droplets in the emulsion. The influence of the process variables was examined through Pareto charts. For Pattern 1 (Figure 6), both time and speed as well as their interaction significantly affected the size distribution of the oil particles with estimated effects of 28.84, 3.47 and 35.54. Similar results were observed for Pattern n.2 for which the non-linear effect of the mixing time was higher than that of the mixing speed (13.28 and 5.80). Also, the non-linear interaction greatly affected the particle size of the oil droplet with an estimated effect of 13.75. The higher effect of the mixing time suggests a speed threshold beyond which further increases do not improve emulsification efficiency.

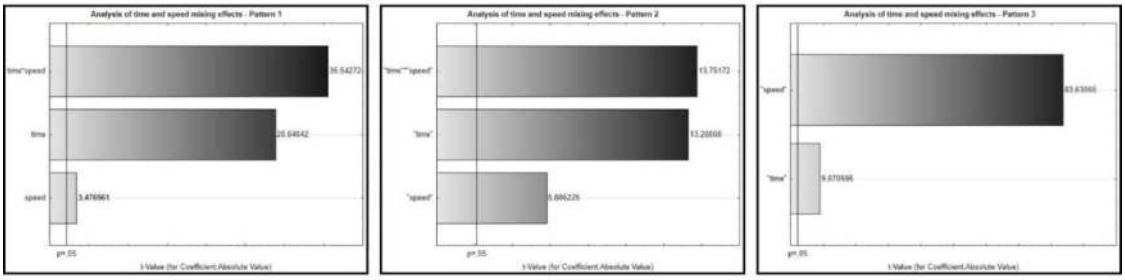


Figure 6 *Pareto charts highlighting the effects of mixing time and speed for each pattern.*

For pattern 3, both time and speed influenced the size distribution of the oil droplets in the emulsion, but speed resulted in being the dominant factor with an estimated effect of 83.63. Time is highly influential on its own, while speed contributes to a lesser extent. In addition, by analyzing the viscosity of the oil-in-water emulsion the effect of the three mixing paths was observed when comparing similar mixing time and speed (data not shown). These findings underscore the importance of designing mixing systems that exploit time-speed interactions rather than relying solely on increased rotational speed.

5. Conclusions and Future Perspectives

Despite the growing interest in digital approaches and automation of food processing, significant challenges remain for fully realizing the potential of such emerging opportunities. This doctoral research offers new information regarding the digital design and automation of food manufacturing with a particular focus on the controlled dynamic shape of food during baking/cooking and the use of innovative mixing paths of oil-in-water emulsion systems performed by a Delta Robot. After a first series of experiments in which surface grooves on pasta samples were created manually, a lab-scale automated system capable of generating precise grooves on sheeted dough was tested demonstrating the opportunity to precisely control the shape morphing of food products. For the first time, three digital models consisting of mixing patterns for oil-in-water emulsions were developed and tested by using Delta Robots. The mixing movements affect the main properties of the emulsion, i.e. the particle size of oil droplets and the viscosity.
Looking forward, advancements in artificial intelligence, machine learning and mathematical models are expected to enhance the adaptability of digital approaches and robotic food manufacturing to respond more effectively to complex challenges of the food systems such as customization, food loss, sustainability, etc.

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Integrating food reformulation strategies and emerging technologies to promote healthy diet

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Tutor: Prof. Antonio Derossi

This doctoral thesis aims to apply emerging technologies and food reformulation strategies by using matrices derived from food by-products of plant origin and from underutilized plants as alternative sources of nutrients, developing innovative foods with enhanced nutritional and sensory properties and promoting the adoption of a healthy diet.

Integrazione di strategie di riformulazione degli alimenti e tecnologie emergenti per promuovere una dieta salutare

Questa tesi di dottorato ha l'obiettivo di integrare strategie di riformulazione, utilizzando anche sottoprodotti derivanti dell'industria alimentare o risorse poco utilizzate, e tecnologie emergenti per lo sviluppo di prodotti innovativi con migliorate caratteristiche nutrizionali e sensoriali, al fine di incentivare l'adozione di una dieta salutare.

Key words: food reformulation, nutrient enhancement, emerging technologies, circular economy, healthy diet

1. Introduction

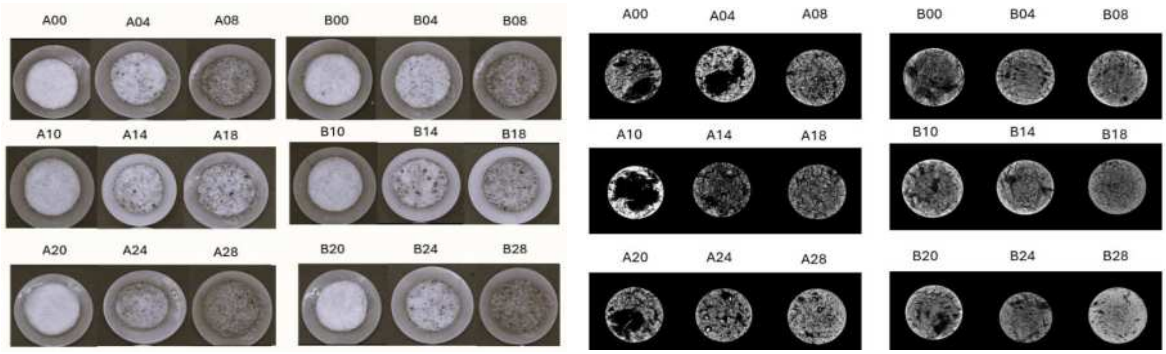
According to the PhD research project previously outlined by Marinaro (2023), this oral communication presents the principal findings from the following activities:
A1) Development of novel food products incorporating ancient wheat varieties and glasswort powder;
A2) Valorization of okara for the formulation and development of innovative buns;
A3) Integration of 3D food printing and by-products to elevate the nutritional quality of air-fried snack products.
Global food waste poses a significant challenge in the face of rising malnutrition and increasing pressure on natural resources (FAO, 2019). To address these issues, there is a growing interest in sustainable nutrient sources and technological innovations to enhance the nutritional quality of foods while reducing components linked to non-communicable diseases (Yusta-Boyo et al., 2024). In this context, and as examples glasswort - traditionally valued for its salty flavor - has emerged as a natural salt substitute in snack formulations, enabling sodium reduction without compromising taste and contributing valuable antioxidants and minerals (Alfheeaid, 2022). Another interesting source of nutrients is okara. Its nutritional composition typically consists of 20–30% protein and 30–50% dietary fiber making it particularly suitable for improving the health profile of various formulations (Santos et al., 2019), including bakery products and plant-based alternatives. Moreover, its neutral taste and high water-holding capacity enable its integration into diverse matrices without compromising sensory quality. Furthermore, the use of emerging technology such as 3D Food printing has been indicated by FAO in a recent list of the forty-four technological innovations in the food sector during the next 20 years (FAO, 2025). This technique enables the fabrication of customized, ready-to-eat snacks with appealing sensory attributes (Derossi, 2024). The study also investigated the influence of printing parameters on oil absorption during air frying—a post-processing method shown to replicate the crispiness of traditional frying while significantly reducing fat content (Téllez-Morales, 2024).

2. Development of novel food products incorporating ancient wheat varieties and glasswort powder

Fresh glasswort was locally collected (Margherita di Savoia, BT, Italy), washed, and dehydrated at various temperatures. The description of kinetic dehydration of glasswort and the assessment of the most important quality was performed to define the optimal conditions able to ensure water activity < 0.4 while protecting the original color of the glasswort. Dehydration experiments were performed by using a domestic drier (Klarstein). An ancient and a modern wheat variety - Autonomia B and Bolero - were utilized to prepare the samples. The dried glasswort was ground at 10 rpm for 5 seconds (Grindomix GM 200, Retsch, Germany), sieved at 600 μm, and then stored at room temperature until the experiments. For the reformulation of savory snacks, a Box-Behnken Design (BBD) was employed to evaluate the effects of salt (0%, 1%, 2%) and glasswort powder (0%, 4%, 8%) concentration on the main quality parameters of the savory biscuit-like snacks. A total of 18 experiments were conducted. Doughs were prepared with wheat flour (60%), olive oil (15%), water (20%), salt, and glasswort, then shaped and baked at 180°C for 20 minutes. Moisture content and water activity were measured using gravimetric methods (AOAC–925.10) and a dew point water activity meter (AquaLab 4 TE), respectively. Color measurements were performed

by using a colorimeter (CR 400, Minolta). Below are the main results. The description of the dehydration kinetic allowed us to define treatments at 70°C for 7.2 hours as the best conditions to ensure safety and to protect the original color of the glasswort. Particularly, Glasswort powder exhibited color parameters of 45.84±3.5, 5.36±1.8 and 15.36±1.4 respectively for L*, a* and b*. A final moisture content of 10.37±0.2 and water activity of 0.483±0.00 were measured. Furthermore, the granulometric analysis showed cumulative volume fraction of 10%, 50% and 90% for sizes of 58.5±4.3, 272.4±26.7 and 533±56.4 µm, respectively. Regarding the color of the snacks products, the results indicate that glasswort had a significant impact on luminosity, showing estimated linear and nonlinear effects respectively of -15.78 and -4.00. In contrast, salt addition did not show any noticeable influence. By studying the surface plots obtained by using the BBD approach, it was clear the great effects of glasswort addiction on the luminosity of the samples which reduced from 68 to 53 by incorporating the 9% glasswort powder in the food formula. The visual aspect of snacks is shown in Figure 1a. In reformulation strategies of food another very important aspect is the effect of the ingredients on the hardness of the samples which were analyzed for both the wheat varieties (Figure 2). Statistical analysis shows that - for the Bolero variety - glasswort had a softening effect, with negative linear and quadratic effect of -9.20 and -3.18, respectively, while the salt had a minor positive hardening effect (+2.06) (data not shown). The 3D surface plot effectively illustrated the changes in sample hardness, showing that samples without glasswort exhibited the highest hardness value (~80 N). In contrast, increasing the glasswort content above 6% resulted in a consistent decrease of the hardness, reaching values below 48 N. Similar trends were observed for the samples formulated by using Autonomia B variety, where both linear and quadratic effects of glasswort addition on hardness were significant, with estimated effects of –10.85 and –5.57, respectively. Salt content influenced hardness only through a non-linear, positive effect, with an estimated coefficient of 3.09. Microtomographic images (Figure 1b) revealed the existence of larger pores in snacks formulated with Autonomia B flour compared to those made with Bolero, leading to which showed a denser and more uniform structure. Total porosity values were in the range of 70-90% and 55-80%, respectively, for Autonomia B and Bolero. Increasing the amount of glasswort reduced the total porosity (%) in Autonomia B samples, suggesting that the high fiber content contained in such ingredients could reinforce the dough leading to a more compact structure. In Bolero-based snacks, the dense structure was further reinforced by the combined effect of glasswort and the wheat's inherently stronger gluten network. Sensory analysis showed that the addiction of the glasswort significantly enhances saltiness perception of the snacks even with low salt content (Fig. 3). Samples with high glasswort but low salt (e.g., A14, A18, B14, B18) were perceived as saltier than those higher in salt concentration. In addition, the snacks perceived to be more salty - i.e. A28 and B28 - were evaluated with a low level of overall liking. Moderate glassworts improved both saltiness perception and acceptability, as shown for samples A14 and B14. Bolero-based snacks generally showed a more positive response to glasswort in terms of saltiness and acceptability compared to Autonomia B.

Fig .1. *Photographic images of the samples formulated with Autonomia B and Bolero wheat varieties (a). Representative binary 2D cross-sectional images of baked snacks (b). Autonomia B (A), Bolero (B), first number indicates salt concentration, second number indicates glasswort concentration.*



In both Bolero and Autonomia B samples (Tab.1), by increasing the glasswort content a significant decrease in RDS was observed suggesting a slower glycemic response. Concurrently, SDS content increases, particularly in Bolero samples (B14 and B18), indicating a shift toward more slowly digestible starch. TDS follows a similar decreasing trend with higher glasswort content, especially in Bolero samples. Notably, RS levels remain very low, and no statistically significant differences were observed across all formulations. Total starch content (TS) decreases with the addition of glasswort, more markedly in Bolero samples. This could reflect the dilution effect of glasswort's non-starch components or interactions affecting starch availability.

Fig. 2. *Surface response plot describing the effects of NaCl and glasswort on the hardness of snacks formulated with wheat variety Bolero (a) and Autonomia (b).*

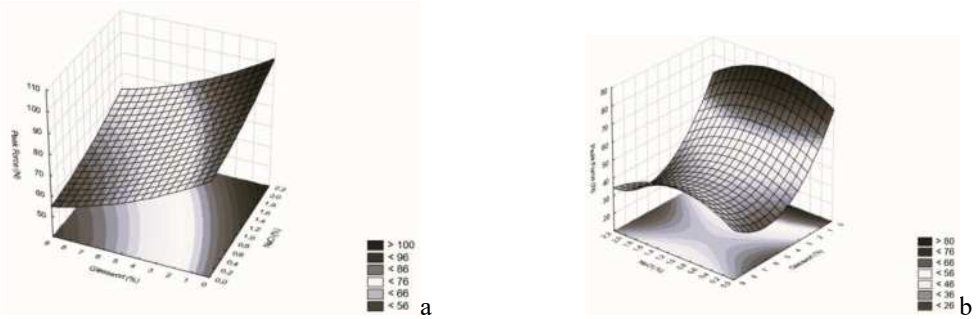
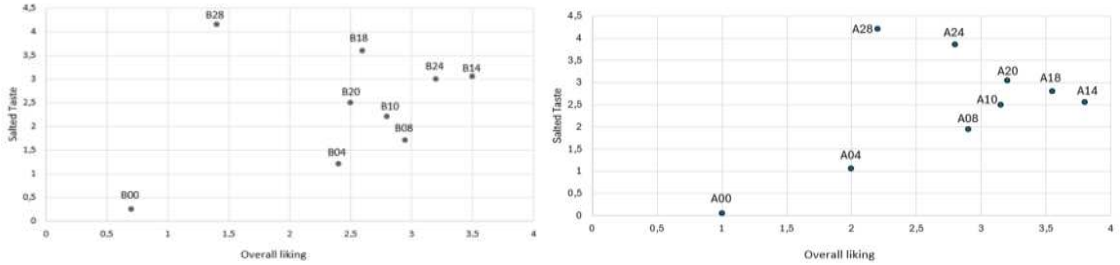


Fig.3. *Saltiness perception and appreciability of glasswort snacks made with Bolero (B) and Autonomia (A).*



***Tab. 1**. Digestible starch fractions (g starch/100 g sample d.b.)*

Sample	RDS	SDS	RS	TDS	TS
A10	50.3±2 A	5.85±2.4 A	0.26±0.02 A	54.96±0.04 A	55.22±0.2 A
B10	55±0.14 a	2.7±0.5 a	0.17±0.03 a	57.5±0.3 a	57.7±0.4 a
A14	46±0.3 AB	11.8±0.05 B	0.3±0.01 A	54±2.3 A	54.6±1.5 A
B14	43±1 b	9.3±1 b	0.4±0.06 a	51.2±2.8 b	51.6±2.9 b
A18	43±1 B	10.6±0.04 B	0.34±0.03 A	51±1.3 A	51.2±0.9 A
B18	46.14±3 b	4±0.2 c	0.3±0.09 a	47.9±0.2 b	48.9±0.1 b

Statistically significant at $p<0.05$

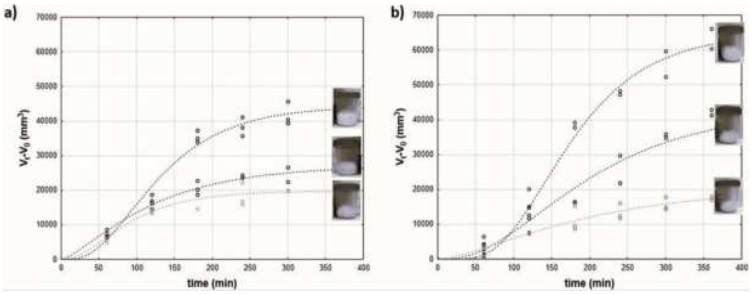
3. Valorization of okara for the formulation and development of innovative buns

Powdered okara was supplied by Dukan Boutique (France). A physic-chemical characterization revealed the color parameters of 76.5±1.2, 2.1±0.3 and 18.4 ±0.5, for L*, a* and b* respectively, a moisture content and aw values of 6.87±0.4 gH2O/100g w.b and 0.457±0.00, and a particle size of 60.28±0.8, 158.06±1.8, 296.12±5.8 µm for 10%, 50% and 90% distribution. Three dough formulations were developed: a control with no okara (F0), and 5% (F5) and 10% (F10) okara powder replacing wheat flour (w/w). Each formulation was fermented at 30°C using *Saccharomyces cerevisiae* alone (L1) or in combination with *Lactiplantibacillus plantarum* (L2). At selected time intervals, volume increment was calculated on photographic images and the kinetic of proofing was evaluated with the Chapman equation:

$$\Delta V(t) = \Delta V_{max} \times [1 - e^{(-bt)}]^c \quad \text{(Eq.1)}$$

Based on the evaluation of the proofing performances and on the protein content, analyzed with the method reported in Caporizzi et al. (2025), selected dough were further baked at 180°C and then used to assess the physical properties of the baked buns, immediately after baking and during storage for 7 days at 25°C. Specifically, water activity, moisture content with the gravimetric method (AOAC 925.10), textural properties (TAX-T2 texture analyzer, Stable Micro Systems, Surrey, UK) and microstructural characteristics (SkyScan 1174, Brüker, SkyScan, Kontich, Belgium) were investigated. Results showed that okara increased protein content (Tab.3) but reduced leavening performance, especially at high concentration of 10%. In Fig.4 it is reported that there is an evident reduction in the maximum volume increment with increasing levels of okara in the dough formulation. However, 5% of the substitution provided acceptable leaving behavior. Physicochemical analyses revealed a gradual decrease in water content and activity during storage in the control sample, reaching values, respectively, of 17.5 g H20/100g and 0.804 after seven days. In contrast, F5 exhibited an initial reduction until day 3, followed by a slight increase at day 7 (19.03g H20/100g). Textural properties were mostly unchanged between F5 and F0 samples, though chewiness increased slightly (from 4.201±0.717 N*mm for F0 to 5.455±1.902 N*mm for F5). Microstructural analysis showed a more compact crumb with smaller and less connected pores in F5 with respect to control (F0), with porosity values of 79.68% and 75.29% respectively (data not shown).

Tab.2 *Protein content of raw materials and doughs*



Sample	% crude protein
Soft Wheat Flour	9.07±0.01[b]
Okara powder	27.1 ± 0.2[a]
L1_F0	9.46±0.06[b]
L1_F5	9.83±0.05[a]
L1_F10	9.88±0.04[a]
L2_F0	8.99±0.10[d]
L2_F5	9.32±0.03[c]
L2_F10	9.40±0.01[bc]

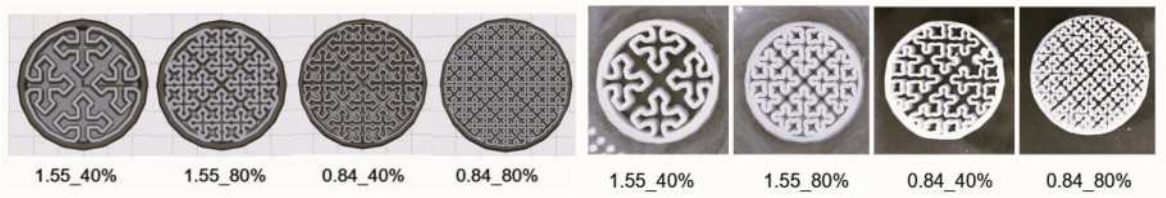
Statistically significant at $p<0.05$

Fig. 4*. Volume increment during leavening with L1- S. cerevisiae (a) and L2- a mix of S. cerevisiae and L. plantarum (b).*

4. Integration of 3D printing and by-products to elevate the quality of air-fried snacks

The okara powder (Dukan New Boutique, France) was mixed with water (ratio of 20:80 v/w) by using a lab-scale mixer (Cooking Chef, Kenwood Ltd., UK) for 10 minutes at speed 6. For the air-frying process, a blend of vegetable oils was used (Fratelli Mantova, Italy), consisting of oleic sunflower oil (65%), avocado oil (20%), and rice oil (15%). The printing variables were optimized using the software Ultimaker Cura. The experiments were designed by modulating two nozzle diameters (0.84 mm and 1.55 mm) and two infill densities (40% and 80%) resulting in a total of four experimental conditions, labeled as follows: 0.84 mm_40%; 0.84 mm_80%; 1.55 mm_40%; 1.55 mm_80%. (Fig. 4).

Fig.4. *Digital models and photographic images of printed samples.*



For the air-frying process, oil was sprayed onto the top surface of the sample from 5 cm. The quantity of a single spray was preliminarily defined to maintain the oil-sample ratio of 0.1:1 g/g. Single or double sprayes of the oil were conducted according to the weight of the 3D printed object, respectively considering the two infill levels above reported. Air frying experiments were conducted by using a domestic system (Ariete, 4631, Italy) at 180°C. Baking kinetic was described by using a Weibullian model:

$$xw(t) = xw_0 * \exp(-k * time^n). \quad \text{(Eq. 2)}$$

where xw is the moisture content (g H2O/g w.b.) at time *t*, xw0 is the moisture content of the raw sample, *k* is the dehydration rate (min-1), and *n* is a shape factor. The estimated kinetic parameters of air-frying processing for all experimental conditions are reported in table 6. Then, the baking time required to obtain the final moisture content of 5 g H20/100 g w.b. was estimated and validated (Figure 6). The samples were then analyzed for their oil absorption using the gravimetric method (A.O.C.S, 1989). Increasing the infill level from 40% to 80% a significant increase in oil absorption was observed from 23.24±2.4 to 37.16±4.8 g oil/100 g d.b. (Figure 5). This result suggests that denser structures (80%) absorbed more oil, probably for greater top surface area available for oil contact during spraying and frying. A similar trend it was observed for samples obtained by using the larger nozzle (1.55 mm) with values of 20.3±5.3 g oil/100 g d.b. and 9.6±1.8 g oil/100 g d.b. respectively for infill level of 80 and 40%. Furthermore, the use of a smaller nozzle diameter (0.84 mm) resulted in higher oil uptake compared to the larger one (1.55 mm), for both infill levels.

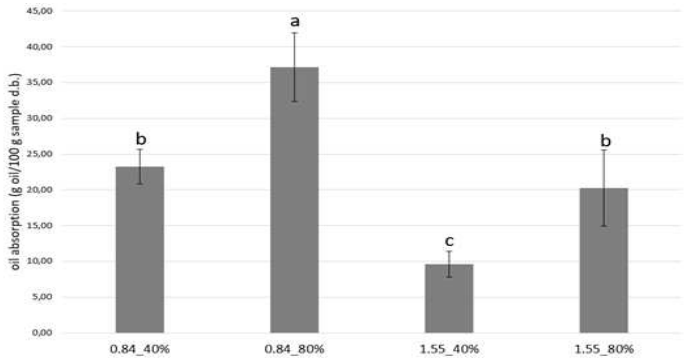
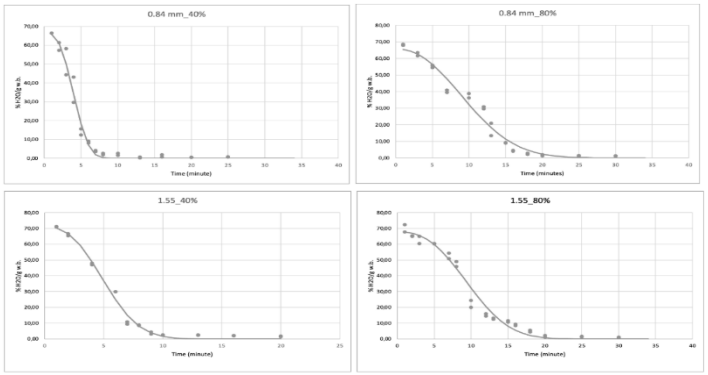


Fig. 5. *Oil absorption (g oil/100 g sample d.b.) of printed snacks*

Fig.6 *Dehydration kinetic of air-fried samples developed by using 3D food printing.*



Tab.6 *Kinetic parameters derived from data fitting.*

Samples	k	n	Estimated time(min) for moisture content = 5 (gH2O/100g w.b.)
1.55mm 40%	0.01119	2.53443	9
1.55mm_80%	0.00189	2.59400	16.30
0.84mm_40%	0.01053	2.99183	6.30
0.84mm_80%	0.00424	2.22560	18

5. Conclusions and Future Perspectives

These findings demonstrate that the integration of food reformulation strategies with emerging technologies can offer effective solutions for the development of innovative and health-oriented food products. Functional ingredients derived from plant-based by-products - such as glasswort and okara - contributed to improved nutritional profiles of traditional bakery items, supporting salt reduction, increased protein content, and reduced starch digestibility. Furthermore, the application of advanced technologies, i.e. the 3D food printing, underscores the potential of digital and innovative approaches to modulate oil absorption during air-frying promoting more healthy products. Collectively, these strategies not only align with current trends in consumer health awareness but support the broader goals of sustainability by promoting the valorization of food by-products and fostering circular food production systems.

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Application of IoT and Machine Learning Technologies for Monitoring Hop Cone Ripening

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This study aims to predict the optimal hop cone ripening using Smart Agriculture (SA) technologies, including Internet of Things (IoT) and Machine Learning (ML) models. Two ML algorithms were developed to classify ripening stages (immature, mature, overripe) using a soft voting classifier. To this end, the identification of the optimal harvest window required a three-year morphological and chemical characterization of hops at different ripening stages. Finally, in the fourth year, the ML algorithms forecasted the optimal harvest maturity in the first half of September, coinciding with the peak accumulation of brewing-relevant chemical compounds.

Applicazione di Tecnologie IoT e Machine Learning per il Monitoraggio della Maturazione di Coni di Luppolo

Questo studio mira a identificare il momento ottimale di maturazione dei coni di luppolo, attraverso tecnologie intelligenti in agricoltura, quali Internet of Things (IoT) e Machine Learning (ML). Sono stati sviluppati due algoritmi di ML e classificate le fasi di maturazione (immaturo, maturo, troppo maturo) utilizzando un classificatore a voto ponderato. Quindi, i coni di luppolo a diversi stadi di maturazione sono stati caratterizzati morfologicamente e chimicamente per tre anni. Nel quarto anno, gli algoritmi di ML hanno previsto la raccolta nella prima metà di settembre, in coincidenza con il picco di accumulo dei composti chimici di interesse birrario.

Key words: *Humulus lupulus* L.; bitter acids; Essential oils; smart agriculture; ripening prediction

1. Introduction

Hop (*Humulus lupulus* L.) is a perennial, climbing, and dioecious plant whose female inflorescences are used in the brewing industry for the presence of lupulin, a resinous substance rich in bitter acids and essential oils (EOs). The biosynthesis and accumulation of bitter acids and EOs start with cone development and continue during the maturation (Bailey et al., 2009); however, there are no on-farm reliable methods for the identification of the optimal ripening degree, which is assessed by farmers solely by visual and olfactory evaluation. Identifying the optimal hop maturation is crucial for farmers as harvesting too early or too late can result in incomplete accumulation or degradation of bitter acids and EOs, respectively (Darby et al., 2017). In the context of SA, technologies like IoT and ML algorithms can assist farmers in decision-making by collecting large amounts of data through a heterogeneous set of technologies and by identifying patterns within that data, respectively (Atzori et al., 2010; Murphy, 2012). In this regard, recent studies have demonstrated the feasibility of using these technologies for on-field assessment of fruit ripening, with applications in crops such as papaya and grape (Pereira et al., 2018; Santos Costa et al., 2019). Although SA technologies have been applied to hop to monitor the plant growth and health (Degadwala et al., 2023; Řeřicha et al., 2025), for the best of our knowledge, no ML models currently exist for predicting hop ripeness. Therefore, the aim of this study is to predict the optimal harvest window for ripe hop cones by testing two ML models using environmental data collected from field devices, alongside results from a three-year chemical and morphological characterization of hops.

2. Materials and Methods

2.1 Experimental crop set up

The experiment was conducted in the time-period 2021-2024 at "Azienda Agricola Ludovico Lucchi" situated in Campogalliano, Modena (Emilia Romagna region, Italy) (44° 42' 19.9" N, 10° 50' 26.1" E), on hop plants cv. Cascade. The crop was monitored through an IoT network composed of a weather station and different IoT sensors, including tensiometers, volumetric sensors, and foliar sensors. The measured parameters included atmospheric variables (air temperature, humidity, pressure, dew point, rainfall, evapotranspiration, solar radiation, wind speed and direction), soil conditions (moisture and temperature at surface and depth), and foliar wetness.

2.2 Hop sampling

Hop cones were sampled in the time period 2021-2024. For each sampling, about 250 g of fresh cones were collected for morphological analyses, and, subsequently, the cones were dried and used for chemical analyses. Hops were collected at different ripening stages, and the number of samplings and dates are shown in Table 1. Samplings in 2021 and 2022 were performed following the BBCH scale for hops (Rossbauer et al., 1995), starting

when cones were at growth stage 7 (BBCH code 71, "initial cone development") and ending at growth stage 9 (BBCH code 92, "senescence cone over ripeness"). In 2023, three samplings were conducted at BBCH codes 81,89 and 92 corresponding to unripe, ripe, and overripe cones, respectively. In 2024, one sampling was carried out at optimal ripeness (BBCH code 89).

Table 1 *Time schedule of hop samplings in the period 2021-2024*

Year	N. samplings	August	September	October
2021	6	18-23-26	3-8-15	
2022	9	8-17-26	2-8-15-21-27	10
2023	3	24	8-15	
2024	1		15	

2.3 Hop cone morphological and chemical analyses

For each sampling, morphological measurements were carried out on approximately 20 samples, for which length and width were measured using a calliper. The bitter acids extraction was conducted following the method described by Galaverni et al. (2024) and their quantification was performed using High Performance Liquid Chromatography (HPLC). For the quantification, a calibration curve was obtained by dilution of ICE-4 standard, in line with the official method (Analytica-EBC, method 7.7). EOs were extracted by steam distillation for 4 hours with a Clavenger apparatus to determine the oil content, and the yield was determined as the ratio between the weight of extracted EO and the dry weight of the used cones (w/w %).

2.4 Data analysis

Morphological and chemical data were analysed using XLSTAT software and, after verifying normality (Shapiro-Wilk test) and homoscedasticity (Levene's test), subjected to one-way ANOVA followed by Tukey's post-hoc test. Agronomic indicators, such as NHH curve, Growing Degree Days (GDD), and Heat Units (HUs) were calculated for further correlations with data collected by sensors and the results of morphological and chemical analyses, following methods described in previous literature (Derscheid and Lyte, 1981; Machado et al., 2004; Ferrante and Mariani, 2018).

2.5 Optimal harvesting prediction

Two different ML models were developed: (i) a Multiple Linear Regression (MLR) model with a soft voting classifier, and (ii) Principal Component Regression (PCR) model with a soft voting classifier. Each algorithm combines two types of ML models: (i) a regressor, capable of predicting input features; and (ii) the soft voting classifier, that is a supervised multi-class classifier able to distinguish input in classes (immature, mature, overripe), averaging the class probabilities predicted by different classifiers. For the regression task, the data were split into different sets: data collected before 2023 was used for training, while data from 2023 were reserved for testing. For the classification task, data collected during the hop-growing seasons from 2020 to 2023 were used as the training set, while data from 2024 served as the test set.

3. Results and Discussion

3.1 Hop cone analyses

During the 2021–2022 period, a significant cone elongation was observed during the first three samplings, after which no further significant changes occurred (Table 2). A similar growth pattern was observed for cone width.
In 2023, no variations were noted in the cone length, but a significant decrease in the cone width. Notably, the cone elongation is an important parameter to consider when evaluating the cone ripeness as it ceases only at physiological maturity (Čeh et al., 2012). Similarly, the width contraction generally occurs during the final ripening stages, at which point the cones completely close the bracts and are ready to be harvested (Rossbauer et al., 1995). Although there were no significant variations in bitter acid content in 2021, levels increased during cone maturation in 2022, peaking in the first half of September. Our morphological and chemical results align with Kavalier et al. (2011), who reported concurrent increases in cone volume, mass, and bitter acid accumulation. In contrast, alpha acid content declined in 2023, potentially due to unfavourable weather conditions (e.g. high temperatures and low precipitation) (Srečec et al. 2008). The EO yield significantly increased across the sampling dates in all years, consistent with previous literature (Lafontaine et al., 2019).

Table 2 *Morphological and chemical features of hops at different ripening stages in different years*

Year	Function	Day	Length (cm)	Width (cm)	α-acids (%)	β-acids (%)	EO yield (%)
2021	Training	08/18	0.76 ± 0.06^{c}	0.56 ± 0.05^{c}	6.38 ± 0.49^{a}	5.93 ± 0.44^{a}	0.38 ± 0.00^{e}
		08/23	1.81 ± 0.10^{b}	1.02 ± 0.03^{b}	6.33 ± 0.13^{a}	6.60 ± 0.47^{a}	0.82 ± 0.00^{d}
		08/26	2.34 ± 0.07^{a}	1.20 ± 0.03^{a}	6.62 ± 0.13^{a}	6.62 ± 0.12^{a}	1.39 ± 0.01^{c}

		09/03	2.61 ± 0.17^{a}	1.19 ± 0.04^{a}	6.40 ± 0.42^{a}	6.35 ± 0.48^{a}	1.89 ± 0.00^{b}
		09/08	2.57 ± 0.10^{a}	1.20 ± 0.04^{a}	6.62 ± 0.04^{a}	6.41 ± 0.06^{a}	1.95 ± 0.00^{ab}
		09/15	2.75 ± 0.10^{a}	1.16 ± 0.03^{a}	6.25 ± 0.12^{a}	6.64 ± 0.13^{a}	2.05 ± 0.08^{a}
		08/08	0.76 ± 0.06^{c}	0.76 ± 0.05^{d}	0.97 ± 0.00^{c}	1.35 ± 0.06^{c}	0.21 ± 0.02^{d}
		08/17	1.85 ± 0.09^{b}	1.02 ± 0.03^{c}	3.41 ± 0.35^{b}	5.35 ± 0.62^{b}	0.34 ± 0.01^{d}
		08/26	2.34 ± 0.07^{a}	1.21 ± 0.03^{b}	5.55 ± 0.11^{a}	7.41 ± 0.17^{ab}	1.20 ± 0.03^{c}
		09/02	2.61 ± 0.17^{a}	1.20 ± 0.04^{b}	5.72 ± 014^{a}	7.48 ± 0.22^{ab}	1.73 ± 0.39^{bc}
2022	Training	09/08	2.56 ± 0.10^{a}	1.20 ± 0.03^{b}	5.18 ± 0.13^{a}	7.58 ± 0.20^{ab}	2.10 ± 0.00^{b}
		09/15	2.75 ± 0.10^{a}	1.17 ± 0.03^{b}	5.30 ± 0.75^{a}	7.82 ± 1.13^{a}	2.33 ± 0.11^{ab}
		09/21	2.53 ± 0.09^{a}	1.15 ± 0.02^{bc}	4.30 ± 0.35^{ab}	6.19 ± 0.50^{ab}	2.48 ± 0.04^{ab}
		09/27	2.78 ± 0.08^{a}	1.43 ± 0.02^{a}	5.06 ± 0.47^{a}	5.52 ± 0.47^{b}	2.20 ± 0.28^{ab}
		10/05	2.45 ± 0.07^{a}	1.27 ± 0.03^{a}	5.06 ± 0.86^{a}	6.54 ± 0.86^{ab}	3.00 ± 0.35^{a}
		08/24	2.35 ± 0.06^{a}	1.36 ± 0.02^{a}	7.22 ± 0.42^{a}	5.50 ± 0.42^{a}	1.11 ± 0.03^{c}
2023	Test	09/08	2.46 ± 0.09^{a}	1.12 ± 0.02^{b}	6.98 ± 0.14^{b}	5.62 ± 0.14^{a}	1.86 ± 0.05^{b}
		09/15	2.63 ± 0.13^{a}	1.04 ± 0.02^{c}	3.79 ± 0.11^{c}	5.63 ± 0.11^{a}	1.99 ± 0.04^{a}

For each column, different letters within days in the same year indicate significant differences at $p<0.05$ by Tukey's test

3.2 Algorithm outputs

The performances of the MLR and PCR models were evaluated on the test set (2023) and are summarized in Table 3. Furthermore, for the first algorithm, the adopted soft voting classifier achieved a F1-score of 0.929 and an accuracy of 0.929. For the second algorithm, the soft voting classifier obtained a F1-score of 0.884 and an accuracy of 0.881 on the test set.

Table 3 *Performance (in terms of MAE, MSE and R^2) of MLR and PCR models on the test set*

Algorithm	MAE	MSE	R^2
MLR	0,9976	2,6795	0,9517
PCR	0,5834	0,7471	0,7522

3.3 Algorithm predictions

The predictions (from August 10) of the optimal harvest time in 2024 are shown in Figure 1. The first algorithm (MLR model) predicted the harvest period between September 2 and 16, while the second (PCR model) estimated the ideal window from September 10 to 25.

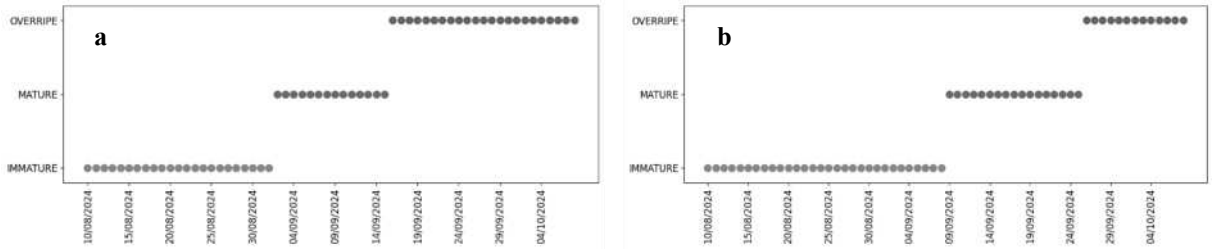


Figure 1 *2024 optimal harvesting period predictions by first algorithm (MLR and soft voting classifier) (**a**) and second algorithm (PCR and soft voting classifier) (**b**) from August 10, 2024*

In 2024, hops were collected on September 15 at their optimal ripeness, with alpha and beta acid contents of 3.96 ± 0.13% and 3.87 ± 0.16%, respectively, and an EO yield of 1.20 ± 0.04%. Although the results for the EO yield were in line with those typical for the Cascade variety (John I. Haas, 2016), slightly lower bitter acids levels were found, which is consistent with the predicted decline in yield and alpha acids content by 2050 because of climate change (Mozny et al., 2023). Notably, the forecasted optimal harvesting periods aligned with the peak accumulation of brewing-relevant chemical compounds, indicating that ML models developed so far for predicting optimal maturation in other crops can be effectively applied to hops as well (Pereira et al., 2018; Santos Costa et al., 2019).

4. Conclusions and Future Perspectives

This study proposed two ML-based algorithms for the prediction of the optimal harvest time of hops. The algorithms forecasted the ideal harvest window during the first half of September, which was confirmed by the chemical analyses of hops sampled in the same period and presenting bitter acids content and EO yield in line with those typical of the cv. Cascade. These results suggest that AI-based prediction models can be useful tools in the

identification of the optimal ripening time of hops, which could support farmers in decision-making processes. Since the proposed approach obtained good performances, future research activities may include the collection and integration of new types of information to improve the prediction and classification tasks. For instance, new trials may be conducted on different hop cultivars with different maturation kinetics or under different climatic conditions or growing areas.

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Innovative Chemical, Physical and Microbiological Strategies to Extend the Shelf-Life of Bakery Products

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The main objective of this PhD project is to develop innovative strategies to extend the shelf life of bakery products through the use of high-value ingredients derived from food by-products. The research focuses on integrating environmentally friendly packaging systems and sustainable preservation technologies. Particular attention is given to the chemical, physical, and microbiological aspects that influence product degradation. Ultimately, the goal is to reduce food waste while enhancing the nutritional and functional value of baked goods.

Strategie innovative chimiche, fisiche e microbiologiche per aumentare la conservabilità dei prodotti da forno

L'obiettivo principale di questo progetto di dottorato è sviluppare strategie innovative per prolungare la shelf-life dei prodotti da forno attraverso l'impiego di ingredienti ad alto valore nutraceutico ottenuti da sottoprodotti alimentari. La ricerca si concentra sull'integrazione di sistemi di packaging ecosostenibili e tecnologie di conservazione a basso impatto ambientale. Particolare attenzione è rivolta agli aspetti chimici, fisici e microbiologici che influenzano il deterioramento del prodotto. L'obiettivo finale è ridurre lo spreco alimentare migliorando al contempo il valore nutrizionale e funzionale dei prodotti da forno.

Abstract

Bakery products are an essential part of the human diet but are highly perishable due to physical and microbiological degradation. Extending their shelf life is a technological challenge with significant environmental implications, particularly concerning food waste reduction. This study explores an integrated approach combining chemical, physical, and microbiological technologies aimed at extending the shelf life of bakery products in an economically and environmentally sustainable way. Chemical modifications were introduced through pH reduction using organic acids and citrus pectins. Physical strategies included water migration monitoring with advanced sensors developed in collaboration with CERN and the use of infrared (IR) radiation for the pasteurization of pre-packaged baked goods. On the microbiological side, selected lactic acid bacteria were used in controlled fermentations to produce bioactive powders rich in metabolites and bacteriocins. The combined strategies demonstrated strong potential for improving the microbial stability and quality of bakery products, offering promising applications in the industrial bakery sector.

1. Introduction

Bakery products play a central role in the global food supply as key sources of carbohydrates. Despite their importance, they are subject to a relatively short shelf life due to staling, moisture migration, and microbial spoilage. Extending the shelf life of these products is crucial not only from a technological standpoint but also in light of environmental sustainability goals, particularly the reduction of food waste.

Recent advances in food technology have led to the development of several approaches to tackle this challenge, involving chemical modifications to formulation, physical treatments aimed at preserving moisture and microbial stability, and microbiological techniques leveraging fermentation and natural antimicrobials. However, there remains a need for integrated strategies that combine these approaches to achieve greater effectiveness without compromising product quality or sustainability.

This study aims to evaluate and combine innovative chemical, physical, and microbiological technologies to extend the shelf life of bakery products in a sustainable and scalable manner.

2. Materials and Methods

2.1 Chemical Technologies: pH Modulation

Model bakery doughs were prepared and modified with varying concentrations of citric acid (0.2–1%) and citrus pectin (1–3%). The pH was measured using a benchtop pH meter (±0.01 accuracy). Microbial proliferation was monitored at 25 °C over 10 days using total viable counts (TVC) and mold/yeast enumeration (ISO 21527-1).

2.2 Physical Technologies: Moisture Migration and IR Pasteurization

Water migration was evaluated through both:

- Destructive moisture content analysis (oven-drying at 105 °C for 24 h).

- Non-invasive sensors developed in collaboration with CERN, capable of real-time moisture profiling across crust and crumb.

For infrared (IR) pasteurization, pre-packaged bakery items were exposed to medium-wavelength IR radiation (3–4 µm) for durations of 30–90 seconds. Post-treatment microbial loads and sensory attributes (texture, color, aroma) were analyzed.

2.3 Microbiological Technologies: Bioactive Powders

Lactic acid bacteria (LAB) strains (Lactobacillus plantarum, Pediococcus acidilactici) were cultured in optimized MRS media and fermented under controlled conditions (30 °C, anaerobic, 48 h). Fermentation broths were freeze-dried and the powders incorporated at 5% into bakery dough. Antimicrobial activity was tested in vitro against spoilage organisms (*P. commune; P. verrucosum; P. brevicompactum; P. decumbens*) using agar diffusion assays and in vivo shelf-life trials.

3. Results and Discussion

3.1 pH Modulation

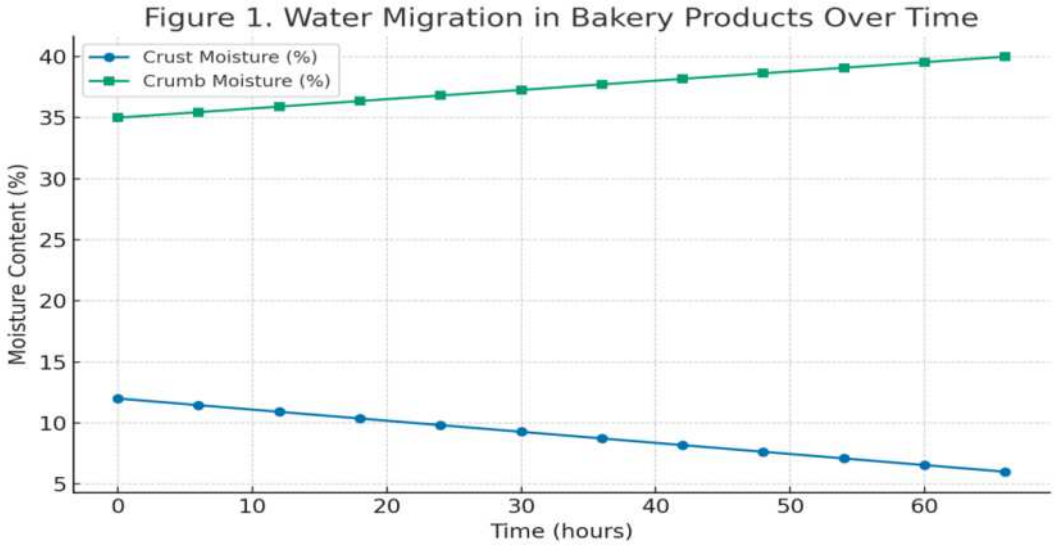
Doughs formulated with organic acids showed a significant reduction in microbial growth, particularly when pH was maintained below 4.5 ($p < 0.01$). The synergistic use of pectin improved the consistency and buffering capacity of the matrix, enhancing microbial inhibition over time.

Table 1. Microbial Load at Different pH Levels

pH Level	TVC (log CFU/g, Day 5)
5.5	7.2
5.0	6.0
4.5	4.1
4.0	2.3

3.2 Moisture Migration Analysis

Real-time monitoring of water migration using CERN sensors provided dynamic insights into internal moisture redistribution. The most pronounced migration occurred within 48 hours post-baking, affecting crust crispness and internal softness. Results correlated strongly with gravimetric analysis ($R^2 = 0.92$).



3.3 IR Pasteurization

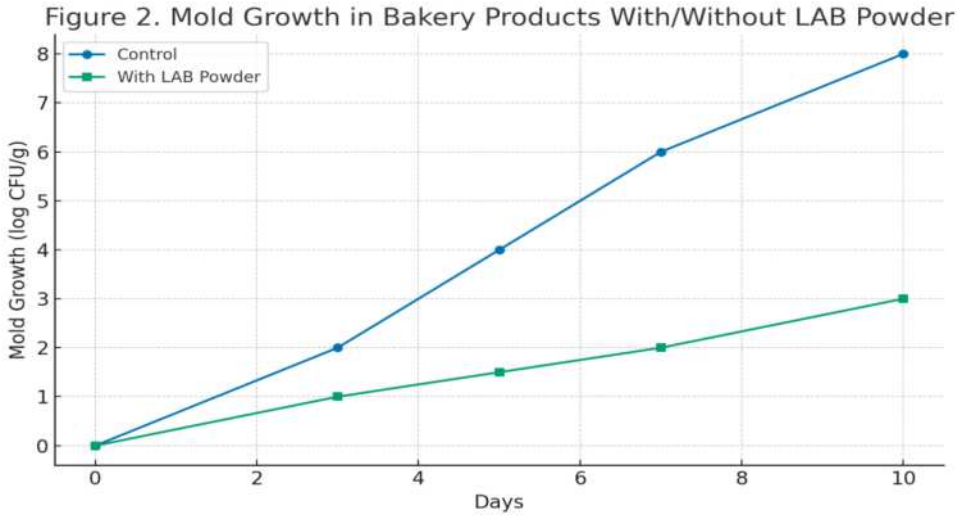
IR-treated samples (≥60 s) exhibited microbial reductions exceeding 2 log CFU/g for yeast and mold populations. Sensory tests indicated minimal quality loss, with only mild surface browning observed in the longest treatments. This suggests IR as a viable post-packaging intervention for microbial stabilization.

Table 2. Effect of IR Treatment on Microbial Load and Sensory Properties

IR Treatment Time (s)	Microbial Reduction (log CFU/g)	Sensory Score (1-10)
30	1.0	8.5
60	2.2	8.0
90	2.8	7.2

3.4 Fermentation and Bioactive Powders

LAB-derived powders demonstrated strong antimicrobial activity, especially from L. plantarum, which inhibited Aspergillus growth entirely in vitro. Bakery items containing LAB powders had extended shelf life by up to 7 days under ambient storage, with acceptable sensory properties.



4. Conclusions and future perspectives

This research demonstrated that an integrated approach involving formulation adjustments, physical treatments, and microbiological strategies can effectively extend the shelf life of bakery products in a sustainable manner. The use of organic acids and pectins to modulate pH, the application of infrared (IR) technology for mild thermal treatment, and the incorporation of LAB-based functional powders represent promising tools for improving microbial stability while maintaining product quality. Furthermore, the real-time monitoring of water migration using advanced sensors opens new avenues for predictive shelf-life modeling.

Future studies should explore the scalability of these solutions at the industrial level, considering economic feasibility, consumer acceptance, and regulatory compliance. Additional research is also needed to investigate the synergistic effects of combining preservation technologies and optimizing packaging materials in alignment with circular economy principles. Ultimately, this work contributes to the development of bakery products that are not only longer lasting but also aligned with environmental and nutritional goals, supporting a more resilient and sustainable food system.

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Applications of MOX Sensors for Volatile Organic Compound Detection in Agri-Food sector

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This PhD thesis explores the use of innovative IoT nanosensors based on metal oxide semiconductors (MOX) to monitor quality and safety in the agri-food supply chain. Various food matrices, including meat, fruits, and baked goods, were analyzed. Volatile profiles were assessed using MOX sensors and validated through gas chromatography-mass spectrometry (GC-MS). The studies aimed at evaluating freshness and detecting alterations in raw materials. Results demonstrate the versatility and effectiveness of MOX-based sensing systems. The research highlights their potential as rapid, non-destructive tools for real-time quality control across different stages of food production and storage.

Applicazioni dei sensori MOX per il rilevamento di composti organici volatili nel settore agroalimentare.

Questa tesi di dottorato esplora l'uso di nanosensori IoT innovativi basati su semiconduttori di ossidi metallici (MOX) per il monitoraggio della qualità e sicurezza nella filiera agroalimentare. Sono state analizzate diverse matrici alimentari, tra cui prodotti ittici, carne, frutta, prodotti da forno e mangimi. I profili volatili sono stati valutati mediante sensori MOX e validati con GC-MS. Gli obiettivi degli studi spaziavano dalla valutazione della freschezza all'identificazione di alterazioni delle materie prime. I risultati dimostrano la versatilità ed efficacia dei sensori MOX, evidenziandone il potenziale come strumenti rapidi e non distruttivi per il controllo qualità in tempo reale.

Key words: Volatilome; Electronic Nose; IoT nanosensors; innovative technique and agri-food sector.

1. Introduction

This oral presentation outlines the main results of four research activities carried out as part of a PhD project focused on the use of innovative technologies for monitoring volatile organic compounds (VOCs) in the agri-food sector. In particular, an electronic nose based on metal oxide semiconductor (MOX) sensors was employed, in combination with conventional analytical techniques such as solid-phase microextraction gas chromatography–mass spectrometry (SPME-GC-MS):

A1) Analysis of the volatile profile of raw poultry carcasses and cooked meat from animals fed with different diets, including those containing insect-based meals, to evaluate the impact on product quality and safety (explained in detail in Section 4.1 and 5.1).
A2) Evaluation of the effect of two controlled atmosphere conditions (different O_2 and CO_2 concentrations) on the shelf-life of table grapes, identifying volatile markers associated with freshness and spoilage (explained in detail in Section 4.2 and 5.2).
A3) Real-time monitoring of dough leavening using an electronic nose integrated into domestic mixers, with the aim of optimizing the process and supporting automated fermentation control systems (explained in detail in Section 4.3 and 5.3).
A4) Investigation of cold fermentation in sourdough through microbiological analysis and VOC profiling, to improve quality control and sensory standardization in artisanal bakery products (explained in detail in Section 4.4 and 5.4).

2. Applications of MOX Sensors in Agri-Food Monitoring

Metal oxide semiconductor (MOX) sensors represent a highly promising technology for the real-time detection of volatile organic compounds (VOCs) and analytically relevant gases across a wide range of application fields (Poeta et al. 2025a). Their operating principle is based on changes in electrical resistance induced by redox reactions occurring on the sensitive surface of the sensor (Poeta et al. 2023). These variations are influenced by the chemical composition of the active material and the operating temperature, parameters that can be optimized to enhance sensitivity and selectivity. Thanks to their low energy consumption, miniaturization potential, and compatibility with silicon-based technologies, MOX sensors can be easily integrated into portable, wearable, and IoT systems (Firooz et al. 2009). In the agri-food sector, these sensors are particularly valuable for quality control, as they enable the detection of volatile markers associated with freshness, spoilage, or adulteration (Siepmann et al. 2019). They thus offer an effective solution for rapid, non-destructive, and continuous monitoring along the production chain. Beyond agri-food applications, MOX sensors are also used in the biomedical field for non-invasive

diagnostics through breath analysis (Hansen et al. 2005). Finally, in environmental monitoring, MOX sensors enable the detection of atmospheric pollutants, providing a compact, cost-effective, and easily deployable alternative to conventional laboratory instruments, which are often bulky and expensive (Isaac et al. 2022).

3. Experimental Procedure

In this PhD thesis a multi-step protocol was developed for the fabrication and application of metal oxide semiconductor (MOX)-based gas sensors aimed at quality control in the agri-food sector. The sensors produced were subsequently employed in various research projects, each with specific objectives, including the monitoring of food freshness, the traceability of fermentation processes, and the early detection of spoilage or contamination. The process involved: i) the formulation of a screen-printable paste by combining the sensing oxide, glass frit, and organic vehicles; ii) deposition onto alumina substrates via screen-printing; iii) thermal pre-treatment followed by sintering to ensure microstructural stabilization; iv) integration into a sensing device, such as the S3+ electronic nose platform; and v) validation through gas exposure tests and correlation with GC-MS analysis. This procedure allowed for the optimization of sensor sensitivity and selectivity toward volatile markers of interest.

4. Materials and Methods

During the PhD program, both conventional analytical techniques and innovative methodologies were employed to investigate volatile compounds. Among the traditional approaches, gas chromatography coupled with mass spectrometry (GC-MS) was used, utilizing a GC 2020 system with an MS-QP2020 mass spectrometer (Shimadzu) and a MEGA-5MS column. Compound identification was performed by comparing the obtained spectra with those in the NIST11, NIST11b, and FFNSC2 libraries. In parallel, an innovative sensor-based approach was developed using electronic noses equipped with metal oxide (MOX) sensors. In studies A1, A2, and A3, the S3+ device was employed, developed in collaboration with NANO SENSOR SYSTEMS Srl, a spin-off of the University of Brescia. This instrument integrates six custom MOX sensors housed in a stainless-steel chamber heated to 500 °C and is equipped with a fluid dynamic system to ensure homogeneous distribution of volatile compounds. Data were acquired in real time and transmitted to a dedicated web platform, highlighting the device's IoT capabilities (Figure 1).

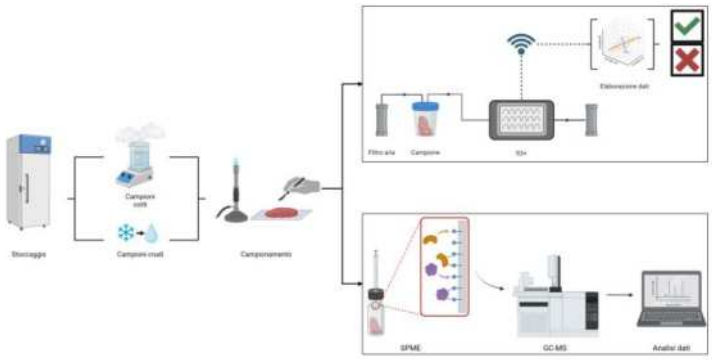


Figure 1. Schematic representation of the analytical protocol based on GC-MS and electronic nose for volatile compound analysis.

For study A4, a portable electronic nose was employed. This compact device integrates five MOX sensors from different manufacturers to enhance selectivity. It also includes environmental sensors (temperature, relative humidity, and atmospheric pressure), a headspace sampling system with a pump and solenoid valve and communicates via Bluetooth with a mobile application for configuration and control. The system features a fast response time of approximately two seconds and a low energy consumption of 684 mW.

4.1 Impact of Feeding Live and Dehydrated Insect Larvae on Chicken Meat

A study was conducted on the volatile profiles of raw and cooked poultry meat from broilers (n=192) fed four different diets: conventional (CONTROL), sustainable without soy (ST), and sustainable with live (LL) or dehydrated (LD) Black Soldier Fly larvae (Genzardi et al. 2024) After reaching 174 days, breast fillets were collected, frozen, and later analyzed (raw or cooked). A total of 48 meat samples were analyzed via GC-MS and S3+ e-nose, resulting in 624 analyses. Each sample was tested in triplicate for GC-MS and 10 times with the e-nose, under controlled and hygienic conditions.

4.2 Quality monitoring of table grapes stored in controlled atmosphere

A study was conducted to assess how different controlled atmosphere (CA) conditions affect the shelf-life of *Italia* table grapes (Poeta et al. 2025b). Bunches were selected post-harvest and stored at 2 ± 1 °C under three conditions:

ambient air (Ctrl), 3% O_2/10% CO_2 (CA-1), and 3% O_2/30% CO_2 (CA-2). For each condition, 16 samples (~1 kg each) were analyzed over 0, 10, 20, 30, and 40 days.
Quality was evaluated through both sensory and instrumental methods, including visual appearance, berry browning (b*), and rachis water content (RWC). Enzymatic activity (PPO and PDO) was measured to assess tissue degradation. VOCs were analyzed using SPME-GC-MS and the S3+ electronic nose equipped with custom IoT nanosensors (Palumbo et al. 2024).

4.3 Real-Time Monitoring of Dough Preparation in Kitchen Machines

This study analyzed the volatilome produced during dough leavening using three wheat flours with different strength levels (W200, W250, W390). Doughs were prepared under standardized conditions and leavened for 1.5 hours at room temperature. Volatile compounds were monitored continuously using an electronic nose and SPME-GC-MS, with measurements divided into pre- and post-leavening stages. The integrated analysis allowed for clear differentiation of fermentation phases and revealed aroma differences linked to flour strength (Genzardi et al. 2025).

4.4 Monitoring the Olfactory Evolution of Cold-Fermented Sourdough

In this study on sourdough volatilome, dough was prepared using 80% flour–water mix and 20% sourdough inoculum, with a blend of whole wheat and strong flour. Cold fermentation was conducted at 5 °C using a single sourdough batch. Samples were taken at six time points up to 24 hours and analyzed for pH, microbiology, and volatile emissions using an electronic nose and SPME-GC-MS. Measurements were conducted under controlled conditions to ensure accuracy and avoid external interference (Poeta et al 2025c).

5. Results and Discussion

In each project, the volatile fraction of various agri-food samples was analysed using both traditional techniques (SPME-GC-MS) and innovative approaches (electronic nose based on MOX sensors), with analytical conditions, such as sensor doping and operating temperature, specifically tailored to the organic components under investigation.

5.1 Analysis of Volatile Compounds in Poultry Samples Using GC-MS and MOX Sensors

The volatilome of raw and cooked chicken meat from four dietary groups (CONTROL, LL, LD, ST) was analyzed using SPME-GC-MS and the S3+ electronic nose. Cooking resulted in a reduction of aldehydes, alcohols, and ketones, along with an increase in esters, carboxylic acids, and ethers. Hexanal and 1-octen-3-ol emerged as markers of lipid oxidation. Several volatile compounds were diet-specific, and ketones associated with microbial spoilage were detected in cooked samples from the ST group.
The use of the S3+ device, combined with Linear Discriminant Analysis (LDA), enabled effective sample classification, achieving an accuracy of 86.5% for cooked samples and 81.8% for raw ones (Figure 2A and 1B). These findings confirm the reliability of MOX sensors in distinguishing samples based on both cooking status and feeding regime. This non-destructive approach, suitable for integration into IoT systems, represents a promising strategy for improving traceability and sustainability in the poultry production chain, particularly through the use of alternative feeds such as insect larvae.

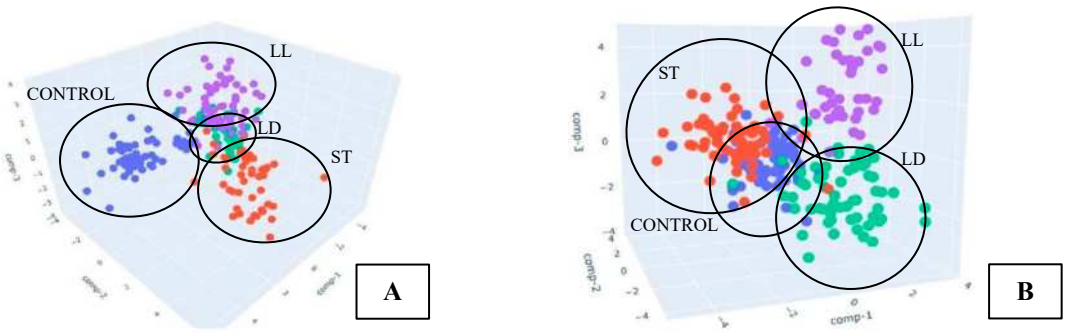


Figure 2. (A,B) LDA in 3D, representing cooked (A) e raw (B) samples with CONTROL diet, ST, LD, and LL.

5.2 Quality and sensory attributes of table grape stored in controlled atmosphere

Controlled atmosphere (CA) storage with low O_2 and high CO_2 (CA-1 and CA-2) improved the visual quality of *Italia* table grapes by limiting browning, water loss, and mold development. Control samples showed higher enzyme activity (PPO, PDO) and berry browning, along with greater weight loss. However, CA also induced metabolic stress, evidenced by increased respiration rates and accumulation of fermentation-related compounds like ethanol and succinic acid, especially in CA-2. GC-MS and S3+ electronic nose analyses confirmed these effects. PCA (Figure 3) and LDA (Figure 4) showed clear separation of treatments over time, highlighting ethanol

and 1-hexanol as potential stress markers, while hexanal and anethole were linked to freshness. CA-2 was the most detrimental condition.

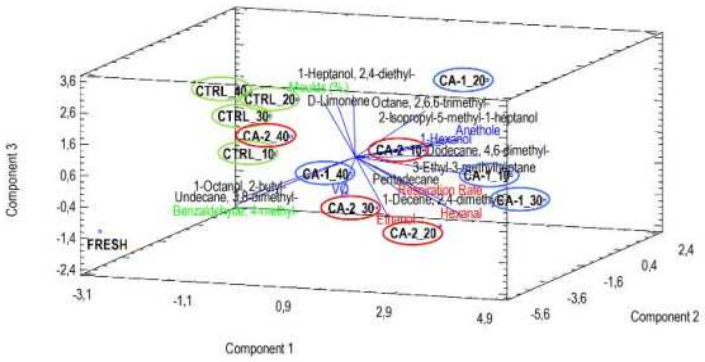


Figure 3. PCA (65.3% of the total variance, with the first three principal components accounting for 31.4%, 21%, and 13% of the variance, respectively) plots of table grapes analysed at harvest (Fresh, 0d) and after 10, 20, 30 and 40 d of storage in air (control, CTRL_10, CTRL_20, CTRL_30, CTRL_40), 3%/10% O_2/CO_2 controlled atmosphere (CA-1_10, CA-1_20, CA-1_30, CA-1_40) and 3%/30% O_2/CO_2 controlled atmosphere (CA-2_10, CA-2_20, CA-2_30, CA-2_40) at 5 °C.

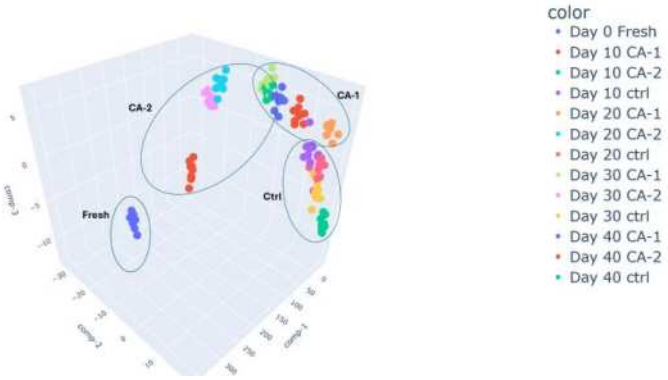


Figure 4. 3D LDA of grape storage under different modified atmospheres and at different time points. The model achieved an accuracy of 86.53%, demonstrating its effectiveness in distinguishing between storage conditions and sampling days.

5.3 Dough Preparation in Kitchen Machines

In the dough preparation study using a kitchen machine, LDA analysis of electronic nose data revealed clear differences in VOC profiles between flours of different strengths (W200, W250, W390) during leavening. 3D LDA analysis (Figure 5) clearly distinguished between the pre- and post-leavening phases for each flour type, confirming the sensor system's ability to monitor fermentation progression. In parallel, SPME-GC-MS identified 108 VOCs, with specific compounds varying according to flour type and fermentation stage. Alkanes were most abundant, while alcohols increased post-leavening, especially ethanol. Some compounds were specific to either phase or flour, influencing the aroma. Results from GC-MS aligned with electronic nose data, confirming its effectiveness for real-time, non-invasive monitoring of dough fermentation.

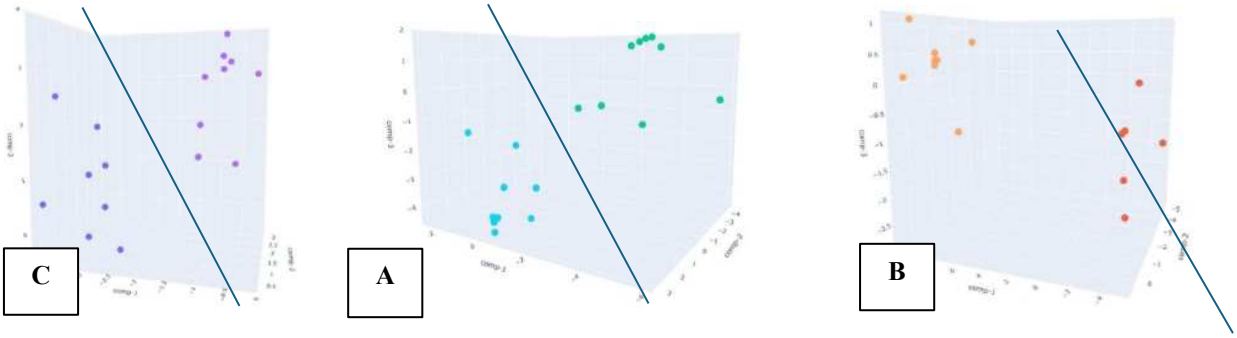


Figure 5. (A,B,C) LDA plots showing dough samples prepared with three flour types: (A) W390 – PRE-leavening (right) vs. POST-leavening (left); (B) W250 – PRE-leavening (left) vs. POST-leavening (right); (C) W200 – PRE-leavening (left) vs. POST-leavening (right).

5.4 Volatile Organic Compounds of Cold-Fermented Sourdough

In the sourdough fermentation study, LDA applied electronic nose data revealed clear temporal separation of samples collected at different fermentation times, especially between 0 h and later stages (Figure 6). GC-MS analysis confirmed changes in VOC composition: terpenes dominated early stages, while ethanol, acetic acid, and esters (e.g., ethyl acetate) increased over time, enhancing fruity and sweet aromas. Microbiological analysis showed growth of *Lactobacillus spp.* and yeasts, with pH dropping from 5.8 to 3.8. Low-temperature fermentation (5 °C) favored a stable microbial balance and aroma development.

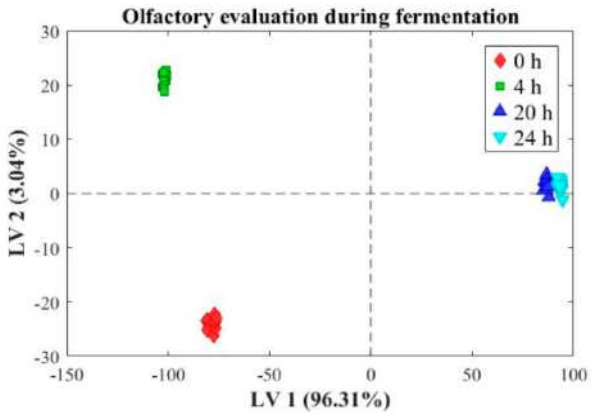


Figure 6. LDA of the olfactory evolution of dough fermentation.

6. Conclusions and Future Perspectives

This PhD work confirmed the potential of MOX sensors as compact, sensitive, and versatile tools for real-time food quality monitoring. Successfully applied in case studies on grapes, poultry, and dough fermentation, they proved effective under practical conditions. Despite limitations like humidity sensitivity and signal drift, MOX sensors remain advantageous. Future developments combining AI and IoT will enhance their intelligence, stability, and applicability across agri-food, environmental, and health sectors.

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Oral presentation sessions
PhD students – 3rd year
Abstracts

PARALLEL SESSION 8
Processing and Reformulation Strategies for Product Innovation

Technological and Nutritional Properties of Pigmented Whole Wheat Foods: The Role of Innovative Technologies

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In this study, the effects of two baking methods – conventional baking and ohmic heating – on the preservation of bioactive compounds, especially anthocyanins, in pigmented wholemeal bread were evaluated. The primary aim was to investigate the influence of baking techniques on the nutritional and technological properties of bread, focusing on the processing properties of the raw material and the preservation of anthocyanins.

Proprietà tecnologiche e nutrizionali di alimenti integrali pigmentati: il ruolo delle tecnologie innovative

Questo studio ha valutato l'impatto di due metodi di cottura - convenzionale e riscaldamento ohmico - sulla ritenzione di composti bioattivi, in particolare degli antociani, in pani integrali realizzati a partire da sfarinati di frumento tenero pigmentati. L'obiettivo principale è stato quello di valutare l'impatto di diverse tecniche di cottura sulle proprietà nutrizionali e tecnologiche del pane prodotto da frumento pigmentato, focalizzandosi sui parametri coinvolti nella lavorazione della materia prima e sulla ritenzione degli antociani a seguito della cottura.

Keywords: Pigmented wheat, anthocyanins, ohmic heating, conventional baking

1. Introduction

The PhD project aims to improve the nutritional value and the content of bioactive compounds in genetic lines of pigmented wheat, focusing on anthocyanins. As anthocyanins are sensitive to various process conditions, such as temperature, pH variations, and moisture (Dangi *et al*., 2023), they require appropriate operational parameters to ensure their integrity and stability in cereal-based foods. Indeed, the challenges associated with the pigmented wheat use remain multifaceted. These concern various aspects, including agronomic variability, the complex metabolic dynamics of anthocyanins, differences in grain quality, significant losses of bioactive substances during processing, their interactions with other nutrients, and sensory acceptance by the end consumer.
Considering the numerous topics of interest that involve various interdisciplinary aspects, in line with the objectives of the PhD project, the activities so far have focused on the chemical and nutritional characterization of different genetic lines, to identify the most promising ones both from a technological point of view and in terms of their anthocyanin content. Selected genetic lines of hard and soft wheat were then milled to obtain wholemeal flours for the production of cereal-based foods. Delving into the most useful processing technologies to preserve the naturally occurring compounds in pigmented wholemeal flour, some lines of research included the production of foods such as cookies and bread (Pasqualone *et al*., 2013; Bartl *et al*., 2015; Francavilla & Joye, 2022; Gamel *et al*., 2023; Koksel *et al*., 2023), muffins (Li *et al*., 2007), and dry or fresh pasta (Ficco *et al*., 2016). Despite the nutritional benefits and advances in identifying the best methods to improve anthocyanin retention, the loss of anthocyanins during cooking remains significant. Studies to optimize processing technology for improving the retention of bioactive compounds have shown that the baking process can influence the anthocyanin content and the nutritional and functional properties of pigmented bread. Based on conventional bread production, it was found that the combination of high temperatures and short baking times can help to preserve the anthocyanin content (Bartl *et al*., 2015; Francavilla & Joye, 2022). Different baking methods (e.g., convection, steam oven, conveyor belt) have different heat transfer coefficients, which can influence the uniformity of heat transfer in the dough and consequently the preservation of anthocyanins. Accordingly, the research aimed to investigate the factors that affect the stability of anthocyanins in pigmented wholemeal bread during the baking process, making conventional baking and ohmic heating comparable in terms of technological conditions.

2. Ohmic heating and conventional baking

Ohmic heating, known as resistance heating, is a technology for inactivating the microbiological spoilage of heat-sensitive foods. This technology, which is already recognized as an alternative to classic pasteurization and sterilization, is based on the use of alternating electric current. Moreover, ohmic heating is now being used as a new method in the baking process for producing baked goods. It has been evaluated for the production of gluten-free bread, where the absence of gluten-forming proteins makes it difficult to produce a leavened product that meets consumer expectations. More recently, it has also been applied to conventional cereal-based bread (Waldert *et al*., 2025). The flow of electric current through a food matrix (resistor), which has a specific electrical conductivity, generates energy dissipated as heat through the Joule effect, facilitating the cooking of the treated food (Asráin-Redín *et al.*, 2024). Unlike conventional baking methods, which are based on convection, conduction, and radiation, and often result in uneven heat distribution, ohmic heating is a volumetric technique

that enables rapid and uniform heating throughout the product. This method significantly reduces cooking time and energy consumption due to its high electrical-to-thermal energy conversion efficiency. In conventional baking, the heat penetrates the dough gradually from the surface to the core, resulting in slower and less uniform processing. In contrast, with ohmic heating, heat is generated immediately and uniformly within the food matrix, typically starting from the core and progressing outwards.

3. Materials and Methods

3.1 Materials

Wholemeal flour from the commercial Austrian soft wheat cultivar 'Purpur' ('PUR') and wholemeal flour obtained by recombining specific milling fractions of soft wheat cultivar 'Zora' ('ZA') from Azienda Agricola AURIS (Dairago, MI), selected for their high anthocyanin content, were used. The PUR flour and the ZA flour contained 39.41 ± 0.45 µg Cy-3-glc/g db. and 93.05 ± 0.60 µg Cy-3-glc/g db. of anthocyanins, respectively.

3.2 Dough recipe and preparation

The recipe was adapted from Rumler *et al*. (2023) for making wholemeal bread dough. The dough was prepared by mixing 100% wholemeal pigmented flour with 1% sugar, 1.8% salt, and 3% dry yeast (based on the flour). The amount of water was adjusted to the optimal absorption of the individual flour, as determined by the farinographic analysis (~ 65% at 26 ± 1 °C, measured with a temperature meter). All dry ingredients were mixed using a laboratory kneader (KitchenAid Professional, © KitchenAid, Benton Harbor, MI, USA). The dough was placed in a fermentation chamber (MANZ Backtechnik GmbH, Creglingen, Germany) set to 30 °C and 85% relative humidity (RH). After the first fermentation, the dough was divided into 500 g portions and placed into an oiled baking tin for conventional baking, while for the ohmic heating, a specific chamber was used and submitted to a second fermentation step (30 °C, 85% RH). For both baking methods, optimizing the process involves manual adjustments, such as changes to voltage input and time for ohmic heating, as well as the time-temperature for conventional baking, utilizing real-time monitoring of the optimal baking temperature. Conventional breads were baked in a conventional deck oven (model 60/rW, MANZ Backtechnik GmbH, Creglingen, Germany). The ohmic baking experiments were conducted using a pilot-scale ohmic heating unit (DIL, Quakenbrück, Germany) with bipolar rectangular current and voltage pulses at a pulse repetition frequency of 12 kHz (Waldert *et al*., 2025). After baking, the bread was cooled at room temperature for two hours and immediately analysed. Two loaves were prepared in triplicate (n = 6) for each baking method. A portion of each loaf was used for immediate analysis. The remaining samples were frozen, freeze-dried, and milled using a Rotor Beater Mill SR 300 (particle size ~ 500 µm) and then stored at 4°C for further analysis.

3.3 Bake loss and volumetric analysis

Baking loss was measured gravimetrically, and calculated as the mass ratio of dough (m_d) to bread (m_b after baking), after two hours of cooling, using the following Equation (1):

1) $\text{Baking Loss (\%)} = ((m_d - m_b)/m_d)) \cdot 100$

The specific volume of bread was measured by using the Perten BVM 6600 Bakery Ingredient Analyzer (PerkinElmer Instruments AB, Hägersten, Sweden) and calculated as the following ratio: Volume of bread (expressed in cm^3)/weight of bread (g).

3.4 Texture analysis

Crumb firmness and relative elasticity (REL) were determined using a modified texture analysis procedure, aligning with Bender *et al*. (2019) and established instrumental methods (AACC Method 74–09.01). A Stable Micro Systems™ TA-XT+ Texture Analyzer (Godalming, UK), featuring a 5 kg load cell and a 36mm Ø Cylinder Probe (SMS P/36R), was used for all uniaxial compression measurements. Loaves were sectioned into 3 cm-thick slices using an automatic bread slicing device (electronic, Robert Bosch GmbH, Gerlingen, Germany). Crumb firmness is the maximum force (N) recorded during compression. Relative elasticity (REL) was calculated as: $REL\ (\%) = (F_{120} / F_{MAX}) \times 100$, where F_{MAX} is the maximum force recorded and F_{120} is the force measured 120 seconds after reaching F_{MAX}, during the probe relaxation phase.

3.5 Bread colorimetric indices and crumb pore analysis

The IRIS visual analyzer (model VA400, Alpha MOS, Toulouse, France) was utilized to assess the color of the crust and crumb. Colorimetric indices (L*a*b*) were measured according to the CIELAB color space.
Crumb porosity was analysed using ImageJ software (version 1.54p, National Institutes of Health, Bethesda, MD, USA). The analysis focused on a 2 × 2 cm square evaluation of the selected crumb from a slice photo taken with a Nikon D-90 digital camera (Tokyo, Japan) combined with a Digi-Eye® system (Verivide, Leicester, UK). According to the method described by Bender *et al*. (2018), the analysis included quantifying pore number, average pore size (mm), total pore area as a percentage of bread area (% porosity), and standard deviation of pore size to

assess pore uniformity.

3.6 Anthocyanins analysis
Total anthocyanin content was quantified using the differential pH colorimetric method, as described by Iannucci *et al*. (2022).

3.7 Statistical analysis
Multiple sample analysis was performed, and the results are presented as mean ± standard deviation. Analysis of variance (ANOVA) and Fisher's least significant difference (LSD) were performed with SPSS Version 23.0 (IBM SPSS Statistics, Armonk, NY, USA), and differences at $p<0.05$ were considered significant. Statistical significance analyses were conducted separately for each pigmented flour.

4. Results and Discussion

4.1 Experimental trials
Preliminary trials were conducted to make the two different baking methods comparable, optimizing the recipe formulation (based on water content) and the parameters of conventional baking (time and temperature) and ohmic heating (holding time, power input, treatment chamber size). The tests were defined based on recent studies in which ohmic heating was applied to gluten-free raw materials (Bender *et al*., 2019), including pigmented cereals such as rice (Evangelista *et al*., 2025) and conventional wheat (Waldert *et al*., 2025). Finally, the bread was baked in a conventional oven at 220 °C on the top and bottom for 32 min. For ohmic heating, stage 1 with a fixed voltage of 500 V was evaluated as sufficient to achieve the desired heating by applying an input power of 1 kW for 150 seconds, followed by an additional holding time in stage 1 at 0.3 kW for 30 seconds. Considering the time required to reach optimum cooking, ohmic heating resulted in a 90.63% reduction in cooking time compared to conventional baking.

4.2 Quality parameters of bread
The wholemeal bread produced (Figure 1) generally has a more compact crumb than bread made with refined flour or gluten-free raw material. The choice to use wholemeal flour is due to the fact that anthocyanins are mostly located in the peripheral layers of wheat caryopsis. The presence of bran hinders the formation of an already weak gluten in these pigmented flours, which reduces the extensibility of the dough and the ability to hold gas during rising, especially in conventionally baked bread.

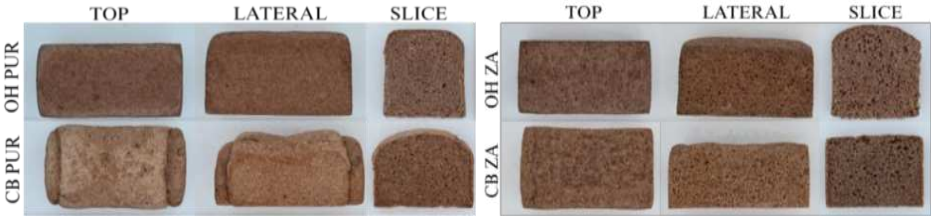


Figure 1. *Effect of ohmic baking and conventional baking on the surface and pores of 'PUR' (on the left) and 'ZA' pigmented bread (on the right).*

Indeed, uniaxial compression results (Table 1) show values consistent with what would be expected for a wholemeal pigmented bread (Koksel *et al.*, 2023; Koksel *et al.*, 2025) and comparable between the two different baking methods. All wholemeal loaves show a dense crumb with an intermediate elasticity indicative of bread that is not too crumbly.

Table 1. *Crumb firmness and relative elasticity (REL) of ohmic and conventional wholemeal pigmented bread.*

Sample	F_{MAX} (N)	F_{120} (N)	REL (%)
OH PUR	25.43±1.20^{a}	12.73±0.55^{a}	50.10±2.06^{a}
CB PUR	24.68±1.97^{a}	12.03±0.83^{a}	48.83±2.46^{a}
OH ZA	28.12±1.64^{A}	14.88±0.96^{A}	52.90±1.05^{A}
CB ZA	28.42±2.11^{A}	14.79±0.97^{A}	52.11±1.98^{A}

For PUR bread, different lowercase letters (a, b) in the same column indicate a statistically significant difference ($p<0.05$) between baking methods, while for ZA bread, this is shown by different uppercase letters (A, B).

While it is generally assumed in literature that ohmic heating leads to a greater number of pores and higher porosity in bread compared to conventional baking, our results for wholemeal bread (Table 2) offer a different perspective.

An increased number of pores was observed in ohmic-baked bread, consistent with the existing literature. However, the porosity values for breads made from the same flour were comparable regardless of the heating method. The inclusion of bran significantly influenced pore characteristics, especially in conventionally baked breads 'PUR' and 'ZA'. In these cases, a high value for pore uniformity indicated an uneven distribution of pore sizes within the crumb. This can be attributed to the different heat transfer mechanisms. The rapid, volumetric heat distribution of ohmic heating traps the water vapor in the dough, which promotes its expansion and simultaneous gelatinization of the starch, which ultimately stabilizes the structure of the bread. In contrast, conventionally baked wholemeal dough tends to collapse structurally, which probably contributes to uneven pore development.

Table 2. *Physical quality parameters of ohmic and conventional wholemeal pigmented bread.*

Sample	Baking loss (%)	Specific Bread Volume (cm^3/g)	Number of pores	Pore average size (mm)	Porosity (%)	Pore uniformity*
OH PUR	6.9 ± 0.26^{a}	2.4 ± 0.05^{a}	136.0 ± 10.60^{a}	1.37 ± 0.11^{b}	46.49 ± 0.90^{a}	2.96 ± 0.73^{b}
CB PUR	6.3 ± 0.19^{b}	2.1 ± 0.03^{b}	76.36 ± 6.87^{b}	2.52 ± 0.24^{a}	47.70 ± 0.59^{a}	9.02 ± 1.39^{a}
OH ZA	7.6 ± 0.36^{A}	2.3 ± 0.05^{A}	116.64 ± 7.53^{A}	1.63 ± 0.11^{B}	47.28 ± 0.86^{A}	3.77 ± 1.03^{B}
CB ZA	8.0 ± 0.72^{A}	2.0 ± 0.03^{B}	69.46 ± 6.94^{B}	2.88 ± 0.33^{A}	49.43 ± 1.24^{A}	8.02 ± 2.60^{A}

*Pore uniformity: Standard deviation of average pore size; for PUR bread, different lowercase letters (a, b) in the same column indicate a statistically significant difference ($p<0.05$) between baking methods, while for ZA bread, this is shown by different uppercase letters (A, B).

The larger average pore size observed in conventionally baked bread, compared to ohmic-baked bread, is primarily due to the structural breakdown of the dough during conventional heating, resulting in coarser porosity. Conversely, ohmic heating promotes improved dough development, resulting in a greater specific volume and improved uniformity of porosity. In conventional and ohmic baking, two opposite mechanisms of water migration occur, which are related to the different thermal gradients that characterize the two processes. This difference in water transport subsequently affects the weight loss after baking during cooling, which is due to the different water redistribution within the product matrix (Derde *et al*., 2014; Bender *et al*., 2019; Waldert *et al*., 2025). However, the conditions used in these trials resulted in quite similar baking loss values between the two heat treatments, for the same flour, within two hours of cooling. In the case of wholemeal bread, the behavior could still be influenced by the presence of bran, which could lead to different dynamics of water migration. The baking loss, as highlighted in the work discussed by Waldert *et al*. (2025), often involves not only the evaporation of water during baking but is also partly related to product losses, especially during ohmic heating. In this experiment, this condition occurred more frequently in ZA breads than in PUR breads. Analysis of the colorimetric indices (Figure 2) shows that all the bread loaves have a dark brown color with a predominance of the b* index conferred by the presence of anthocyanins combined with the bran of the flours.

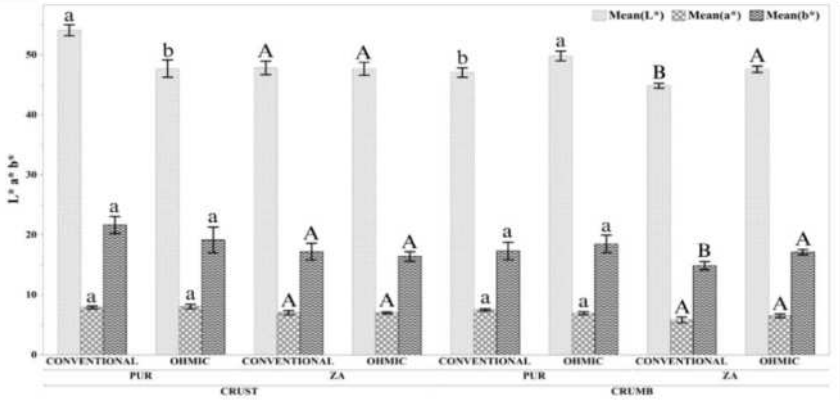


Figure 2. *Colorimetric indices of ohmic and conventional baking of wholemeal pigmented 'PUR' and 'ZA' bread.*

Statistical significance was assessed between the two baking methods (ohmic and conventional), considering the data for each specific colorimetric index (L^*, a^*, b^*) and distinctly for the 'crust' and 'crumb' factors; for PUR bread, different lowercase letters (a, b) for the same colorimetric index indicate a statistically significant difference ($p<0.05$) between baking methods, while for ZA bread, this is shown by different uppercase letters (A, B).

The rate at which ohmic baking occurs, the mode of heat transfer, and water dynamics lead to crustless bread, which would help reduce susceptibility to the Maillard reaction (Lund & Ray, 2017), instead of being associated with crust formation during conventional baking. This would result in a difference in crust color between the two baking methods. Despite this, the technological conditions adopted, combined with the use of a lid, prevented the formation of a distinctly developed crust in both ohmic and conventionally baked bread. This is evidenced by the absence of statistically significant differences in the colorimetric indices (L^*, a^*, b^*) detected for the crust, where

all bread loaves were homogeneous between the two types of baking treatment. The only difference is found in the L* index of the 'PUR' bread crust between the conventional and ohmic baking, and this is related to the presence of lighter areas (as visible in Figure 1) due to partial contact with the surface of the lid used during conventional baking. For the crumb, on the other hand, the only difference is found for the lower L* index in conventional baking compared to ohmic baking for both PUR and ZA. As a result, the crumb is slightly darker, possibly related to the partial collapse of the bread dough during baking.
Characterization analyses of total anthocyanin and total phenolic content in the bread loaves made during the trials are currently underway. First results indicate that ohmic heating appears to have a protective effect on anthocyanins compared with traditional baking. This preliminary result illustrates how exposure to elevated temperatures over a short period (180 seconds in ohmic baking) may help to preserve the anthocyanins in pigmented wholemeal bread. In addition, ohmic treatment may increase the bread loaf's anthocyanin availability after applying a medium-intensity electric field.

5. Conclusions

The optimization of the process conditions made it possible to produce comparable bread in the two different baking processes (conventional baking and ohmic heating). The use of ohmic heating also enabled a reduction in baking time and the associated energy consumption, as well as a potential increase in anthocyanin retention compared to conventional baking.

6. References

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Valorization of dairy production in inland areas: product and process innovation

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This research project aims to enhance and promote traditional dairy products from the inland areas of the Molise region. This project has been possible through a collaboration with the historic Di Nucci dairy, in Agnone. The dairy products have been analyzed from nutritional, microbiological, and genomic perspectives. In addition, we evaluated some bioactive compounds in raw milk and dairy products from the company. This research not only supports the valorization of traditional products but also promotes innovation, particularly in the development of functional dairy foods.

Caratterizzazione dei prodotti caseari tipici delle aree interne del Molise

L'obiettivo del progetto di ricerca è la valorizzazione dei prodotti caseari tipici delle aree interne della regione Molise. Il progetto è stato reso possibile grazie alla collaborazione con il caseificio storico Di Nucci, situato ad Agnone. I suoi prodotti sono stati infatti caratterizzati da un punto di vista nutrizionale, microbiologico e genomico. È stato inoltre condotto uno studio sui composti bioattivi presenti nel latte crudo e nei prodotti lattiero-caseari dell'azienda. Ciò è utile non solo per valorizzare i prodotti tipici, ma anche per la loro innovazione attraverso lo sviluppo di alimenti funzionali.

Keywords: milk, dairy products, nutritional analysis, bioactive compounds, microbiological analysis, genomic analysis.

1. Introduction

In line with the PhD thesis project previously outlined, this oral presentation highlights the main results of the following activities:

- nutritional analysis of raw milk and dairy products from the company, with a focus on their bioactive compound content;
- microbiological analysis of the whey starter and dairy products;
- genomic analysis of both the whey starter and the final dairy products.

2. State of the Art

The proposed research focuses on the valorisation of traditional dairy production in the inner areas of the Molise region. The concept of 'typicality' is closely tied to the product origin, specifically, the connection between the product and its territory, through the origin of raw materials or the location of processing activities. While tradition is a strength, it can also present challenges, particularly when it aims at improving product quality and fostering innovation in sectors that heavily rely on empirical and artisanal methods. Collaboration between research institutions and the dairy industry has led to product and process innovations making it possible to deliver foods with a balanced quality-to-price ratio, high nutritional value, and guaranteed safety standards. Re-evaluating and promoting traditional dairy production are increasingly important to meet the evolving demands of the European market, also enhancing the competitive advantage of these products. In line with the objectives of the PhD project, the initial phase of the research involved the nutritional characterization of the company's typical dairy products. This step is essential to identify ways to improve and innovate these products, especially in terms of enhancing their nutritional profile. Additionally, microbiological and genomic analyses were conducted to investigate the key shifts of the microbial evolution during production and ripening. The understanding of the origin and composition of the microbiota, and the role those different microorganisms play in flavour development and product differentiation, remains a central topic of research. Earlier studies using traditional, culture-based methods aimed to assess microbial changes influenced by the production techniques and ripening stages. However, with the growth of high-throughput sequencing technologies, research has shifted toward a more comprehensive profiling of the dairy microbiota. This approach allows us to explore the structure, function, and dynamics of microbial

communities across different layers of a single product, moving toward a holistic understanding of the biomolecules involved in shaping the final characteristics of traditional cheeses (Vacca *et al*., 2024).

3. Materials and Methods

Nutritional analysis

Samples were collected from raw milk, semi-ripened Caciocavallo (a cheese made exclusively with 100% raw milk from Molise whey starter, salt, and rennet), and from Stracciata, a fresh stretched-curd cheese made with the same ingredients. The milk was aliquoted and stored at -18°C. The cheese samples were frozen, grated, and divided into aliquots, then also stored at -18°C prior to chemical and nutritional analysis. The following parameters were analyzed: moisture, ash, protein, and fat content, following AOAC (2000) methods. Additionally, carotenoids, retinols, and tocols were quantified using the protocols reported in Panfili *et al.* (2003, 2004, 2008).

Microbiological analysis

Samples of whey starter, Stracciata, and semi-ripened Caciocavallo cheeses underwent microbiological analysis. Stracciata, a fresh stretched-curd cheese, was monitored throughout its storage period of up to 8 days, while semi-ripened Caciocavallo was analyzed during different ripening stages. The characterization of the key microbial groups was performed using culture-based methods. Streptococci were counted on M17 agar, after incubation for 72 hours at 45 °C, while lactococci were counted on M17 agar, after 72 hours at 22 °C. *Enterobacteriaceae* were enumerated on Violet Red Bile Glucose Agar after 48 hours at 37 °C, and fecal coliforms on Violet Red Bile Lactose Agar after 48 hours at 44 °C. *Micrococcaceae* were counted on Mannitol Salt Agar after 48 hours at 30 °C. Finally, lactic acid bacteria were quantified on MRS agar after anaerobic incubation for 72 hours at both 22 °C and 45 °C. These analyses followed the methodology described by Coppola *et al*. (2000).

Cheese microbiota analysys

DNA was extracted from cheese samples using the Norgen DNA Food Isolation Kit, with some modifications to the standard protocol. As a comparison, the extraction was carried out using an automatic extractor. In that case, workstation Chemagic STAR (Hamilton, Perkin Elmer, USA) was used, and the extraction protocol was performed with Chemagic STAR DNA BTS kit (Perkin Elmer, USA) according to the manufacturer's instructions. The purified DNA was stored at 4 °C for short-term use, or at -20 °C for longer storage. Quantification of the extracted DNA was performed using both Nanodrop™ and Qubit™ technologies. Taxonomic analyses of microbiota compositoin were conducted using the Illumina MiSeq platform. Specifically, the V3–V4 regions of the 16S rRNA gene were amplified from the DNA extracts using the universal fusion primers PCR1F_343 and PCR1_R784, as described by Nadkarni et al. (2002). Negative control was included to detect potential contamination, containing all reagents required for 16S rRNA amplification and library preparation. Libraries were quantified using a Qubit fluorometer. The resulting FASTA files underwent quality control using FastQC, and subsequent analyses were performed in R by using the DADA2 (Callahan et al. 2016) and phyloseq (McMurdie & Holmes, 2013) packages. DADA2 was used following author's recommendations to obtain counts of amplicon sequence variants (ASV) for each sample. After removing chimeric ASV, taxonomic assignment was carried out by comparing sequences against the SILVA v138.2 database. (Quast et al. 2013) For a first inspection of the dataset, The NMDS analysis on Bray-Curtis was carried out on rarefied ASV counts to assess differences in all samples microbiome composition resulting from manual versus automated DNA extraction methods. Following the observed differences between the two DNA extraction methods, analyses within (alpha diversity) and between (beta diversity) microbial variation are currently being conducted separately for each method.

5. Results and Discussion

Nutritional analysis

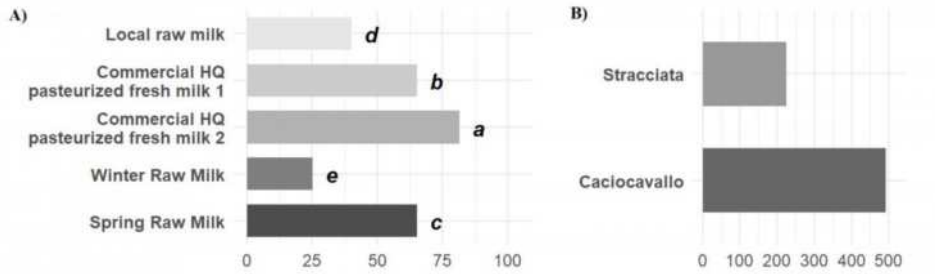
In the following tables and figures, the results on the nutritional analysis (Table 1), content of tocols (Figure 2), carotenoids (Figure 3) and retinols (Figure 4) are reported.

Table 1. *Compositional analysis of raw milk and dairy products from the company (g/100 g fresh weight f.w.).*

	Moisture (%)	Ash (%)	Protein (%)	Fat (%)
Raw Milk	88.5 ± 0.1	0.7 ± 0.0	3.1 ± 0.06	3.7± 0.0
Caciocavallo	36.4 ± 0.1	4.1 ± 0.0	27.1 ± 0.9	32.7 ± 0.2
Stracciata	66.2± 0.4	1.2 ± 0.0	15.4 ± 0.4	18.8 ± 0.2

Values are expressed as mean ± standard deviation (n=3)

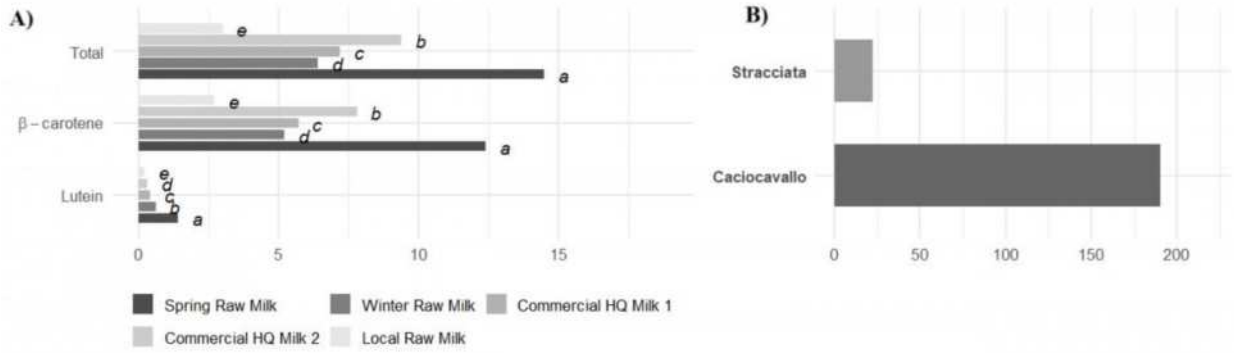
Figure 1. *A) a-Tocopherol content of raw milk from the company, commercial and local milk (µg/100 ml f.w), B) a-tocopherol content of stracciata and caciocavallo from the company (µg/100 g f.w).*



Values are expressed as mean ± standard deviation (n=3). Statistical significance was attributed to p values < 0.05 and was obtained by using the least significant difference (LSD) test.

Among the tocols, only α-tocopherol was detected, which is the primary contributor to vitamin E activity. This activity is expressed as Tocopherol Equivalents (TE) (mg/100 g).

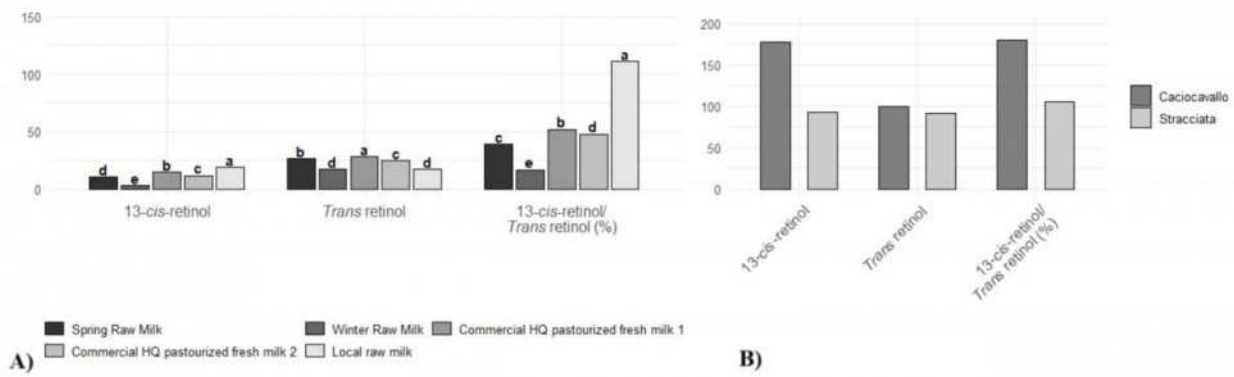
Figure 2. *A) Main carotenoid content of raw milk from the company, local and commercial milk (µg/100 ml f.w.). B) Beta-carotene content of stracciata and caciocavallo from the company (µg/100 g f.w).*



Values are expressed as mean ± standard deviation (n=3). Statistical significance was attributed to p values < 0.05 and was obtained by using the least significant difference (LSD) test.

Lutein and β-carotene were the main carotenoids detected in the milk, accounting for approximately 10% and 80% of the total carotenoid content, respectively. β-carotene was present at the highest levels, particularly in milk collected during spring, when the animals graze on pasture. It would be valuable to analyze the bioactive compound content in the forages to better understand their contribution to the carotenoid levels in the milk.

Figure 3. *A) Retinol content of raw milk from the company, commercial and local milk (µg/100 ml f.w). B) Retinol content of stracciata and caciocavallo from the company (µg/100 g f.w).*



Values are expressed as mean ± standard deviation (n=3). Statistical significance was attributed to p values < 0.05 and was obtained by using the least significant difference (LSD) test.

The main retinol compounds detected were 13-*cis* and *all-trans* retinol, representing about 14% and 77% of the

total retinol content, respectively. The ratio between 13-*cis* and all-*trans* retinol is also reported. All-*trans* retinol is considered to have 100% vitamin A activity, expressed as Retinol Equivalents (RE) (mg/100 g). In raw milk from the company (spring and winter), the ratio of 13-*cis* to all-*trans* retinol is lower than local raw milk; this fact may contribute to a higher vitamin A activity. In highly pasteurized milk samples, the increased ratio might result from the heat treatment applied. For commercial raw milk Caciocavallo and Stracciata cheeses, the higher ratio could be linked to the microbial presence and its changes during ripening and storage (Panfili *et al*. 2008).

Microbiological analysis

The results of the microbiological analyses are summarized in Table 2.

Table 2. *Microbial loads (logarithmic scale) relating to the whey starter, stracciata and caciocavallo samples at the different storage times.*

	Whey starter	Stracciata t0	Stracciata t5	Stracciata t8	Caciocavallo t0	Caciocavallo t15	Caciocavallo t30	Caciocavallo t90
Lactic Acid Bacteria at 45°C	7.4 ± 0.1	6.8 ± 0.1	6.7 ± 0.2	6.3 ± 0.6	6.3 ± 0.0	6.4 ± 0.3	6.5 ± 0.5	5.3 ± 0.1
Lactic Acid Bacteria at 22°C	5.0 ± 0.0	4.5 ± 0.3	6.1 ± 0.1	6.8 ± 0.5	5.8 ± 1.5	7.4 ± 0.1	7.3 ± 0.1	7.3 ± 0.1
Thermophilic Streptococci	8.3 ± 0.0	8.1 ± 0.0	7.1 ± 0.3	7.2 ± 0.6	6.7 ±0.1	6.8 ± 0.1	6.4 ± 0.1	5.3 ± 0.1
Lactococci	5.0 ± 0.0	5.3 ± 0.1	5.4 ± 0.1	6.1 ± 0.3	5.6 ± 0.1	7.6 ± 0.0	7.4 ± 0.1	7.4 ± 0.0
Enterobacteriaceae	<10 UFC	<10 UFC	3.0 ± 0.2	4.2 ± 0.1	<10 UFC	<10 UFC	<10 UFC	<10 UFC
Faecal coliform bacteria	<10 UFC	<10 UFC	<10 UFC	<10 UFC	<10 UFC	<10 UFC	<10 UFC	<10 UFC
Micrococcaceae	<10 UFC	<10 UFC	<10 UFC	4.4 ± 0.1	<10 UFC	3.7 ± 0.2	3.9 ± 0.3	3.1 ± 0.0
Total coliform bacteria	<10 UFC	<10 UFC	3.3 ± 0.1	3.7 ± 0.1	<10 UFC	<10 UFC	2.9 ± 0.0	2.3 ± 0.1

t0=0 days; t5=5 days; t8= 8 days; t15=15 days; t30=1 month; t90=3 months.

In the early stages of storage, for both Stracciata and Caciocavallo, we observed a diverse microflora primarily composed of thermophilic and mesophilic lactobacilli, as well as thermophilic streptococci and lactococci. This finding aligns with the literature, which reports that natural whey starters are mainly populated by thermophilic lactic acid bacteria (Coppola *et al*.,2000). The absence of coliform bacteria confirmed the good hygienic quality of all products.

Cheese microbiota 16S analysys

In the NMDS analysis, it is possible to observe differences between the extraction automatically and manually conducted (Figure 4). This suggests that the extraction methods produce different microbial profiles. The next step will be the further exploration of the microbial differences in Stracciata and Caciocavallo during time. The plots deriving from the biostatistical analyses will be evaluated to analyze differences and to choose results deriving from one of the two extraction methods to make a comparison with the subsequent analysis through the Nanopore.

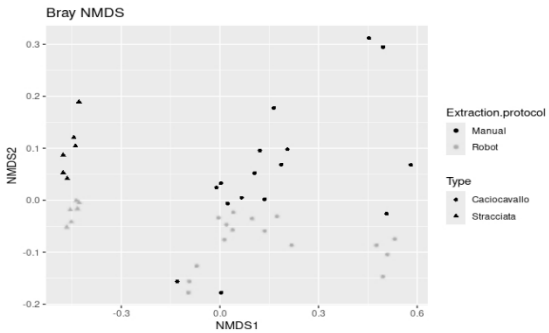


Figure 4. *Microbial community differences in analyzed dairy products revealed by NMDS : Manual vs. Automated DNA Extraction*

6. Conclusions and Future Perspectives

This research project focused on characterizing the company's typical products, demonstrating how niche dairies can produce foods with high nutritional value. This value can be further enhanced by adding ingredients rich in bioactive compounds, ideally sourced locally, which could lead to the development of innovative functional foods. One promising example is the use of wild edible plants native to the Molise region. Such an approach would also support the sustainable development of rural areas, aligning with the goals of the National Strategy for Inner Areas (SNAI). Furthermore, the application of Nanopore technology will enable a more detailed study of the microbiota, helping us to better understand its impact on the organoleptic, sensory, and safety qualities of the products.

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Application of Liquid Nitrogen for Pistachio Skin Removal

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This PhD research investigates the efficacy of a cryogenic pistachio peeling process using Liquid Nitrogen (LN_2), focusing on optimizing peeling performance, preserving kernel integrity, reducing environmental impact, and enhancing the valorisation of the resulting by-product as a source of bioactive compounds.

Applicazione dell'Azoto Liquido per la Pelatura del Pistacchio

Il presente lavoro di dottorato analizza l'efficacia di un processo criogenico di pelatura del pistacchio mediante azoto liquido (LN_2), con particolare attenzione all'ottimizzazione dell'efficienza di pelatura, alla conservazione dell'integrità del seme, alla riduzione dell'impatto ambientale e alla valorizzazione del sottoprodotto ottenuto come fonte di composti bioattivi.

Keywords: Antioxidant activity; Lipid Oxidation; Lipid Thermostability; Liquid nitrogen; Pistachio peeling; Skin bioactive compounds;

1. Introduction

In accordance with the PhD thesis project, this oral communication reports the main results of the following four activities directed to:

A1) Comparative assessment of two distinct peeling processes applied to different pistachio samples, evaluating the efficiency and the product response.
A2) Analysis of the physico-chemical properties of pistachio kernels following the peeling processes, including colour, moisture content, water activity and structural integrity.
A3) Monitoring of quality parameters, such as the oxidative stability.
A4) Characterization of bioactive compounds in pistachio skins resulting from both peeling methods.
A5) Application of analytical techniques for chemical group identification,
A6) Analysis of thermal behaviour and thermal stability.

2. The Role of Liquid Nitrogen: Properties and Potential in the Food Sector

Liquid nitrogen (LN_2) is a cryogenic fluid widely employed in various industrial sectors, and its relevance in the food industry has grown steadily in recent decades (Zhao, Powell-Palm, et al. 2021). Chemically composed of two nitrogen atoms connected by a strong triple bond, it is an inert, colourless, odourless, non-toxic, and non-flammable substance with a boiling point of -196°C. These extreme thermophysical characteristics make LN_2 especially suitable for applications requiring rapid and uniform cooling. For example, in the rapid cryogenic chilling of meat and seafood products, immersion or spray-freezing in LN_2 helps to preserve protein integrity, limit moisture loss and minimize lipid oxidation across diverse matrices. In the food sector, this translates into a range of advantages during processing, preservation, and handling of perishable goods (Arriaga et al., 2024). One of the most established applications of LN_2 in the food sector is in deep freezing. Unlike conventional freezing methods that form large, potentially cell-damaging ice crystals, LN_2 enables ultrafast cooling that produces microcrystals, preserving the structural integrity, texture, and colour of food matrices. This rapid transition also helps reduce oxidative reactions, moisture migration, and microbial growth of food product, thereby extending shelf life and maintaining organoleptic and nutritional properties. Furthermore, since LN_2 is inert, it does not leave any residue or alter the flavour or safety of the treated product (Pesce et al., 2025b). Beyond preservation, LN_2 offers logistical and environmental advantages. Its ability to function in passive cooling systems (without continuous electrical input) makes it ideal for temperature-sensitive transportation scenarios, especially in regions with limited infrastructure (Arriaga et al., 2024). Additionally, its use can reduce the reliance on traditional refrigerants with higher environmental impact. As industries seek sustainable and high-performance alternatives, LN_2 emerges as a strategic resource for innovation in food technology (Jin et al., 2025).

3. Innovative Applications of Liquid Nitrogen: From Processing to Cryogenic Peeling

While LN_2 is widely recognized for its role in freezing, its applications in the food industry are expanding into more innovative and value-added domains. Among these, its use in the preservation of fish, meat, and plant-derived products is particularly notable. In seafood and muscle foods, LN_2 treatment helps reduce lipid oxidation, minimize drip loss, and maintain tissue integrity. In plant products, though the response varies by botanical origin and structure, LN_2 has shown potential to preserve crispness, colour, and volatile compounds better than thermal methods (Pesce et al., 2025b). LN_2 is also being explored in advanced applications such as microencapsulation of bioactives, production of fine food powders (Pascual-Pineda et al., 2019; Jung and Rhee, 2020). These uses leverage the rapid phase change of LN_2 to achieve textures, structures, or preservation levels not attainable through conventional means. Among the most promising and industrially relevant innovations is cryogenic peeling (Zhao, Chen et al., 2021; Pesce et al., 2025a). This method involves exposing food products to LN_2, causing a sudden thermal shock that fractures the outer layers, typically the skin or testa, due to differences in thermal expansion between surface and underlying tissues. Once returned to ambient conditions, the skin detaches easily, enabling efficient, mechanical-free removal. Compared to conventional steam, chemical, or abrasive peeling methods, cryogenic peeling requires no added water or reagents, significantly reduces processing time, and minimizes the degradation of heat-sensitive compounds. Moreover, traditional peeling methods often compromise the phenolic content or structural integrity of the skins or kernels, limiting the functional value of by-products. LN_2-based peeling better preserves antioxidant and nutritional components, enabling potential reuse of the peels in nutraceuticals, animal feed or bioactive ingredient recovery. As food industries seek both technological efficiency and waste valorisation, cryogenic peeling presents itself as a sustainable, scalable, and high-quality solution (Pesce et al., 2025a). Overall, the integration of liquid nitrogen into both conventional and novel food processing lines marks a significant step toward precision, sustainability, and product enhancement in modern food technology.

4. Experimental Procedure

In this PhD thesis, the experimental procedure was based on a comparison of two peeling methods, traditional hot-water peeling and the experimental LN_2 treatment from several points of view. In order to evaluate the effect of LN_2 treatment pistachio kernels obtained from different peeling treatments, compared with unpeeled sample, were characterized for their physico-chemical properties: i) moisture content; ii) water activity; iii) peroxide value; iv) total acidity; v) colour parameters, while the skins acquired from each treatment were evaluated for their bioactive composition and functional activity: i) total polyphenol content; ii) flavonoid concentration; iii) total anthocyanin levels; iv) antioxidant capacity. Finally, both kernel and skin fractions were subjected to: i) Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) for functional-group identification; ii) Thermogravimetric Analysis (TGA) to assess moisture content and decomposition profiles, iii) Differential Scanning Calorimetry (DSC) for thermal-behavior analysis and iv) High resolution field emission scanning electron microscopy (FE-SEM) for detailed surface-morphology and microstructural examination.

5. Materials and Methods

Natural pistachio (PN), the unpeeled pistachio, was compared with kernels obtained by traditional hot-water peeling (P-H_2O) and corresponding skins (S-H_2O), produced by immersing pistachios in hot water (95 ± 1 °C) for 2 minutes; skins were then hand-peeled, and both fractions were dried at 70 °C for 2 hours. LN_2-peeled pistachios (P-LN_2) and skins (S-LN_2) were prepared by placing nuts on a perforated grid ~1 cm above liquid nitrogen vapour for 10 minutes, after which skins were hand-peeled with no further drying required. Physico-chemical properties, bioactive composition and antioxidant activity of pistachio samples were performed according to Pesce et al. (2025b). ATR-FTIR analysis were done using Bruker FTIR spectrometer Alpha with a Diamond Crystal ATR accessory. TGA and DSC were performed on an SDT Q600 instrument (TA Instruments) equipped with the DSC-TGA Standard module. Approximately 10 mg of each pistachio sample were placed in a platinum crucible and loaded into the analysis chamber. The temperature was ramped from ambient (25 °C) to 1000 °C at a constant heating rate of 10 °C·min^{-1}. An inert atmosphere was maintained by purging with nitrogen (N_2) at a flow rate of 100 mL·min^{-1}; no secondary purge gas was used. FE-SEM was performed with a JEOL JSM-7600F microscope (JEOL Ltd., Japan). Statistical analysis was conducted using MinitabTM software (version 20.0). One-way analysis of variance (ANOVA) was performed on mean values and Fisher's test was conducted to compare the difference between P-H_2O and P-LN_2 (differences between sample means were considered significant at $p < 0.05$).

6. Results and Discussion

6.1 Physico-chemical and functional parameters of Bronte pistachio and skin

Table 1 highlights the differences in physico-chemical parameters of Bronte pistachio kernel obtained from P-H_2O

and P-LN_2, essential for maintaining product stability and preventing contamination. The P-LN_2 treatment produced pistachio kernels with optimal UR% and Aw levels without requiring additional water use or drying processes. Notably, pistachios treated with P-LN_2 exhibited significantly lower peroxide values (PV), indicating reduced oxidation compared to P-H_2O-treated kernels, in line with those reported that Pesce et al. (2025b). Additionally, the P-LN_2-treated kernels displayed better colorimetric properties, retaining brightness and yellowness, which are often reduced by high-temperature oxidation (Khoshnoudi-Nia et al., 2019). Table 1 also presents the results for pistachio skins, where the LN_2 treatment significantly outperformed H_2O in terms of bioactive compound content and antioxidant activity. The high values in S-LN_2 were attributed to higher concentrations of polyphenols and anthocyanins, which are highly susceptible to degradation under heat. The findings suggest that cryogenic treatment with liquid nitrogen is a promising method for preserving both the quality of pistachio kernels and the bioactive compounds in their skins, offering potential for sustainable use of pistachio skin as a functional ingredient in food products (Hughey et al., 2012; Pesce et al., 2025b).

Table 1. Physico-chemical parameters of Bronte pistachio kernel obtained from different peeling treatments; bioactive compounds and antioxidant activity of pistachio skin obtained from different peeling treatments.

	PN	P-H_2O before drying	P-H_2O after drying	P-LN_2	S-H_2O	S-LN_2
UR (%)	3.26±0.24^{c}	35.18±0.35^{a}	6.95±1.38^{b}	2.07±0.20^{c}	-	-
Aw	0.51±0.02^{c}	0.90±0.01^{a}	0.61±0.07^{b}	0.54±0.02^{bc}	-	-
PV (meqO_2/kg of oil)	7.33±0.36^{b}	-	26.41±0.05^{a}	6.64±0.47^{b}	-	-
TA (%)	2.10±0-20^{c}	-	3.13±0.50^{b}	9.03±0.60^{a}	-	-
L*	-	-	38.84±0.59^{b}	45.27±0.58^{a}	-	-
a*	-	-	-5.99±0.53^{a}	-9.47±0.23^{b}	-	-
b*	-	-	21.8±0.29^{b}	23.40±1.00^{a}	-	-
C*	-	-	22.61±1.38^{b}	25.05±0.57^{a}	-	-
h°	-	-	105.35±1.29^{b}	112.20±0.31^{a}	-	-
TPC (mgGAE/g of skin)	-	-	-	-	13.33±2.56^{b}	69.84±8.15^{a}
Flavonoids (mgQUE/g of skin)	-	-	-	-	0.92±0.02^{b}	3.14±0.12^{a}
Anthocyanins (mgCy-GE/g of skin)	-	-	-	-	24.75±4.04^{b}	406.65±39.96^{a}
DPPH (mgTE/g of skin)	-	-	-	-	20.69±1.10^{b}	122.30±0.64^{a}
FRAP (mgTE/g of skin)	-	-	-	-	2041.68±73.52^{b}	19760.34±382.90^{a}

Data are expressed as mean value±standard deviation. Values followed by different letters are significantly different according to Fisher's least significant difference test ($p < 0.05$).

6.2 Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)

The FTIR analysis of pistachio samples (PN, P-H_2O, and P-LN_2) showed in Figure 1 evidenced that P-H_2O presented significant oxidative changes, including lipid peroxidation and the formation of secondary oxidation products like aldehydes. This was evident from shifts in the O-H and C=O stretching bands, suggesting degradation of unsaturated fatty acids and proteins (Valasi and Pappas, 2021; Valasi et al., 2020). In contrast, P-LN_2 preserved the chemical and structural integrity, with no signs of oxidation or degradation, confirming the effectiveness of cryogenic preservation (Okumus and Temiz, 2025).The analysis of pistachio skins showed in Figure 2 revealed that S-H_2O underwent hydrolysis and oxidative degradation, while S-LN_2 maintained greater spectral stability, preserving their bioactive compounds and structural features. These findings suggest that cryogenic treatment with LN_2 is an effective method for preserving both pistachio kernels and skins, maintaining their nutritional and functional properties, while water treatment accelerates degradation (Okumus and Temiz, 2025; Pesce et al., 2025b).

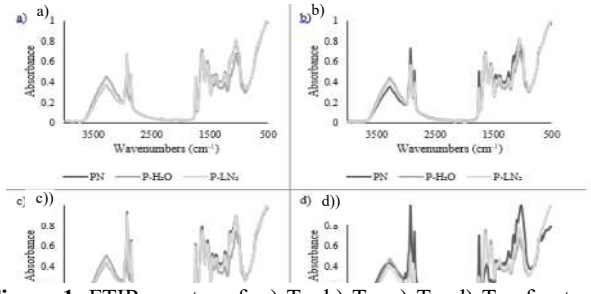


Figure 1. FTIR spectra of: a) T_0; b) T_1; c) T_2; d) T_3 of natural pistachio (PN), H_2O peeled pistachio (P-H_2O) and LN_2 peeled pistachio (P-LN_2). Analyses were conducted at the Department of Analytical Chemistry, Complutense University (CPU), Madrid.

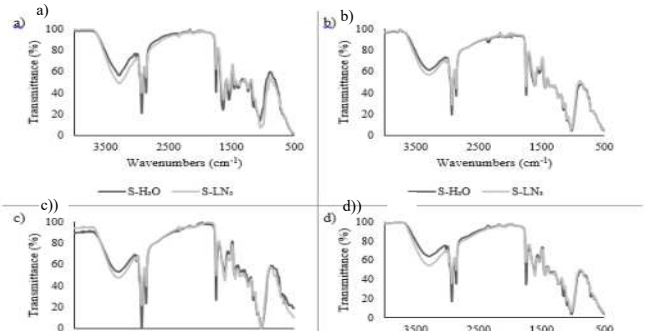
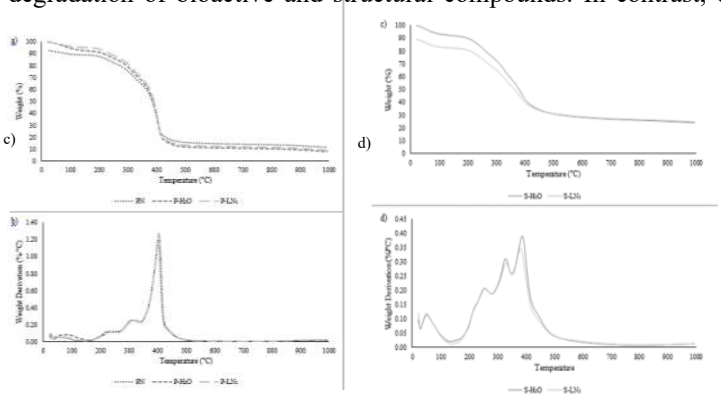


Figure 2. FTIR spectra of: a) T_0; b) T_1; c) T_2; d) T_3 of pistachio skin obtained from H_2O peeling (S-H_2O) and LN_2 peeling (S-LN_2). Analyses were conducted at the Department of Analytical Chemistry, Complutense University (CPU), Madrid.

6.3 Thermogravimetric Analysis (TGA)

TGA and its derivate (DTG) of pistachio kernels and skins (Figure 3) revealed four main degradation phases, moisture/volatile loss (<140 °C), decomposition of simple phenols and tocopherols (190-240 °C), breakdown of cellulose and stable lipids (300–-55 °C), and lignin/saturated fatty-acid degradation (360-720 °C). Hot-water peeled samples (P-H_2O, S-H_2O) showed earlier, more intense mass loss in the first three stages, indicating greater degradation of bioactive and structural compounds. In contrast, cryogenically peeled samples (P-LN_2, S-LN_2) exhibited delayed, more gradual decomposition, with over 60 % of total mass loss occurring in the final stage and higher final residues (kernels 9.4 %, skins 27.9 %), demonstrating superior retention of thermolabile constituents and overall thermal stability (Picerno et al., 2023).



6.4 Differential Scanning Calorimetry (DSC)

The DSC (Figure 4) obtained in a single heating scan from ambient temperature to 500 °C for PN, P-H_2O, P-LN_2 and their isolated skins (S-H_2O, S-LN_2) display only high-temperature exothermic events due to thermal decomposition; no melting or crystallization transitions are observed below 100 °C. This absence of low-temperature lipid phase changes is consistent with the requirement for a preliminary cooling step to -80 °C and analysis of a second heating scan to capture crystallization and melting phenomena (Sanahuja et al., 2011). In PN decomposition begins at approximately 200 °C, reaches a maximum heat-flow rate at 346°C (1.66 W/g) and releases 438.8 J/g of energy. P-H_2O shifts the decomposition peak to 431.6 °C but reduces peak intensity by about 50 % (0.83 W/g) and halves the total enthalpy release to 212.3 J/g, indicating extraction of labile lipid and volatile components (Valdés et al., 2015). P-LN_2 preserves more of the lipid fraction, with an intermediate decomposition peak at 432.3 °C, a peak heat flow of 0.98 W/g and 238.4 J/g of energy released. The isolated skins decompose at lower temperatures, 39.6 °C for S-H_2O and 333.5 °C for S-LN_2, and exhibit broader exothermic profiles, reflecting the multistep oxidative breakdown of polyphenolic and fibrous constituents (García et al., 2013).

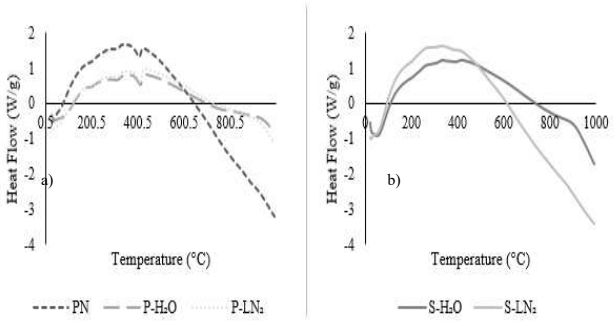


Figure 4. DSC spectra of: a) PN, P-H_2O, P-LN_2; b) S-H_2O and S-LN_2. Analyses were conducted at the Department of Analytical Chemistry, Complutense University (CPU), Madrid.

6.4 Electron microscopy via SEM

The SEM analysis (Figure 5) revealed that hot peeling produces pistachio kernels with smooth, uniform surfaces but also deep detachment lines and micro-indentations, symptoms of thermal and mechanical stress that promote lipid oxidation (Moslehi et al., 2021). In contrast, liquid-nitrogen peeling generates a uniformly rough, porous microtexture with fine, evenly distributed microfractures; despite this increased surface area, peroxide levels remain markedly lower probably due to minimal enzyme activation and slowed oxidative reactions (Chen et al., 2025). Similarly, skins from cryogenic peeling are thin, uniformly jagged sheets ideal for bioactive extractions, whereas hot-peeled skins are thicker, less flexible, and bear remnants of cuticle that introduce structural heterogeneity (Moslehi et al., 2021). Overall, cryogenic pelleting strikes a superior balance between enhanced morphology and chemical stability.

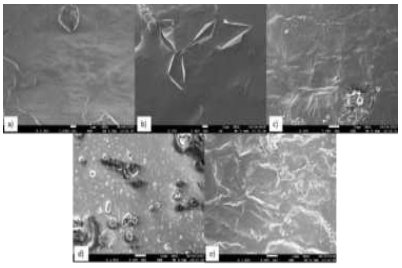


Figure 5. SEM images of: a) PN; b) P-H_2O; c) P-LN_2; d) S-H_2O; e) S-LN_2. Analyses were conducted at the Department of Analytical Chemistry, Complutense University (CPU), Madrid.

7. Conclusions and Future Perspectives

Cryogenic peeling with LN_2 outperforms conventional hot-water treatment by yielding pistachio kernels with better physico-chemical quality (moisture, water activity, peroxide value, total acidity and colour) and skins rich in bioactive compounds (polyphenols, flavonoids, anthocyanins and antioxidant capacity), while preserving structural integrity, thermal stability and microtexture. This process minimizes oxidation and valorises skins for nutraceutical or biodegradable applications. Future works will focus on pilot-scale implementation, techno-economic and life-cycle analyses, extension to other nuts, and DSC freeze–thaw cycling to reveal melting and crystallization transitions currently undetected in single-scan analyses.

This research was conducted in collaboration with Prof. Reynaldo Villalonga and Prof. Alfedo Sanchez, Department of Analytical Chemistry, Complutense University (CPU), Madrid.

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Effect of durum wheat meal in low-moisture texturized vegetable proteins and its application in filled pasta

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This PhD thesis dealt with the study of plant-based protein ingredients mainly obtained with dry fractionation technology. During the first year of this project, a comprehensive characterization of the raw materials derived from legumes and durum wheat by-products was carried out, to assess their techno-functional properties. Then, during the second year, the ingredients were used for new food product development. Specifically, one activity of the thesis aimed to produce texturized vegetable proteins (TVP) through the low moisture extrusion of durum wheat by product. Finally, in the third year, the TVPs were used to develop a filler for fresh ravioli.

Effetto del panello di grano duro in proteine vegetali testurizzate prodotte attraverso estrusione a bassa umidità e loro applicazione nella pasta ripiena

Questa tesi di dottorato ha riguardato lo studio di ingredienti proteici plant-based ottenuti mediante tecnologia di frazionamento secco. Nel primo anno di dottorato, è stata effettuata una caratterizzazione delle materie prime, derivanti da legumi e sottoprodotti di grano duro, ossia panello, al fine di comprenderne le proprietà tecnologiche e funzionali. Nel secondo anno gli ingredienti sono stati impiegati in prodotti alimentari. Una delle attività della tesi ha avuto l'obiettivo di produrre proteine vegetali testurizzate (TVP) attraverso l'estrusione a bassa umidità a partire da panello di grano duro. Infine, nel terzo anno, le TVP sono state utilizzate come ripieno nella produzione dei ravioli.
Key words: texturized vegetable proteins, durum wheat meal, by-products, dry-fractionation, fresh filled pasta.

1. Introduction

Durum wheat meal, that is milling by-product of durum wheat (*Triticum turgidum* L. ssp. *durum*) after germ oil extraction, is mainly composed of proteins and fibers. Through dry fractionation, durum wheat meal can be processed into a protein-rich ingredient, that could be utilized in different food products (Squeo et al., 2022; Squeo et al., 2023). The advantage of using this ingredient is the large-scale production and its availability during all year (Zingale et al., 2022).
The global concerns related to climate change have risen the demand for sustainable, ethical, and health-conscious alternatives to animal-based food (De Angelis et., 2024). Therefore, meat alternatives became increasingly requested by consumers who want to reduce meat consumption or follow a meat-free diet (Zahari et al., 2023). In the last few years, the application of by-products to develop meat alternatives has grown, following the principles of a circular economy (Zahari et al., 2023). Extrusion cooking technology aims to texturize protein ingredients used for alternative meat products. The texturization occurs through thermal and mechanical stress due to heating, screw rotation, and high-pressure conditions in the extruder barrel (Lee et al., 2022; Dey et al., 2021). These conditions denature, reorganize, and align proteins, resulting in a three-dimensional fibrous network formation that resembles meat muscles (Kaleda et al., 2021; Lee et al., 2022). In general, low-moisture extrusion cooking is used to produce texturized vegetable proteins (TVP) due to its simplicity, high productivity, low cost, and energy efficiency (do Carmo et al., 2023). Therefore, TVPs are widely used in the reformulation of traditional meat products such as hamburgers, sausages and meatballs (Baune et al., 2022).
Pasta is a traditional staple food known worldwide and filled with pasta. It is one of the most ancient known, that can be filled with vegetables and meat (Nazari et al., 2024). In order to produce alternatives to meat Grahl et al. (2020), produced a filled pasta, with spirulina-soy-extrudate as alternative protein source to meat and studied its sensory profile. However, the application of TVP in meat alternatives is still little explored in scientific literature. Therefore, in this work a part of the activities carried out in this PhD project is presented. Specifically, the first aim was to understand the properties of the TVPs produced through the low-moisture extrusion and the second was to apply the TVP, in fresh filled pasta. Those activities were carried out in collaboration with the research center TFTAK (Tallinn, Estonia) and the Gastronomia Frost s.r.l. company (Castellana Grotte, Italy).

2. Materials and Methods

2.1 Production and properties of the TVP

Durum wheat meal (DWM) was provided by Casillo Next Gen Food srl (Corato, Italy) and contained 22.8 g/100g of proteins and 55.89 g/100g carbohydrates (dry matter basis), whereas commercial pea protein isolate (PPI) was

purchased from Caremoli S.p.A (Monza, Italy) and had 80.0 g/100g of protein and 1.51 g/100g carbohydrates (dry matter basis). PPI and DWM were mixed in ratio 80:20, 70:30, 60:40 (PPI: DWM w:w %) and PPI was chosen as a control TVP. The extrusion process was conducted with a co-rotating intermeshing twin-screw extruder KETSE 20/40 (Brabender GmbH, Duisburg, Germany) by setting the temperature profile to 40/75/118/136/156/158 °C, the screw speed to 600 rpm and the total moisture content to 30.5%. The conditions were chosen after preliminary experiments. Water was added with a calibrated peristaltic pump and the diameter at the die was 1.6 mm. Before the extrusion, each ingredient was calibrated to determine the powder mass flow rate in the volumetric feeder. The samples were dried in the oven for 50 min at 70 °C and packed into zip-lock plastic bags and stored at room temperature before the analysis.
Water holding capacity (WHC), texture profile analysis (TPA) and quantitative descriptive sensory analysis on the TVP were conducted in TFTAK (Tallinn, Estonia) following the methods described by Kaleda et al. (2021).

2.1.1. Statistical analysis

Each analysis was conducted in triplicate, except for the sensory evaluation, that was performed in two replicate sessions. All the data are expressed as a mean and standard deviation. The means of the samples were elaborated with one-way analysis of variance ANOVA followed by Tukey's HSD (Honestly Significant Differences) test for multiple comparisons at a significance level $\alpha = 0.05$ by using Minitab 19 Statistical Software (Minitab Inc., State College, PA, USA).

2.2 Fresh filled ravioli development

The TVP produced 30% of DWM and 70% of PPI was chosen to produce filled pasta due to its good techno-functional, textural and sensory properties. Fresh filled pasta was produced in Gastronomia Frost s.r.l. Preliminary trials were carried out in order to understand if the recipe used in the company was suitable for the TVP. The main objective was substituting the bovine meat with the TVP. A flow chart of the process for pasta production is shown in Figure 1.

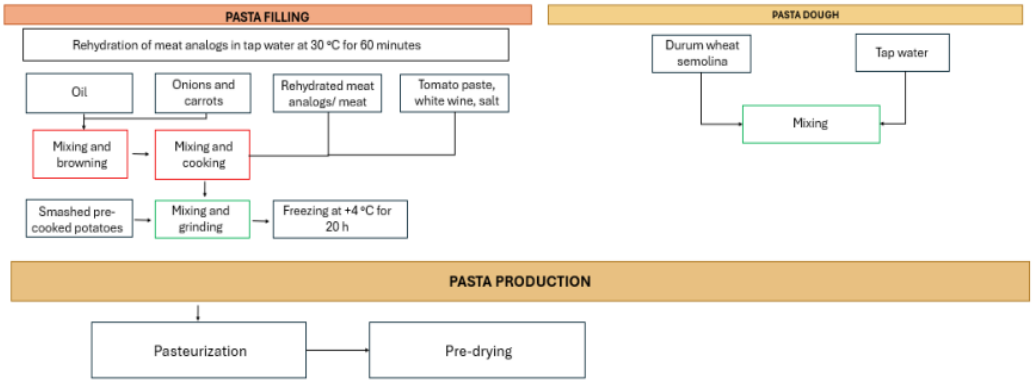


Figure 1 *Flow chart of the process for fresh filled pasta production.*

2.2.1. Texture and consumer test of ravioli

Texture of pasta was determined by a cutting test using a texture analyser, equipped with a 1 kN load cell (Z1.0 TN, Zwick Roell, Ulm, Germany). The cutting test was carried out according to the method AACC 66-50.01 (AACC, 2000). The sample (5.5 × 4 × 2 cm, L × H) was cooked at the optimal cooking time defined as the time needed for the white core in the pasta to disappear and then immediately positioned on the base to be cut. The blade descended at a speed of 1 mm/s until the sample was cut. The maximum force (N) needed to cut the cooked pasta was evaluated. The measurements were performed in eight replicates for each sample.
Consumer test was carried out in order to assess the palatability of innovative pasta. A total 122 pasta consumers 49 male and 73 female, aged from 23 to 61 y were recruited among students and employees of University of Bari and Gastronomia Frost s.r.l. Each consumer received a white dish containing one raviolo (identified by an alphanumerical code), the evaluation sheet and a pencil. Consumer test of pasta was carried out in two different sessions for each sample. Each consumer had to express an overall acceptability judgment from 1 to 9 in which score 1 represented "I do not like it at all" whereas score 9 indicated "I like it very much".

2.2.2. Statistical analysis

The obtained data are expressed as a mean and standard deviation. The means of the samples were processed with one-way analysis of variance ANOVA followed by Tukey's HSD (Honestly Significant Differences) test for multiple comparisons at a significance level $\alpha = 0.05$ by using Minitab 19 Statistical Software (Minitab Inc., State College, PA, USA).

3. Results and Discussion

3.1 Properties of TVP

The TVP produced are displayed in Figure 2. The WHC and textural properties of the TVP are presented in Table 1. WHC indicates the ability to bind water per gram of TVP (Schlangen et al., 2022), and considering the raw material composition, it could be assumed that this property was mainly influenced by the protein content, that decreased by adding DWM in the formulation (Schlangen et al., 2022). WHC may play an important role in the sensory attributes of a TVP, influencing juiciness, tenderness, texture, and mouthfeel (Lee et al., 2022), therefore its decrease could limit those aspects in TVP.



Figure 2 *Visual appearance of texturized vegetable protein (TVP) produced from pea protein isolate (PPI) and durum wheat meal (DWM) blends.*

Texture profile analysis results indicated that the samples PPI_DWC 70:30 and PPI_DWC 60:40 were similar to the control PPI in the hardness parameter, indicating that higher content of DWM did not affect the hardness of the extrudates. Adhesiveness of the samples increased by increasing DWM in the formulation, possibly due to the higher carbohydrate content in the ingredient. On the contrary, springiness in the samples containing DWC tended to decrease, as a result of the lower protein content which caused a reduction of bond formation during texturization (Brishti et al., 2021). Likewise, cohesion which depends on the protein content and to the cross-linking between proteins, decreased at higher DWM content (do Carmo et al., 2023). The decrease in chewiness was related to the higher carbohydrate content, which causes a less chewy structure (do Carmo et al., 2023).

Table 1 *Water absorption capacity (WHC) and texture properties of the TVP produced.*

Parameter	PPI	PPI_DWC 80:20	PPI_DWC 70:30	PPI_DWC 60:40
WHC (g H_2O/ g TVP)	2.82 ± 0.18a	2.14 ± 0.13b	1.80 ± 0.02c	1.75 ± 0.04c
Hardness (g)	2959 ± 374b	4029 ± 492a	3552 ± 595ab	3144 ± 459b
Adhesiveness (g*sec)	-0.06 ± 0.24a	-1.21 ± 1.36ab	-2.5 ± 0.44b	-8.45 ± 2.35c
Springiness (%)	23.62 ± 2.78a	22.57 ± 2.33a	19.14 ± 1.65b	13.37 ± 1.49c
Cohesion	0.57 ± 0.05a	0.56 ± 0.05a	0.5 ± 0.04b	0.40 ± 0.05c
Chewiness	57.26 ± 7.96a	53.35 ± 7.64ab	45.02 ± 3.38bc	41.67 ± 8.16c

PPI—Pea protein isolate; DWM— Durum wheat meal. Data are expressed as a mean ± SD. Different letters in the same row indicate significant differences according to Tukey's HSD test ($p \leq 0.05$).

Sensory properties results are presented in Figure 3. DWM had a little impact on the odor (Figure 3A) besides roastiness. However, this roastiness perception was not associated with the off-odors. Interestingly, the taste of the extrudates did not change by adding DWM (Figure 3B), and no off-taste were perceived, indicating that the by-product could effectively be used as a food ingredient.

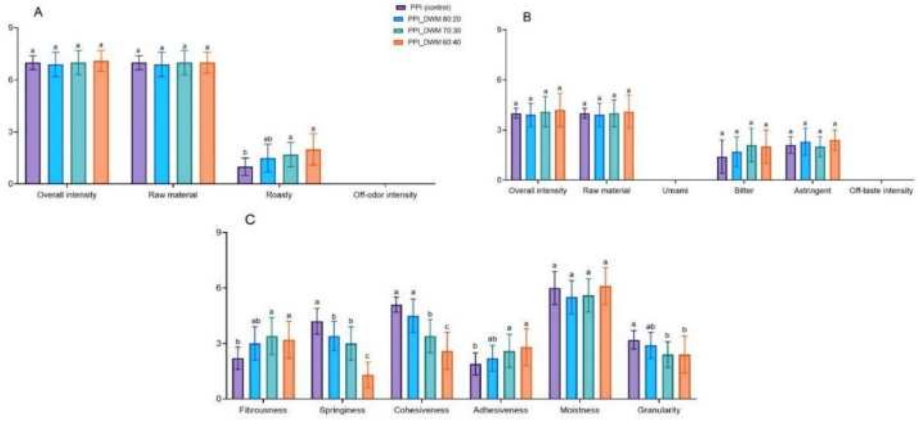


Figure 3 *Results of the sensory analysis of TVPs. A: odor, B: taste, C: texture. The scale was 0–9. PPI—pea protein isolate,*

DWM—durum wheat meal. Data are expressed as a mean ± SD (n = 2). Different letters within each attribute indicate significant differences according to Tukey's HSD test ($p \leq 0.05$).

Texture of the extrudates (Figure 3C) was the most influenced by the addition of DWM. In particular, fibrousness adhesiveness, and granularity had only one score difference between the control PPI and the experimental samples, whereas moistness was not affected by the DWM. Adhesiveness was in line with the texture profile analysis, whereas moistness results contradict the results of WHC, indicating that the increase of DWM did not change the moistness perception. These results could be explained by the small differences in WHC between the samples.

3.2 Filled pasta

Fresh filled pasta was produced by substituting bovine meat with the TVP in the formulation used by the company Gastronomia Frost s.r.l. The research activity started with preliminary trials, in which the TVP was tested to produce the filler of pasta on a laboratory scale, to evaluate its machinability. An internal sensory test was also performed in order to understand the taste of the product. Those tests were necessary to establish if the product needed improvements or was suitable for the scale up.

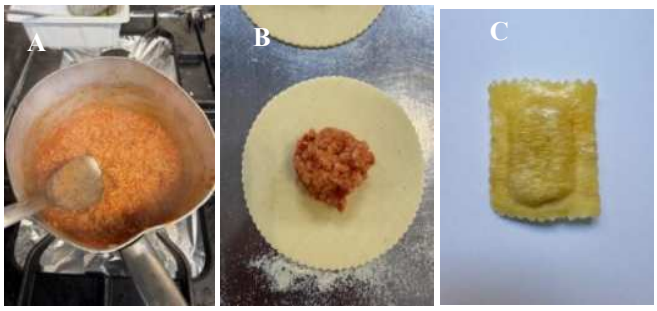


Figure 4 *Preliminary trials of filled pasta with the meat analog (A, B) visual appearance of the final experimental pasta (C).*

Cutting force of pasta is reported in Figure 5. Texture is an important attribute, specifically cutting force is connected to the firmness of cooked pasta (Larrosa et al., 2016). Fresh filled pasta produced with the TVP (Pasta MA) had a lower cutting force compared to fresh pasta filled with meat (Pasta M), as shown in Figure 5. Corrêa et al. (2023) reported that lower fat content could reduce the firmness of meat alternative products. Considering the fat content of bovine meat (16.00 g/100g) compared to the TVP (7.18 g/100g), it could be hypothesized that the higher fat content of bovine meat enhanced the firmness of the pasta M.



Figure 5 *Results of the cutting force of the filled pasta. MA—Meat analog; M—Meat. Data are expressed as a mean ± SD (n = 8). Different letters indicate significant differences according to Tukey's HSD test ($p \leq 0.05$).*

In order to understand consumer perception towards innovative foods, a consumer study was carried out and the results are presented in Figure 6.

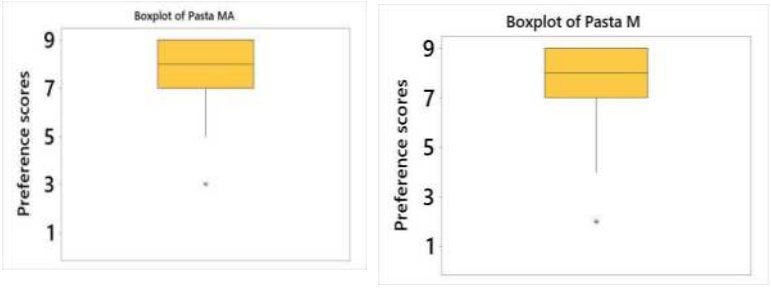


Figure 6 *Results of the consumer test of fresh filled pasta. MA—Meat analog; M—Meat.*

The box plot shows the dispersion of the preference scores, with the line in the middle, representing the median of the data, and the asterisk symbols indicating the outliers. Both the pasta samples had high preferences, indicating that the product was appreciated. This represents a positive output, considering that innovative products are difficult to accept by consumers. Indeed, Guerrero et al. (2009) and Vanhonacker et al. (2013) reported that innovations in traditional food products could fail in attracting new consumers. However, it could be hypothesized that the use of conventional ingredients to produce meat analog i.e., pea and durum wheat, listed in the evaluation sheet did not cause a neophobia in the consumer perception (Guerrero et al., 2009; Grahl et al., 2020).

4. Conclusions and Future Perspectives

The obtained results show that DWM can be effectively employed to produce TVP that, in turn, can be used as ingredient to reformulate traditional food products. Indeed, DWM was successfully incorporated into TVP production and subsequently in filled pasta. Future studies could investigate the application of those TVP in other meat alternatives such as sausages and burger, or in meat analogs produced with high moisture extrusion.

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Plant-based analogues: a sensory-based and consumer-led approach to enhance product acceptance and innovation

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This PhD thesis aims to explore sensory quality and factors influencing the acceptability of new plant-based products developed as alternatives to animal-based products. The sensory, psychological, and behavioral drivers of acceptance of plant-based fish products were explored among adult and child consumers. Moreover, a study was performed to explore the cross-national differences in *food essentialism* - the belief that food categories have intrinsic, immutable "essences" - and to understand how such beliefs shape consumer perceptions of plant-based foods. This PhD project is part of the OnFoods project (PNRR M4C2 – PE10).

Alimenti analoghi a base vegetale: un approccio sensoriale e orientato al consumatore per favorirne l'accettabilità e l'innovazione

Questa tesi di dottorato ha l'obiettivo di valutare la qualità sensoriale e i fattori che influenzano l'accettabilità di nuovi alimenti a base vegetale alternativi a quelli animali. Sono stati indagati i fattori sensoriali, psicologici e comportamentali coinvolti nell'accettabilità dei prodotti analoghi del pesce in adulti e bambini. È stato condotto uno studio cross-nazionale per indagare le differenze interculturali nell'"essenzialismo alimentare", la tendenza a ritenere che i cibi abbiano una "essenza" intrinseca e immutabile, e comprendere come tali convinzioni influenzino la percezione dei consumatori nei confronti degli analoghi. Il progetto di dottorato si inserisce nell'ambito del progetto OnFoods (PNRR M4C2 – PE10).

Key words: Plant-based protein; fish analogues; liking; sensory properties; food essentialism

1. Introduction

In recent years, growing concerns about the environmental impact of animal-derived foods, such as greenhouse gas emissions, pollution, and biodiversity loss, have driven a marked increase in the development and availability of plant-based protein alternatives in the food market. These analogues are specifically designed to replicate the sensory experience of conventional animal-based products, including meat, fish, milk, and eggs. In Europe, consumer interest in alternative protein sources continues to rise, with the market showing a further 5.5% growth between 2022 and 2023 (GFI, 2022). However, despite this positive market growth, the overall acceptability of plant-based analogues remains relatively low, largely due to challenges in achieving desirable sensory qualities (Giacalone et al., 2022). These products often fail to meet consumer expectations in terms of appearance, odour, taste, and texture (Giacalone et al., 2025).

Moreover, although these products are primarily formulated with plant-based ingredients derived from vegetables, legumes, mushrooms, and cereals, they often include a range of additives, such as flavour enhancers, colourants, and preservatives (Kyriakopoulou et al., 2021). This results in extended ingredient lists, which lead consumers to perceive them as processed and, consequently, as less natural and less healthy. However, recent studies have shown that consumer perceptions of processed foods vary significantly and may be influenced by food essentialism, the belief that foods possess intrinsic and unchangeable essences that provide their defining properties (Cheon et al., 2024). A recent study by Cheon et al., (2025) has shown that food essentialism plays a significant role in the acceptance of plant-based analogues, as people with stronger essentialist beliefs tend to perceive plant-based analogues as retaining the fundamental qualities of their animal-based counterparts, such as being natural, unprocessed, and healthy. However, these studies have mainly focused on expected perceptions rather than the actual experiences of consumers under ecologically valid tasting conditions. Therefore, there is no information on how essentialist beliefs influence the perception of sensory attributes of plant-based analogues.

In this context, this PhD project aimed to assess the drivers and barriers to the consumption of new plant-based foods alternatives to animal origin products in different population targets (adults and children). The focus was placed on individual characteristics (e.g., personality traits, sociodemographic and perceptive factors) and on the attitudes towards food (e.g. eating behaviour, consumption frequency, emotions, food essentialism).

In accordance with the PhD thesis project previously described (Appiani, 2024), this oral communication reports the main results from five key activities:

A1) Systematic literature review on the sensory properties and acceptability of plant-based analogues (Appiani et al., 2023);
A2) Online inventory of fish alternative products in the Italian market to identify the most representative items;
A3) Study of adult consumers' liking and sensory drivers of plant-based canned tuna alternatives, including the

influence of socio-demographic and psychological factors (e.g., food neophobia) and attitudes toward plant-based fish alternatives (Appiani et al., 2025);

A4) Study on the sensory drivers of liking, consumer perceptions, and emotional responses associated with the consumption of plant-based breaded fish among children and adults (Appiani et al. under review);

A5) Study of cross-cultural differences in the food essentialism belief and the role of this belief in the consumer's perception of plant-based foods (activity in progress).

2. Material and methods

A1) An extensive literature review focusing on plant-based analogues, including meat, dairy, and egg substitutes, was conducted. 13 articles focusing on meat analogues, 26 on dairy analogues, and 2 on fish and egg substitutes were identified through systematic searches in the Web of Science and Scopus databases.

A2) An inventory of the Italian online marketplace, focusing on plant-based products labelled as fish analogues was performed by examining the e-commerce of 12 supermarkets from major retail chains in northern Italy, along with 7 online stores specializing in vegetarian or vegan products.

A3) A total of 165 consumers (48% women; 38±14 y.o.) were recruited to evaluate eight canned-tuna samples: five formulated with different plant-based (PB) protein sources (e.g. textured soy, pea or wheat protein) and three animal-based control samples. Participants were asked to describe the sensory properties using the Check-All-That-Apply (CATA) method and to assess overall liking (VAS scale, 0-100). Food neophobia, food-related lifestyles (Saba et al., 2019) and frequency of consumption of PB and animal-based products (Michel et al., 2021) were investigated as liking drivers.

A4) 98 children (57% girls; 9-11 years) and 104 adults (52% women 18-60 years) evaluated five plant-based analogues (formulated with wheat, rice or a blend) and two animal-based samples for liking (9-point scale), sensory properties using CATA method, and emotional responses. Emotions elicited by foods were measured using two distinct tools: the EmoSemio approach (Spinelli et al., 2019) was employed to develop a product-specific questionnaire for adults, whereas the Emoji Pair Questionnaire (Sick et al., 2022) was utilized for the younger participants.

A5) A total of 184 Italian (71% female) and 200 Dutch (74.5% female) participants completed an online Food Essentialism Score (FES) survey (Cheon et al., 2024), along with questionnaires assessing nutritional knowledge and environmental awareness. From this sample, a subgroup of 73 Italian and Dutch participants was selected based on their FES scores (low vs. high) to take part in a tasting session involving plant-based products and their animal-based counterparts (meat, milk, fish). Participants were asked to evaluate both overall liking and specific food properties (degree of processing, naturalness, nutritiousness, sensory similarity to the category, health property, familiarity and willingness to consume) (VAS scale, 0-100) under both blind and informed conditions.

3. Results and Discussion

A1) The results of literature review have highlighted that developing plant-based alternatives to meat, dairy, eggs, and fish using plant-derived proteins poses considerable sensory challenges, particularly regarding appearance, aroma, taste, flavor, and texture (Appiani et al., 2023). Notably, the impact of each sensory dimension varies across product categories. For instance, plant-based meat substitutes often require improvements in appearance and texture, while dairy analogues are primarily limited by issues related to aroma and texture. In contrast, fish analogues remain underexplored, with limited data available on their sensory attributes and consumer acceptance. Moreover, most existing studies have focused on adult populations, providing little insight into the perceptions of younger consumers. Finally, only a few studies have adopted a multidimensional approach that considers emotional responses as potential drivers of plant-based analogues liking.

A2) Among the fifty fish alternatives identified, the best-represented brands and market availability products were plant-based canned tuna and breaded fish. These product categories were evaluated in consumer studies (A3 and A4).

A3) Results showed that canned-tuna samples were significantly different in terms of acceptance ($F_{(7,1286)} = 92.96$; $p<0.0001$): the animal-based samples were well accepted in terms of liking (Ls-mean range: 63.4 – 65.6) while plant-based ones received low liking scores (Ls-mean range: 19.8 – 40.4) (Fig. 1). Correspondence Analysis revealed that canned-tuna analogues were characterized by unpleasant appearance, legume/vegetable odor, off-flavour, bitter and sour taste, and a gummy texture (Fig.2), that were identified as drivers of rejection according to Principal Coordinate Analysis.

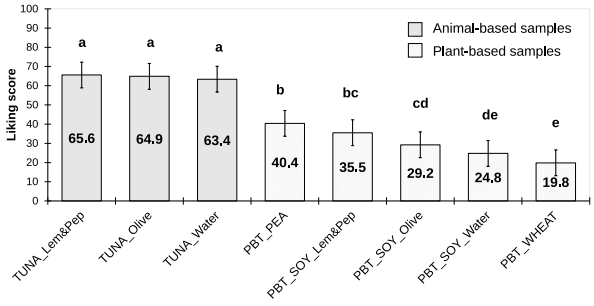


Figure 1 *Ls-mean liking scores by samples. Error bars represent standard error of the mean. Different letters indicate significant differences according to Bonferroni post hoc test.*

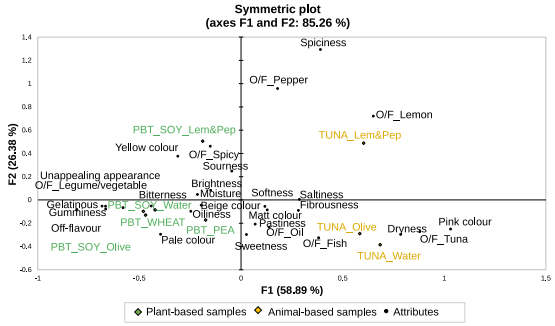
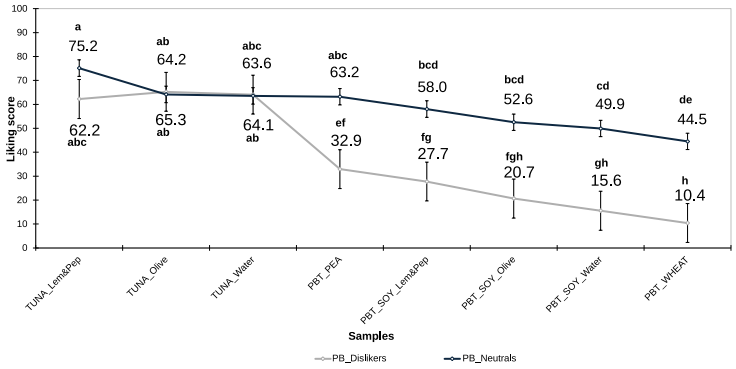


Figure 2 *Correspondence analysis symmetric plot based on CATA descriptions of eight canned-tuna samples (O = Odor; F = Flavor)*

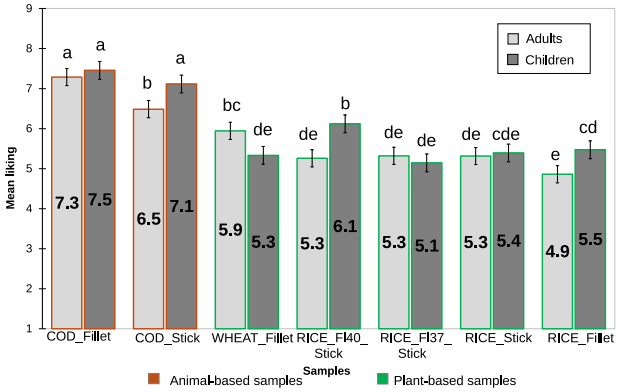
The Hierarchical cluster analysis identified two liking clusters for plant-based tuna samples: the "PB_Dislikers" (73% of consumers) and the "PB_Neutrals" (21% of consumers). PB_Neutrals expressed significantly greater liking across all analogue samples (Ls- mean range: 44.5 – 63.2), suggesting higher acceptance (Fig. 3). Demographic and psychological variables such as age, gender, and food neophobia did not differ significantly between clusters. However, the PB_Neutrals were characterized by greater familiarity with plant-based analogues, reflected in more frequent consumption of plant-based meat, dairy and eggs analogues, and stronger interest in label information and health attributes, which may explain their more favorable responses. Previous studies have demonstrated that regular consumption of plant-based products fosters greater acceptance of their sensory characteristics (Michel et al., 2021). In addition, communicating the health and environmental advantages of plant-based fish may encourage consumers to incorporate these alternatives into their eating habits (Gorman et al., 2023).

Figure 3 *Ls-mean liking scores by samples. Error bars represent standard error of the mean. Different letters indicate significant differences according to Bonferroni post hoc test.*



A4) GLM results revealed a significant effect of the main factor Samples ($F_{(6,1413)}$ =30.4, p <0.0001) and of the interaction *Samples*Age group* ($F_{(6,1413)}$ = 2.8, p = 0.01) on the liking scores (Fig. 4). Both young and adult participants rated animal-based samples significantly higher (Adults: 6.5 – 7.3; Children: 7.1 – 7.5), but they also provided generally positive scores for the plant-based samples (Adults: 4.9 – 5.9; Children: 5.1 – 6.1). Nevertheless, adults showed a stronger preference for the wheat protein-based sample (WHEAT_Fillet), whereas younger participants favored the animal-based (COD_Stick) and the rice flake-based stick (RICE_Fl40_Stick).

Figure 4 *Liking scores ± standard error of mean provided by age groups (children, adults) for each breaded fish sample. Different letters on histograms indicate significant differences according to Fisher's LSD post-hoc test.*



Despite the analogues being generally well-accepted by participants, these samples were significantly differentiated from their animal counterparts in sensory terms by both age groups, who described the samples in a similar manner. The only plant-based sample perceived similarly to animal products by both age groups was the RICE_Fl40_Stick. Plant-based samples were associated with sensory attributes, such as unappealing appearance,

off-flavor, legume and spicy odor and flavor, bitter taste and pasty and gummy filling, which negatively impacted liking, particularly among children. This outcome is consistent with existing literature that identifies odor, taste, and texture as key factors driving food rejection in children (Sick et al., 2019). Emotional responses influenced acceptance in both age groups, with positive emotions linked to animal-based products and negative emotions to plant-based ones. However, the wheat protein sample evoked curiosity and surprise in adults, contributing to its liking (Fig. 5). In contrast, children's preference for the rice-based sample was driven by familiarity and sensory similarity to traditional products (Fig. 6), with emotions providing no additional insights beyond liking.

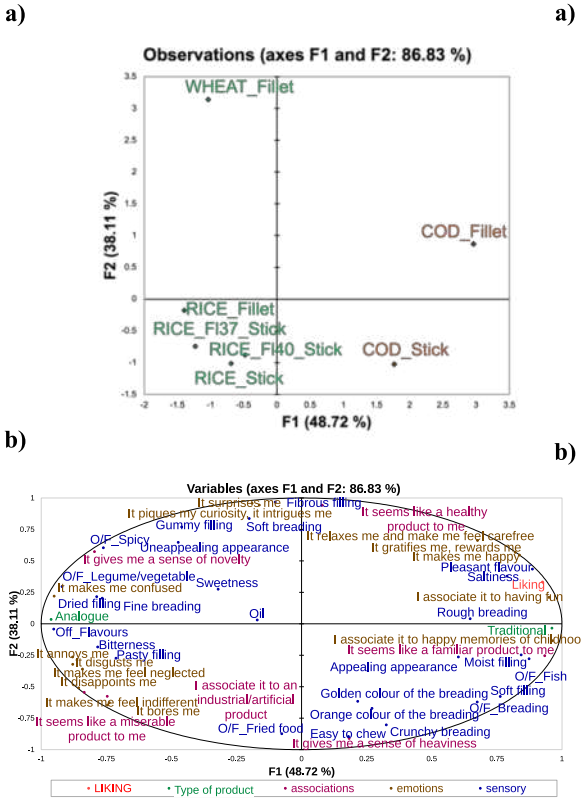


Figure 5 (a-b) *(a) Score plot and (b) correlation circle on the first and second dimensions of the MFA computed on the scores for emotions, CATA attributes and functional/emotional concepts for the adult group.*

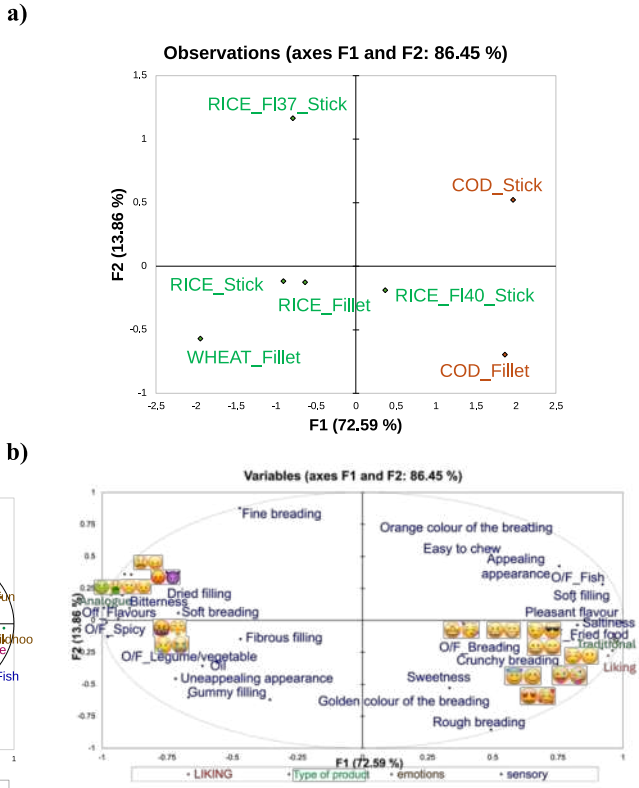


Figure 6 (a-b) *(a) Score plot and (b) correlation circle on the first and second dimensions of the MFA computed on the scores for emotions, CATA attributes and functional/emotional concepts for the children group.*

A5) Data collection for the online questionnaire has been completed. The Food Essentialism Scale (FES) in the European context showed good internal consistency in both countries ($\alpha > 0.80$). Temporal stability of each item and of total FES score was investigated through paired t-test analysis performed over countries that showed no significant differences between the FES constructs. No significant effect of *Gender*, *Age* and *Country* were found on FES score. Interestingly, FES score correlated negatively and significantly with food-related nutritional ($r = -0.33$, $p < 0.0001$), environmental ($r = -0.15$, $p = 0.001$) and sustainable ($r = -0.16$, $p = 0.001$) knowledge. These findings are in line with previous results reported by Cheon et al. (2024).

The second phase of the study is currently ongoing. Paired t-test showed that, under blind conditions (B), the level of food essentialism significantly influenced perceptions of the degree of processing for plant-based meat (Low_FES: 74.4; High_FES: 61.9) and salmon (Low_FES: 75.2; High_FES: 65.4), whereas no significant differences were observed for plant-based milk. However, in informed conditions (I), High_FES consumers tend to shift their perception, evaluating plant-based products as significantly more processed compared to blind tasting conditions (plant-based burger: I-B = 8.68, $p = 0.03$; plant-based salmon: I-B = 6.86, $p = 0.04$). No significant differences between blind and informed conditions were found among Low_FES consumers regarding perceived processing.

4. Conclusions and Future Perspectives

This PhD project, through three main studies, has contributed to a deeper understanding of the factors influencing consumer acceptance of new plant-based foods developed as alternatives to animal-derived products. The outcomes of this project provide valuable insights for food companies interested in developing plant-based analogue products. The results obtained from the three consumer tests show that: A3) Plant-based tuna samples lack sensory appeal, which leads to unfavorable attitudes and reluctance to consume them. The exploitation of

plant-based protein affects all sensory dimensions, particularly odor and taste, underscoring the challenge of replicating the sensory profile of traditional canned-tuna. A4) Age-related differences exist in the liking of plant-based bread fish samples, emphasizing the importance of age-specific preferences and emotional responses in product acceptance. For adults, emotions such as surprise and curiosity contribute positively to product liking, whereas for children, familiarity is a key factor in food acceptance. These findings underline the importance of including children into the development and optimization processes of plant-based analogue products, given their specific needs and expectations A5) The Food Essentialism Scale shows good cross-cultural applicability. In real tasting conditions, food essentialism beliefs appear to influence the perception of certain food properties, such as the degree of processing of some plant-based analogues. However, when provided with product information, consumers with high levels of food essentialism change their evaluations more significantly than those with low essentialism beliefs. Overall, the findings suggest that food essentialism influences consumer judgments and perception of plant-based analogues.

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Bioactive-Rich Mushrooms for Food Reformulation (BIOMUSH-FOOD)

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This PhD project is aimed at the development and optimization of enriched foods with bioactive-rich mushrooms (BRMs). Solid-state fermentations of *Pleurotus ostreatus* and *Ganoderma annularis* on agri-food wastes were monitored using innovative technologies (i.e., digital and thermal imaging analysis and FT-NIR spectroscopy), and the obtained biomasses were characterized. After optimization of BRMs cultivation conditions, the obtained biomasses were included in different food products. In particular, gluten-free breads enriched with 5, 7.5 and 10% BRM powder (on the flours) were produced and characterized, while the development of a 3D-printed vegan nuggets and a miso-like product is still ongoing.

Funghi Ricchi in Composti Bioattivi per la Riformulazione di Prodotti Alimentari (BIOMUSH-FOOD)

Il lavoro è finalizzato allo sviluppo di alimenti arricchiti con funghi contenenti composti bioattivi (BRM). La fermentazione allo stato solido di *Pleurotus ostreatus* e *Ganoderma annularis* è stata condotta impiegando come substrato residui di produzioni alimentari ed è stata monitorata utilizzando tecnologie innovative, quali analisi di immagini digitali e termiche e spettroscopia FT-NIR. Dopo l'ottimizzazione delle condizioni di coltivazione, le biomasse ottenute sono state caratterizzate e incluse in panini senza glutine arricchiti con il 5, 7.5 e 10% di BRM (sul peso delle farine). È ancora in corso lo sviluppo di *nugget* vegani stampati in 3D e di un prodotto simile al miso.

Keywords: Food waste, Solid State Fermentation, image analysis, FT-NIR spectroscopy, gluten-free bread

1. Introduction

An increasing interest in healthy and eco-friendly food options is directing scientific efforts toward identifying new ingredients. In this scenario, mushrooms have seen an increase in their production also due to their content in bioactive and health-promoting substances. Thanks to these valuable characteristics, bioactive-rich mushrooms (BRMs) could be used to reformulate foods to enhance their nutritional profile and provide consumers with good alternative proteins (Bearzi, 2023).
Mushrooms offer a sustainable way to upcycle agri-food waste by converting them into valuable food resources. Using agri-food residues as substrates for mushroom cultivation is an eco-friendly alternative to waste disposal. Edible mycelium can be grown via solid-state fermentation (SSF), which uses moist solid materials without free-flowing liquids (Castorina et al., 2023).
The addition of mushroom powder to food products can represent a value addition strategy, even if dependent on several parameters like the species and fortification conditions, that allow to obtain food products with enhanced nutritional attributes and quality (Dhanapal & Rajoo, 2023; Salehi, 2019).
The overall objective of this PhD research project, part of the OnFoods project, is to integrate mycelium powders of BRMs grown on agri-food wastes in different food formulations to improve their nutritional profile.

2. Experimental Plan

This report presents the main results of the PhD work, divided into the following activities (Bearzi, 2023):
A1) Literature survey about BRMs. The different characteristics of BRMs were studied, focusing on nutritional aspects, bioactive compounds, and cultivation methods and conditions. This first step led to the choice of two BRM species (*P. ostreatus* and *G. annularis*) and three agri-food wastes (fresh cut salad byproducts, brewer's spent grain, and apple pomace).
A2) Optimization of BRM SSF conditions. The results of this activity were partially presented in a previous report (Bearzi, 2024); thus, only a summary is presented here, with a focus on the second part of the research made in collaboration with the IRTA-Fruitcentre (Lleida, Spain).
A3) Food development and optimization. So far, the project has focused on the enrichment of gluten-free bread with BRM powder, even if the development of other products is still ongoing: a miso-like product and 3D-printed vegan nuggets.

A2) Optimization of BRM solid state fermentation conditions
A2.1. Growing of *P. ostreatus* and *G. annularis* on different agri-food wastes

Materials and methods

P. ostreatus (ATCC 96997) and *G. annularis* (DSMZ 9943) were grown in SSF using a reference medium (i.e., malt extract agar; MEA), fresh-cut mixed salad by-products (MS), and brewer's spent grain (BG) as substrates. Mycelium growth was monitored for 14 days for *Pleurotus* and 25 days for *Ganoderma* by digital and thermal Image Analysis as well as FT-NIR spectroscopy. The biomass was analyzed each day for moisture and then freeze-dried and stored until compositional analyses: protein content (Kjeldahl method), lipid content (Soxhlet method), ergosterol (Pedrali et al., 2020), beta-glucan (K-YBGL Kit Megazyme), and polyphenols (Folin-Ciocalteu method).

Results and discussion

The data referring to the growth of *P. ostreatus* has been already presented in detail in a previous report (Bearzi, 2024). Briefly, the three innovative technologies proved effective in monitoring *P. ostreatus* growth. MS and BG guaranteed valuable biomass superficial growth rates (MS r=21.0 %/day, BG r=16.3 %/day, MEA r=18.0 %/day). Interesting results were obtained analyzing the thermal images, because, apart from superficial growth rates, also information about biomass thickness can be extrapolated. Similarly, FT-NIR data collected on the biomass surface by means of the fiber optic probe allowed to follow the growth kinetics for all the three substrates. On the contrary, less clear results were obtained by the elaboration of spectra collected with the integrating sphere on homogenized samples. As for the composition (Tab. 1), the mycelium grown on MS showed promising results in terms of yield and protein content, despite the substrate's poor composition. Comparable ergosterol levels were observed across the BG and MS substrates.

The same protocol was then applied for SSF of *G. annularis.* The growth curves, calculated elaborating the digital images (Fig. 1.a), showed a very similar behavior on BG and MEA, whereas a slower growth was observed on MS. Compared to *P. ostreatus*, *G. annularis* reached lower growth rates (r=14.8 %/day, 8.8 %/day , and 13.7 %/day on MEA, MS, and BG, respectively). Actually, as shown in Fig. 1b, the mycelium of *G. annularis* did not cover the whole surface of the tray even at the end of 25 days of incubation. The thermal analyses showed that despite a precise segmentation of the temperature levels, the mycelium thickness and surface could be measured only in some trays. This can be due to the very thin layer of mycelium developed by *G. annularis* compared to *P. ostreatus*. The same issue arose in the elaboration of FT-NIR spectra, which showed a general growth trend, but not so clear for the SSF trial on MS.

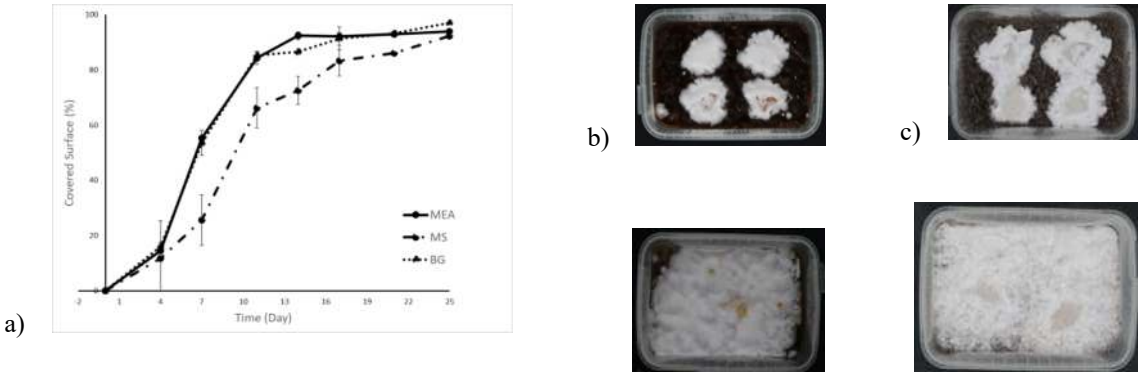


Fig. 1: *a) Growth curves of G. annularis on the different substrates. b-c) Examples of digital images of SSF trays on MS and BG, respectively (7 and 25 days).*

Results of *G. annularis* mycelium yield and composition at the end of SSF trials are shown in Tab. 1. The mycelium obtained on BG showed the highest yield and dry matter. Moreover, in agreement with Wang et al. (2023), it is important to remind that the final composition of the mycelium is correlated to that of the substrate, so, considering the micronutrient, MS exhibited a good profile.

Tab. 1*: Results of yield and composition of P.ostreatus (P) and G. annularis (G) biomasses obtained by SSF trials on different substrates (MEA, reference; MS, fresh-cut salad by-products; BG, brewer's spent grain).*

	PY (%)	BE (%)	Dry matter (%)	Protein (% d.m.)	Lipid (% d.m.)	Ergosterol (mg/100 g d.m.)	Beta-glucans (% d.m.)	TPC (mg/100 g d.m.)
P. MEA	12.5	272	8.7	15.8	1.0	60.9	34.3	211
P. MS	29.2	695	6.8	32.5	1.0	22.2	9.9	121

P. BG	31.2	170	15.9	30.4	5.6	20.3	7.4	153
G. MEA	24.5	532	7.5	17.3	3.0	71.7	22.3	323
G. MS	28.2	723	4.7	31.6	3.9	96.9	11.5	298
g. BG	30.3	165	10.8	34.9	7.6	47.7	6.8	447

PY: production yield; BE: biological efficiency; TPC: total phenol content

Based on the results of this research activity, due to the fastest growth and the high biological yield, the mycelium powder of *P. ostreatus* grown on MS was chosen as ingredient for food reformulation.

A2.2. Optimization of SSF conditions of *P. ostreatus* on apple pomace

By using a third substrate (i.e., apple pomace), an optimization study of the SSF growing conditions of *P. ostreatus* was performed during the abroad period at IRTA-Fruitcentre (Lleida, Spain).

Materials and methods

P. ostreatus mycelium (ATCC 96997) was grown in SSF using apple pomace as substrate. After sterilization, the trays with the substrate were inoculated with *P. ostreatus* mycelium and 50 mL of a sterilized wet solution were added. The trays were then sealed and incubated in a fermentation chamber (FC Range; Seduco Industries, Spain) at 25°C, 65% UR, in the dark, for 14 days. Two SSF trials were conducted, changing the experimental factors as presented in Tab. 2. At 4, 7, 10, and 14 days a picture (in duplicate) of the entire tray was taken to monitor the growth. Afterwards, the content of one tray was dried as a whole, whereas for the other tray the mycelium was separated from the substrate before drying (60°C for 48 h). The dry matter (in duplicate) and protein content (in triplicate) (Kjeldahl method) of the dried samples were evaluated. Significant differences between samples were assessed by one-way ANOVA followed by the LSD test ($p<0.05$).

***Tab. 2**: Experimental factors and levels considered in the two sets of SSF trials of P. ostreatus on apple pomace*

Set	Type of inoculum	Type of box	Wet solution
SSF-1	solid - liquid	tray sealed with lid - tray closed in a bag*- bag* *bag with breathing filter	NS (nutrient solution; Bearzi, 2024)
SSF-2	solid	tray closed in a bag*	NS - 0V (no wet solution) -H_2O (water) – aquafaba (AqF)

Results and discussion

For the SSF-1 set (results not presented), the best growing performances were obtained employing the solid inoculum and the use of the trays closed in a bag. For this reason, the second set of trials SSF-2 was performed only using the solid inoculum and the trays in a bag, to evaluate the effect of different wet solutions. The digital images acquired during the *P. ostreatus* growth (Fig. 2) showed that the addition of a wet solution is needed to obtain a good mycelium development. This is in agreement with Bellettini et al. (2019), who confirmed that moisture is one of the main factors that influence mushroom growth. This aspect also affected the quantity of mycelium that can be collected at the end of incubation.

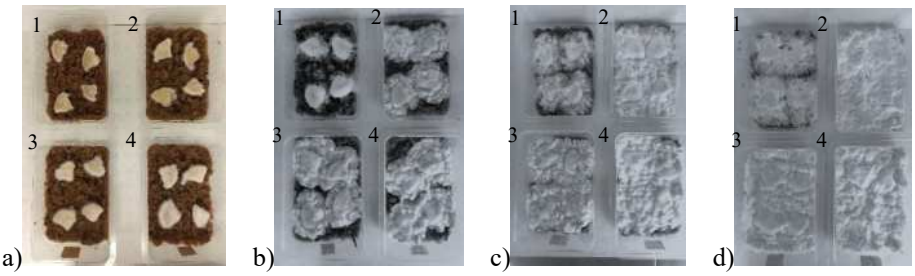


***Fig. 2**: Digital images of P. ostreatus grown on apple pomace after a)4, b)7, c)10, and d)14 days. The trays in the first row are without (1) or with water (2), the trays in the second row have nutrient solution (3) or aquafaba (4).*

Tab. 3 shows the results of the mycelial biomass dry matter and protein content at different sampling times. The mycelium obtained with the addition of AqF showed a day-by-day increase in the dry matter, resulting in the highest value at the end of SSF. The addition of H_2O did not allow a good final dry matter of the mycelium. Regarding protein content, the most promising result was obtained after 14 days with the addition of NS.

***Tab. 3**: Dry matter (mean ± SD; n = 2) and protein content (mean ± SD; n = 3) of the mycelium of P. ostreatus grown on apple pomace with the addition of different wet solutions (see Table 2 for identification) at different sampling times (4, 7, 10, and 14 days). In the same column, values with different superscript letters are significantly different (p ≤0.05).*

Wet solution	Dry matter (%)				Protein content (% d.m.)			
	T4	T7	T10	T14	T4	T7	T10	T14
0V	11.9 ± 0.5[a]	14.0 ± 0.6[b]	17.8 ± 0.2[d]	18.3 ± 0.4[c]	5.2 ± 0.2[a]	4.4 ± 0.3[a]	3.8 ± 0.2[a]	4.6 ± 0.4[a]
H_2O	10.8 ± 0.4[a]	11.9 ± 0.6[a]	14.3 ± 0.1[a]	14.7 ± 0.7[a]	5.9 ± 0.7[a]	4.6 ± 0.6[a]	4.8 ± 0.1[b]	4.4 ± 0.1[a]
NS	11.6 ± 0.6[a]	13.5 ± 0.3[b]	15.7 ± 0.3[b]	16.1 ± 0.2[b]	6.3 ± 0.2[a]	5.8 ± 0.2[b]	7.5 ± 0.1[c]	9.5 ± 0.6[c]
AqF	11.6 ± 0.1[a]	14.2 ± 0.2[b]	16.5 ± 0.1[c]	18.4 ± 0.4[c]	6.3 ± 0.1[a]	6.2 ± 0.3[b]	8.2 ± 0.4[d]	7.7 ± 0.1[b]

Considering that aquafaba is recognized as a food grade product, its use in BRM SSF at industrial level can represent a good valorization strategy.

A3) Food development and optimization

The scientific literature offers limited examples of *P. ostreatus* addition in food products. Most of the works are focused on the use of bioactive extracts instead of biomass. Thus, this work aimed to fill that gap by demonstrating how the whole mycelium biomass can improve the quality and nutritional value of food products. In particular, three applications were considered: gluten-free (GF) bread, miso-like paste, and 3D-printed nuggets. This part of the PhD project is still ongoing (since the PhD course will last until January 2026), thus, only some preliminary results about gluten-free bread are shown, whereas for the other products just the experimental plan is described.

A3.1) Enriched gluten-free bread

Materials and methods

This study investigated the effects of *P. ostreatus* mycelium powder addition (i.e., 5, 7.5, and 10% on flour content) to a GF bread made of rice flour and corn starch. A reference formulation without BRM addition was also produced (STD). GF bread samples were prepared and characterized as reported by Tuna et al. (2023), considering only fresh samples (T0) and samples stored for 24 h at 25°C and 60% relative humidity (T24; data not shown). Briefly, the dry ingredients were pre-mixed for 5 min before adding yeast, oil, and water up to the desired dough consistency of 200 ± 20 BU, considered suitable for GF formulations. The dough was collected, divided into six portions (60 g each), placed into oiled metal molds (100 ×60 ×45 mm) and leavened at 35°C for 40 min. After baking in an electric static oven for 30 min at 175°C, the loaves were cooled for 30 min at room temperature, before being removed from the molds and characterized. Three loaves for each sampling time were analyzed. Significant differences between samples were assessed by one-way ANOVA followed by the LSD test ($p<0.05$).

Results and discussion

Results of GF bread characterization just after production are reported in Table 4. The addition of the BRM mycelium significantly decreased the volume development of the loaves, resulting also in a significantly firmer crumb. However, the addition of 5 and 7.5% of mycelium powder gave quality characteristics similar to those of GF bread containing 15% bean flour (Tuna et al., 2023), with possible beneficial effect on protein, fiber and total phenolic content of the bread (analyses in progress).

Tab. 4: *Results (mean±s.d.) of quality parameters of GF bread enriched with P. ostreatus mycelium powder (STD: reference formulation without addition; M5, M7.5, M10: formulations enriched with 5, 7.5, and 10% of mycelium powder on flour weight). In the same row, values with different superscript letters are significantly different (p ≤0.05).*

Parameter	STD	M5	M7.5	M10
				
Baking loss (%)	22.1±0.8[b]	19.1±0.6[a]	19.8±0.9[a]	19.1±0.9[a]
Crumb moisture (%)	41.9±0.3[a]	45.6±0.2[b]	47.9±0.1[d]	47.5±0.7[c]
Slice moisture (%)	29.3±0.6[a]	34.8±0.8[b]	36.9±0.3[c]	37.5±0.6[c]
Specific volume (mL/g)	5.6±0.2[c]	2.6±0.2[b]	2.8±0.3[b]	1.7±0.2[a]
Max height (mm)	70.2±3.6[c]	34.1±1.0[b]	28.3±0.5[a]	26.1±0.5[a]
Crumb hardness (N)	0.8±0.1[a]	4.6±0.3[b]	4.5±0.4[b]	11.2±0.3[c]

A3.2) Miso-like product

This product is in an early stage of development. The production of sweet miso will follow the recipe suggested by Shockey and Shockey (2019), with some modifications. The koji-rice starter, made by a complex carbon source (rice) and the microorganism (*Aspergillus oryzae*), will be combined in different formulation using soya, the *P. ostreatus* cultivated on apple pomace, and a mix of them. Samples obtained will be fermented in a chamber at

26°C and characterized at 0, 10, 15, 20 and 30 days (microbial count, physico-chemical parameters, composition) to evaluate the best fermented product.

A3.3) 3D-nugget production

The first part of the product development will focus on the optimization of the 3D-printer conditions. The second part will use the *P. ostreatus* mycelium grown on apple pomace to create 3D-printed vegan nuggets according to the work by Marques et al. (2025). The obtained product will be characterized by texture properties.

3. Conclusions and Future Perspectives

The PhD project demonstrated that industrial wastes from fresh-cut salad and brewer's spent grain can serve as valuable substrates for the cultivation in SSF of *P. ostreatus* and *G. annularis*. *P. ostreatus* exhibited superior performance in terms of growth and substrate utilization, whereas *G. annularis* contained higher levels of potentially valuable bioactive compounds.

The analytical technologies employed for monitoring BRM growth delivered promising results, offering fast and reliable methods to evaluate SSF processes.

In terms of food applications, *P. ostreatus* mycelium showed potential for improving the nutritional profile of GF bread, while maintaining acceptable quality characteristics. However, sensory evaluations should be performed to validate enriched formulations.

To maximize the potential of BRMs in food and health-related industries, future research should prioritize the development of scalable production methods and investigation of bioavailability of the nutritional compounds in the final product.

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Oral presentation sessions
PhD students – 3rd year
Abstracts

PARALLEL SESSION 9
Food Microbiology and biotechnology (1)

Innovative Approaches in the Development of Novel Organic Food Products by Applying Careful Processing

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Tutor: Prof. Fabio Minervini; Co-tutor: Dr. Ivana Cavoski

This project aimed to investigate various case studies involving different organic matrices from the Apulia region, to develop innovative food products that exhibit enhanced quality and added value for both small and medium-scale processors, as well as for consumers, through the application of careful processing technologies.

Approcci innovativi nello sviluppo di nuovi prodotti alimentari biologici attraverso l'applicazione di un'attenta procedura di lavorazione

Questo progetto mira a considerare diversi casi di studio che coinvolgono diverse matrici da agricoltura biologica, provenienti dalla regione Puglia, per sviluppare alimenti biologici innovativi che presentino una migliore qualità e un valore aggiunto sia per i piccoli e medi trasformatori che per i consumatori, attraverso l'applicazione di tecnologie di trasformazione attente.

Key words: Vinegar; Organic Fruits; Acetic Acid Bacteria; Organic Food; Fermentation.

1. Introduction

The organic industry is experiencing global growth driven by consumers' demand for healthy and environmentally friendly products, recognizing organic farming's sustainable impact. Despite growth in organic processing, challenges remain, especially regarding regulations that require organic raw materials, limited use of additives, and avoidance of GMOs and irradiation. Small and medium-scale processors often lack knowledge about product value and ingredients interactions. Research is vital for value creation and supporting local organic supply chains (EU Regulation 2018/848).
In the third year of this Ph.D. project, different fruit matrices, from organic farming, were studied as susceptible to being transformed into vinegar as in summer, some fruits (sold as third category fruits) are often discarded despite their nutritional potential. Therefore, converting them into vinegar is a possible solution to decrease their waste.
The specific objectives of the case study were:
A1) To develop the production of organic fruit-based vinegars, through determination of the optimal fermentation parameters (sugar content, temperature and time of fermentation) to convert fruit sugars into ethanol, and then into acetic acid.
A2) To assess the quality of the produced fruit-based vinegars, using commercial apple vinegar as control.

2. Development of organic fruit-based vinegars

Vinegar is an acidic solution produced through the transformation of ethanol and oxygen into acetic acid and water, by acetic acid bacteria (AAB). It is an essential ingredient in European and Asian cuisines, serving various purposes as a condiment, flavor enhancer, preservative, and even a medicinal product (Solieri and Giudici, 2009). Vinegar can be produced from a range of raw materials, such as fruits, grains, and other vegetable matrices. It can be classified based on its raw material, such as fruit-based vinegars (e.g., apple cider vinegar, wine vinegar and sugarcane), and grain-based vinegars known as regular vinegar (e.g., white distilled vinegar and rice vinegar). Each type originates from the fermentation of sugars present in the raw material into ethanol, followed by acetic acid fermentation. The specific raw materials, fermentation methods, and microbial communities involved in production result in vinegars with distinct sensory attributes, including unique flavors and aromas (Ho *et al.,* 2017). Generally, vinegar production involves a two-step process: first, the alcoholic fermentation of the matrix, followed by the acetification. Alcoholic fermentation can be done in anaerobic conditions, either spontaneously or using a commercial inoculum (yeast), while acetification is done in aerobic conditions using either mother vinegar, a natural starter recovered from previous vinegar samples, or commercially available starter of AAB. The sugar content of fruits, expressed as °Brix, holds significant importance in vinegar production, as it directly influences the alcohol concentration attained upon alcoholic fermentation, thereby impacting the ultimate acidity of the vinegar (Chapman and Horvat, 1989). Generally, sugar may be added to the juices to accelerate the fermentation process in case the °Brix of the juices are low.
Recently, there has been a surge in the popularity of fruit vinegars due to their ability to retain antioxidants from fruits resulting in relatively high antioxidant capacities, compared to wine and fruit juices (Ousaaid *et al.,* 2022). Some studies focused on fruit vinegars such as obtained from strawberries (Ubeda *et al.,* 2010), pomegranate

(Ordoudi *et apples*2014), apples (Dabija and Hatnean, 2014), and mango (Adebayo-Oyetoro *et al.,* 2017). The choice of raw material used in vinegar production significantly influences the sensory and chemical characteristics of the final product (Luzón-Quintana *et al.,* 2021). In this sense, fruits from organic farming may differ from those from conventional farming, in terms of nutritional value and phenolics; consequently, the final quality of the vinegar produced from organic fruits may be different. Nowadays, some organic fruits and vinegars are marketed. However, more research is needed to study different fruit matrices and to set a protocol for organic fruit vinegar production, including the optimal parameters for fermentation. This case study focused on the characterization of high-quality organic fruit-based vinegars, obtained by apples, clementine, and peaches from organic farming.

3. Experimental Procedure

A five-step experimental procedure was designed to develop and characterize organic fruit-based vinegars: i) obtainment and standardization of fruit juices and ii) alcoholic fermentation by inoculating fruit juices with *Saccharomyces cerevisiae*; iii) acetic acid fermentation by inoculating the fermented juice with *Gluconobacter oxydans*; iv) evaluation of microbiological safety and sensory properties of the vinegar products; and v) analysis of antioxidant activity.

4. Materials and Methods

Fresh organic apples, peaches, clementines (purchased from NaturaSì, Bari, Italy), and lemons (purchased from Coop, Bari, Italy) were used to prepare juices by pulp extraction and filtration. The pH was adjusted to 5 using lemon juice, and organic sugar was added to reach 18 °Brix. Juices (350 mL) were dispensed in sterilized 500 mL bottles and treated at 100 °C for 1 min. Freeze-dried cultures of *Saccharomyces cerevisiae* DSM 1848 (from beer) and *Gluconobacter oxydans* DSM 7145 (from beer) were rehydrated in YM medium (yeast extract 3 g/L, malt extract 3 g/L, peptone 5 g/L, glucose 10 g/L, pH 5) and YPM medium (yeast extract 5 g/L, peptone 3 g/L, mannitol 25 g/L), respectively. Based on results of growth kinetics, pre-cultures of *S. cerevisiae* (in YM, 20 °C, 42 h, under static conditions) and *G. oxydans* (in YPM, 25 °C, 33 h, under stirring conditions) were used to sequentially inoculate fruit juices at an initial cell density of 7 $\log_{10}$ CFU/mL. After inoculation with *S. cerevisiae*, juices were incubated anaerobically at 20 °C for 14 days until they reached about 5% ethanol. Subsequently, *G. oxydans* was inoculated to initiate acetic acid fermentation, which was run at 15 °C for 21 days.

Microbial cell density during fermentation was monitored at multiple time points by plate counting on Saboraud Dextrose Agar (SDA) (30 °C, 48 h) for yeasts and Glucose-Yeast Extract-Calcium carbonate (GYC) agar (30 °C, 48 h) for AAB. Plate Count Agar (PCA) for total mesophilic aerobic microorganisms (30 °C, 72 h), *Enterobacteriaceae* were enumerated at the end of fermentation on Violet Red Bile Glucose (VRBG) agar, incubated at 37 °C for 24 h. Ethanol and acetic acid concentrations were determined every 24 h through enzyme-based commercial kits (Megazyme).

Descriptive sensory analysis was conducted on three experimental fruit vinegars and a commercial apple vinegar, with 10 trained panellists (aged 22–50), as described by Tesfaye *et al.*, (2002) with modifications. Each vinegar sample (20 mL) was presented in odourless, transparent plastic cups in randomized order at 20 ± 2°C. Seventeen sensory attributes covering appearance, aroma, taste, mouthfeel, and overall acceptability were evaluated using a 15 cm unstructured line scale, with a standardized lexicon provided.

Antioxidant activity was determined *in-vitro* via 2,2-diphenyl-1-picrylhydrazyl (DPPH·) scavenging assay (Caponio *et al.*, 2020) and Ferric Reducing Antioxidant Power (FRAP) assay (Minervini *et al.*, 2019). For DPPH· scavenging activity, 50 µL of sample was added to 950 µL of 0.0031 g/100 mL DPPH· in ethanol, incubated at 25 °C for 30 min in the dark, and absorbance measured at 517 nm. For FRAP, 200 µL of sample was mixed with phosphate buffer and potassium ferricyanide, incubated at 50 °C for 20 min, followed by trichloroacetic acid addition, centrifugation, and reaction with ferric chloride; absorbance was read at 700 nm. Butylated hydroxytoluene (BHT, 0.1–1 g/L) was used as a reference antioxidant.

5. Results and Discussion

5.1 Alcoholic fermentation

Alcoholic fermentation of apple (AV), peach (PV), and clementine (CV) juices was monitored over 14 days post-inoculation with *S. cerevisiae* (Fig. 1). Sugar content steadily declined from 18.0 °Brix until 7.0 (AV), 6.0 (PV), and 7.4 (CV) at day 14, with PV showing the fastest sugar consumption. These results are consistent with the work by Bouatenin *et al.* (2021) who showed that ethanol content obtained from mango and papaya juices after fermentation were significantly different. In our study, ethanol production increased progressively, peaking at 5.94% in PV, followed by 5.25% (AV) and 5.12% (CV), indicating effective alcoholic fermentation across all matrices. An ethanol concentration of about 5–7% is needed by AAB to ferment effectively during the next phase. If the ethanol level is too low, fermentation may be slow or incomplete, whereas excessively high levels can be harmful to the bacteria (Vilela, 2023; Mota and Vilela, 2024). Yeast counts rose rapidly, reaching 8.69–8.91 $\log_{10}$ CFU/mL by days 3–4, then declined to ~4.03–4.25 $\log_{10}$ CFU/mL by day 14. Total mesophilic aerobic

microorganisms followed a similar trend, peaking on day 4 (8.58–8.77 log_{10} CFU/mL) before declining. Overall, PV supported more efficient sugar-to-ethanol conversion and higher yeast activity, suggesting it as the most suitable substrate under these conditions.

5.2 Acetic acid fermentation

Over the 21-day process, ethanol levels declined progressively—from 5.25% to 0.11% in AV, 5.93% to 0.21% in PV, and 5.12% to 0.09% in CV—indicating efficient ethanol oxidation (Fig. 2). Acetic acid concentrations increased in parallel, reaching 5.07% (AV), 5.61% (PV), and 5.02% (CV). The acidity in vinegars should be at least 4% (Moros *et al.,* 2008). The initial relatively high concentration of ethanol and the high acidic conditions of the middle and final stages suggest that most of the bacteria present are AAB (Luzón-Quintana *et al.,* 2021). The growth of AAB was vigorous during the early phase of fermentation, peaking on day 4 at 8.93 (AV), 9.05 (PV), and 8.82 (CV) log_{10} CFU/mL (Fig. 2). Afterwards, AAB population steadily declined to 6.85 (AV), 6.56 (PV), and 6.88 (CV) log_{10} CFU/mL by day 21. Total mesophilic aerobic microorganisms followed a similar trend, increasing during the initial days and peaking between days 4 and 7. PV again showed the highest microbial load, reaching 9.15 log_{10} CFU/mL, suggesting that peach juice offered a more favorable environment for microbial growth during early fermentation. By day 21, microbial counts decreased to 6.42 (AV), 6.99 (PV), and 6.67 (CV) log_{10} CFU/mL, mirroring the decline in AAB and further underscoring the impact of rising acetic acid levels on microbial viability (Fig. 2). Similar trends for AAB during fermentation of black raspberry vinegar was found by Song *et al.* (2016). Importantly, total coliforms were not detected at any point during fermentation, indicating that the acidic, aerobic conditions effectively suppressed potentially harmful bacteria. This confirms the microbiological safety of the process and reflects proper hygienic and fermentation control. In contrast, Dilimen et al. (2021) found coliforms in kiwi vinegars, ranging from 2.38-4.39 log_{10} cfu/mL, but decreasing to 1.00 log_{10} cfu/mL as fermentation progressed.

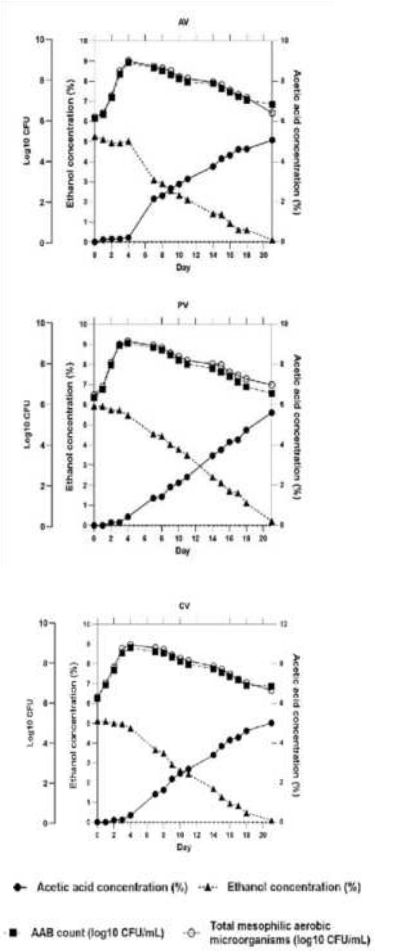


Figure 1 *Alcoholic fermentation dynamics and microbial changes in apple, peach, and clementine vinegars during 14 days.*

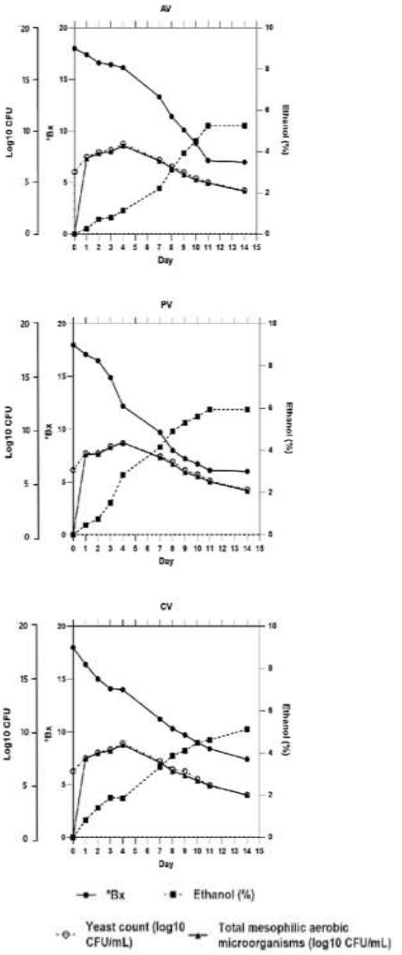


Figure 2 *Acetic acid fermentation dynamics and microbial changes in apple, peach, and clementine vinegars during 21 days.*

5.3 Descriptive sensory analysis of vinegar samples

Descriptive sensory analysis revealed notable differences among vinegar samples (Fig. 3). Apple vinegar (AV) had the highest clarity score (11.2 ± 0.9) and notable acidity (7.5 ± 0.5), contributing to its sharp taste. Peach vinegar (PV) had the lowest acceptability (5.7 ± 1.4), although it showed the highest colour (9.5 ± 0.9), aroma (10.7 ± 0.9), and floral perception (5.4 ± 1.2). Clementine vinegar (CV), with high sweetness (6.3 ± 0.8) and citrus zest notes (5.1 ± 0.9), achieved the highest acceptability (8.0 ± 0.7), reflecting broad preference for its balanced, fruity profile. Commercial organic vinegar (from apple), though low in colour intensity (2.7 ± 0.4), maintained high acceptability (7.2 ± 0.4) and taste quality (6.8 ± 0.6), serving as a well-accepted benchmark. To our knowledge, no previous studies reported a comparison of sensory traits of vinegar samples obtained from different fruits.

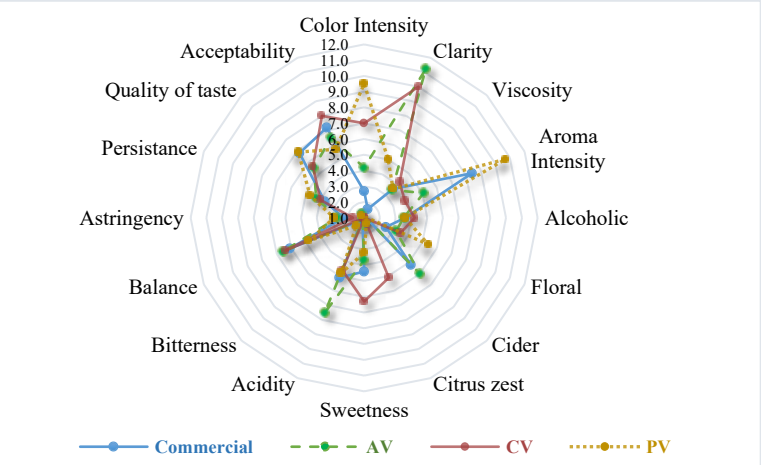


Figure 3 *Descriptive sensory analysis of vinegar samples. Commercial: Commercial Apple Vinegar; AV: Apple Vinegar; CV: Clementine Vinegar; and PV: Peach Vinegar. The data represent the mean scores given by 10 panelists using a 15 cm unstructured line scale.*

5.4 Antioxidants activity of vinegar samples

In-vitro antioxidant activity of fruit vinegars is shown in Fig. 4 and Fig. 5. The positive control (BHT) exhibited a DPPH· scavenging activity of 90.23% and a FRAP value of 2.81 (data not shown). Among the tested samples, CV showed the highest ($P < 0.05$) radical scavenging activity (83.97%), followed closely by PV, 81.56%. Compared to CV and PV, AV displayed a significantly lower ($P < 0.05$) activity (59.21%), while the commercial vinegar showed the weakest ($P < 0.05$) scavenging ability (40.67%) (Fig. 4). Antioxidant activity changes according to the matrix used. For example, Kharchoufi *et al.* (2018) reported that pomegranate vinegar exhibited significantly higher antioxidant activity compared to Rioja wine vinegar, Young Sherry vinegar, Reserva Sherry vinegar, and Gran Reserva Sherry vinegar.

FRAP results followed a similar trend. CV again showed the highest ($P < 0.05$) reducing power (2.68 UA), followed by PV (2.37 UA) and AV (2.08 UA), whereas the commercial sample had the lowest ($P < 0.05$) FRAP value (1.19 UA). Overall, CV and PV showed superior antioxidant capacity across both assays, suggesting a richer profile of bioactive compounds compared to AV and commercial vinegar **(**Fig. 5**)**. According to previous studies, various compounds found in vinegar exhibit notable antioxidant potential due to their ability to scavenge free radicals, chelate transition metal ions, and reduce oxidizing agents (Ousaaid *et al*., 2022).

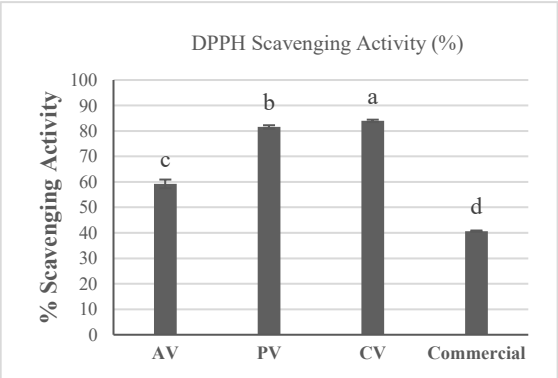


Figure 4 *DPPH scavenging activity of vinegar samples.*

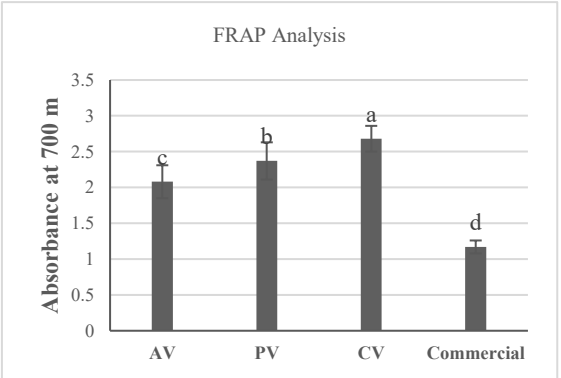


Figure 5 *FRAP absorbance values of vinegar samples at 700 m.*

6. Conclusions and Future Perspectives

This study showed the successful production, at laboratory scale, of fruit vinegars from organic apples, peaches, and clementine through controlled alcoholic and acetic acid fermentation. All three matrices supported efficient microbial growth and metabolite conversion, with peach juice consistently exhibiting higher fermentative performance, microbial activity, and acetic acid yield. Sensory analysis revealed distinct profiles among the vinegars, with clementine vinegar being the most preferred, likely due to its balanced sweetness and citrus notes,

despite peach vinegar displaying stronger aromatic intensity. Antioxidant assays (DPPH· and FRAP) further highlighted clementine and peach vinegars as superior in radical scavenging and reducing power, indicating greater functional potential. Microbiological safety was maintained throughout fermentation, with no total coliforms detected.
Clementine vinegar produced at laboratory scale was more acceptable and showed higher antioxidant activity than commercial vinegar. It would be interesting to scale-up the process, imagining a work-scheme where vinegar processors can receive fruit destined to waste from orchards and use it as raw material for obtaining a valuable organic beverage.

7. Nomenclature

AAB, acetic acid bacteria; *S. cerevisiae, Saccharomyces cerevisiae*; *G. oxydans, Gluconobacter oxydans;* DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; CFU, colony forming unit; AV, apple vinegar; PV, peach vinegar; CV, clementine vinegar; Commercial, commercial vinegar; T, time; BHT, butylated hydroxytoluene; UA, unit of absorbance.

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Microbial Collections as Biorepositories for Sourdough Microbiomes: Microbiological Assessment, Metagenomic Analysis, and Nutritional Profiling

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This doctoral research focused on the comprehensive analysis of three Sardinian Sourdoughs from the microbial collection of the University of Sassari (MBDS-UNISSCC) and the development of appropriate storage methods. The study employed multiple analytical approaches, including microbiological characterisation by means of molecular techniques (RAPD/Rep-PCR and NGS sequencing), biochemical-nutritional, and metabolic profiling using OmniLog® technology. These techniques were applied to monitor the preservation of microbiomes during 12 months of storage under two different conditions: traditional refrigeration (4°C) and cryogenic storage (-80°C), with the microbiota preserved within the fermented dough and as a purified microbial community, respectively.

Collezioni microbiche come *biorepository* per i microbiomi del lievito madre: valutazione microbiologica, analisi metagenomica e profilo nutrizionale

Questa tesi di dottorato si è concentrata sull'analisi completa di tre lieviti madre sardi depositati nella Collezione Microbica dell'Università di Sassari (MBDS-UNISSCC) e sullo sviluppo di metodi di conservazione adatti a questa comunità microbica. Lo studio ha utilizzato approcci multi-analitici, tra cui la caratterizzazione microbiologica mediante tecniche molecolari (RAPD/Rep-PCR e sequenziamento NGS), caratterizzazione biochimico-nutrizionale dei lieviti madre e dei pani ottenuti, e metabolica mediante la tecnologia OmniLog®. Queste tecniche sono state impiegate anche nel monitoraggio della conservazione dei microbiomi, durata 12 mesi, in due diverse condizioni: refrigerazione tradizionale (4°C) e conservazione criogenica (-80°C), con il microbiota conservato rispettivamente all'interno della matrice di lievito madre e come comunità microbica isolata dal substrato.

Key words: Sourdough; microbiome preservation; microbial collection; nutritional profiling.

1. Introduction

In accordance with the PhD thesis project previously described (Coronas, 2023), this oral communication reports the main results of the following four activities directed to:

A1) **Test performance study for microbiome analysis** (SOPs for genetic characterisation of microbiomes with High-throughput sequencing (HTS) technologies, including sample preparation, sequencing protocol, databases and bioinformatic pipelines).

A2) **Optimization of analytical methods to follow the quality of microbiomes during storage** (Intermediate results of the optimization of analytical methods for quality and compliance of the microbiomes).

A3) **Definition of conditions and protocols for long-term preservation of microbiomes** (Optimisation of suitable methods to control the quality of the microbiomes during storage (after 6 and 12 month) (A4.1, A4.3);
Definition of the best conditions to safely store microbiomes. Design of appropriate methodologies to propagate the conserved microbiome prior to utilisation and proof of concept related to safe deposit of microbiomes (A4.2)).

A4) **Application of the knowledge acquired to safely store and reutilize microbiomes.**

1.1 Sourdough-derived bread and its associated health benefits

Bread, a fundamental element of human nutrition for millennia, represents a complex food system whose nutritional value and digestibility are significantly influenced by fermentation processes (Canesin and Cazarin, 2021). In this context, Sardinian bread-making is distinguished by a rich tradition of sourdough bread production, originating from unique agricultural and cultural practices. The fermentation of bread using sourdough is a complex microbiological process involving a dynamic community of yeasts and lactic acid bacteria. These microorganisms not only contribute to the organoleptic characteristics of the final product but also play a crucial role in modifying the chemical and physical properties of the ingredients, with potential direct implications on the digestibility of the product (Akamine et al., 2023). Lactic acid bacteria and yeasts produce specific enzymes such as phytase, protease and amylase that degrade molecules such as phytic acid, improving the absorption of essential minerals such as iron, zinc, magnesium and calcium (Čižeikienė et al., 2015). During fermentation, wheat proteins undergo a partial pre-digestion process by microbial enzymes, with partial hydrolysis fragmenting the complex protein chains into shorter, easily digestible peptides, potentially reducing the presence of protein fractions associated with food intolerances (Di Cagno et al., 2002). The sourdough microorganisms metabolize part of the complex carbohydrates, producing organic acids that slow the absorption of sugars and result in a more gradual

release of glucose into the bloodstream, with potential benefits for controlling the insulin response (Gobbetti et al., 2014). Furthermore, during fermentation, bioactive compounds are synthesized, including lactic acid, acetic acid, bioactive peptides and exopolysaccharides with potential prebiotic properties. These compounds can perform antioxidants, immunomodulatory and potentially beneficial functions for intestinal health (Hakim et al., 2023). These transformations have a profound effect not only on the organoleptic characteristics of the final product, but also on its functional structure and overall digestibility. Despite the numerous studies that have investigated the benefits of sourdough fermentation, there are still knowledge gaps regarding the specific mechanisms that influence bread digestibility, especially with reference to specific geographical contexts and cultural traditions such as that of Sardinia.

1.2 Preservation methods for the sourdough microbiome

Traditional bread-making, that relies on the use of type I sourdoughs, is being rediscovered in artisanal and industrial bakeries due to the unique taste, texture, and potential health benefits of the baked products. These properties are attributed to the diverse microbial communities found in sourdough, comprising both yeast and bacteria. Over time, these communities undergo significant taxonomic and functional rearrangements due to extrinsic and intrinsic factors, including changes in technological conditions and incidental contamination by flour and environmental microbiota. As a result, significant deviations in the characteristics of baked products can occur. Developing proper preservation methods and establishing clear access and benefit-sharing protocols for sourdough microbiomes are thus key to safeguarding and innovating traditional foods. All these activities, including documenting food biodiversity and developing microbiome-based datasets, can be conducted within the context of microbial culture collections, such as Microbial Biological Resource Centres (mBRCs). These infrastructures will play a key role in preserving fermented foods and their microbial communities. In this context, 109 Sourdough samples collected from 64 small villages are preserved in the MBDS-UNISSCC collection at -80°C after backslopping. Descriptive records exist for 78 samples, regarding original preparation methods, provenance, time and temperature of fermentation and bakery production, that are mainly focused on traditional breads and baked goods.

2. Experimental Procedure

This PhD thesis is divided into three main phases, consisting of: i) characterisation of 3 selected sourdoughs (microbiological analyses, identification of isolates, metabarcoding through high-throughput sequencing (HTS) technologies, biochemical analysis); ii) biochemical and nutritional characterisation of prototype breads obtained with the 3 sourdoughs (quantification of organic acids, sugars, total free amino acids, assessment of digestibility); iii) Set up of preservation methods appropriate to the sourdough microbiomes under study (viable count, catabolic footprint using Omnilog technology, genomic metabarcoding during the tested storage conditions: refrigeration at 4°C and cryopreservation at -80°C); iiii) propagation and reuse of the stored sourdough microbiome through its reconstitution (viable count, High-throughput sequencing (HTS) and main technological parameters).

3. Materials and Methods

This study comprehensively characterized 3 Sardinian sourdoughs (SD_A, SD_B, SD_C) through culture-dependent microbiological analysis following standardized protocols. Particularly, for each sourdough, 24 bacterial and 24 yeast strains were identified by means of 16S rDNA and ITS sequencing and characterized by molecular fingerprinting (RAPD-PCR for bacteria, Rep-PCR for yeasts). Four bread prototypes were produced using these sourdoughs and a commercial yeast (control), then subjected to standardized *in vitro* digestion by mimicking oral, gastric, and intestinal phases to evaluate digestibility parameters. Analytical assessments included quantification of sugars and organic acids via HPLC, protein, amino acid profiling, peptide characterization by RP-FPLC, and determination of *in vitro* protein digestibility expressed as percentage of digested versus total protein. Additional evaluations encompassed total and resistant starch content, hydrolysis index and predicted glycaemic index calculations, and quantification of bioactive compounds including phytic acid and fructans.

A standardized microbiome extraction protocol was developed, that consisted of the resuspension and homogenization of 500mg of sourdough in PBS, followed by differential centrifugation (500 rpm for 20s, 100 rpm for 20’ and 15000 rpm for 40’) and resuspension in PBS. Two preservation strategies were evaluated: traditional refrigeration of whole sourdough at 4°C (limited to 2 months due to mold contamination) and cryogenic preservation of the extracted microbiome at -80°C in PBS with 20% glycerol (up to 12 months). To evaluate the suitability of the proposed extraction and preservation methods, culture-dependent enumeration of lactic acid bacteria on MRS agar and yeasts were conducted on WL agar with selective antibiotics. Further, high-throughput community analysis by sequencing of bacterial V3-V4 16S and fungal ITS2 regions, and metabolic profiling by using the OMNILOG ™ platform with BIOLOG PM1 plates, were carried out. Post-storage resuscitation of the preserved microbiomes (-80°C) involved glycerol removal through repeated centrifugation and washing steps followed by revitalization using sterilized wheat flour substrate, with comprehensive analyses performed at 0, 6,

and 12 months.

4. Results and Discussion

4.1 Microbiological and biochemical characterization of sourdoughs (SD_A, SD_B, SD_C)

Significant variation in lactic acid bacteria (LAB) counts, ranging from 8.69 to 9.83 log CFU/mL were observed in the 3 different sourdoughs, with SD_C presenting the highest microbial cell concentration. In SD_A samples, *Fructilactobacillus sanfranciscensis* (35% of total isolates) was predominant among the bacteria, followed by *Levilactobacillus brevis* and *Lactiplantibacillus plantarum*; *Kazachstania pseudohumilis* (20% of total), *Pichia kudriavzevi* and *Torulaspora delbrueckii* were the dominant yeast species. From the RAPD-PCR amplification products (M13 primers for bacteria and GTG5' rep-PCR for yeast) cluster dendrograms were generated using Pearson's coefficient (presence and intensity of bands and coefficient of 90% similarity for the bacteria and 80% for yeasts). 11 and 10 different clusters were identified for bacteria and yeast, respectively. In sample SD_B, the microbial population consisted of 7.74±0.02 Log CFU/g for yeasts and 8.54±0.08 Log CFU/g for bacteria with a yeast: bacteria ratio of 1: 6.5. All bacterial isolates were identified as *F.sanfranciscensis* (50% of the total culturable microbial community analyzed), while the yeasts belonged to the species *Kazachstania humilis* and *Saccharomyces cerevisiae*. In SD_C, compared to the other two sourdoughs, more yeasts and bacteria species were identified, with 31% of the bacterial isolates corresponding to *Pediococcus parvulus*, followed by *L. brevis, Lacticaseibacillus paracasei, L. plantarum* and *Schleiferilactobacillus harabinensis*. As for yeasts, 34% of the isolates were identified as *P. kudriavzevii*, followed by *S. mikatae, S. cerevisiae, S. kudriavzevii, T. delbruecki* and *Wickerhamomyces anomalus*.

Differences in the microbial communities resulted also in differences in the technological properties of the 3 sourdoughs. Lactic acid levels were significantly lower in SD_B (57.4 mM) compared to the other two samples (88.8 mM in SD_A and 89.8 mM in SD_C). The relative fermentation quotient (FQ) was therefore significantly lower in SD_B (0.9) compared to SD_A (1.4) and SD_C (1.5). Another key difference was the presence of mannitol, detected only in SD_B. Finally, TFAA was significantly higher in SD_C (1408 mg/Kg) compared to the other two samples, while peptides were significantly higher in SD_B (45.7 mg/g) compared to SD_A (29.0 mg/g) and SD_C (28.8 mg/g).

Table 1 *Microbiological and biochemical characteristics of sourdoughs*

Analysis	SD_A	SD_B	SD_C
Presumptive LAB (Log CFU/mL)	9.17±0.06 [a]	8.69±0.31 [a]	9.83±0.01 [b]
Yeasts (Log CFU/mL)	7.90±0.04 [ab]	7.19±0.40 [a]	8.29±0.08 [b]
DY	160	145	150
pH	3.78±0.01 [a]	3.88±0.05 [b]	4.02±0.02 [c]
TTA (mL NaOH 0,1M)	19.8±0.4 [a]	20.9±1.1 [a]	18.8±0.6 [a]
Lactic acid (mM)	88.8±0.3 [b]	57.4±12.2 [a]	89.8± 6.6 [b]
Acetic acid (mM)	61.6±0.4 [a]	63.9±3.4 [a]	60.0±0.7 [a]
FQ	1.4±0.0 [b]	0.9±0.2 [a]	1.5±0.1 [b]
Maltose (mM)	ND	ND	ND
Glucose (mM)	ND	ND	ND
Fructose (mM)	ND	ND	ND
Mannitol (mM)	ND	16.17±3.84	ND
TFAA (mg/Kg)	1106±97[a]	946±6 [a]	1408±20 [b]
Peptides (mg/g)	29.0±1.7 [a]	45.7±1.7 [b]	28.8±3.6 [a]

In summary, SD_C is distinguished by higher microbial load (LAB and yeasts), higher pH and elevated TFAA content, while SD_B is characterized by the presence of mannitol and higher peptide content.

4.2 Biochemical and Nutritional Characterisation of Breads fermented with the 3 sourdoughs

The biochemical characterization of bread samples revealed significant differences in fermentation-related parameters and nutritional composition. Control bread (BCont – leavened by commercial baker's yeast) exhibited significantly higher pH (5.64) and lower total titratable acidity (2.95 mL NaOH 0.1M) compared to sourdough breads (BSD_A: pH 4.89, TTA 4.50; BSD_B: pH 4.68, TTA 5.75; BSD_C: pH 4.83, TTA 4.85), reflecting the acidification effect of lactic acid fermentation. BSD_A demonstrated significantly higher in vitro protein digestibility (78.88%) compared to other samples. BCont showed the highest predicted glycemic index (68.08%) versus BSD_B (59.08%) and BSD_C (60.39%), indicating partial starch degradation during sourdough fermentation (Tab.2). Sourdough-fermented breads exhibited significantly reduced phytic acid content compared to BCont, confirming the phytase activity of lactic acid bacteria in improving mineral bioavailability, while fructans levels were paradoxically higher in sourdough samples, suggesting limited fructan degradation or potential microbial synthesis. Post-digestion analysis revealed that BSD_B and BSD_C contained elevated glucose and lactic acid concentrations, with BSD_B uniquely containing mannitol, while total free amino acid (TFAA) content was significantly higher in BSD_C (687.8 mg/Kg) compared to BCont (492.9 mg/Kg). The amino acid profile analysis demonstrated that digested BSD_B and BSD_C samples (DBSD_B, DBSD_C) contained elevated levels of both essential and non-essential amino acids compared to controls, indicating enhanced proteolysis and improved protein digestibility through sourdough fermentation, with the higher lactic acid content correlating with increased amino acid availability for intestinal absorption.

Table 2 *Biochemical and nutritional characteristics of bread made with different sourdoughs and control bread (baker's yeast only)*

Analysis	BCont	BSD_A	BSD_B	BSD_C
DY	152	150	148	149
pH	5.64±0.14 ^{c}	4.89±0.06 ^{b}	4.68±0.03 ^{a}	4.83±0.10 ^{b}
TTA	2.95±0.07 ^{a}	4.50±0.00 ^{b}	5.75±0.35 ^{c}	4.85±0.17 ^{b}
IVPD	70.18±1.71 ^{a}	78.88±2.21 ^{b}	68.35±1.54 ^{a}	68.10±1.52 ^{a}
pGI %	68.08±1.95 ^{b}	67.08±2.03 ^{b}	59.08±0.87 ^{a}	60.39±0.49 ^{a}
TFAA (mg/Kg)	492.9±10.5 ^{a}	594.2±32.3 ^{ab}	619.5±24.4^{b}	687.8±27.1 ^{b}

4.3 Assessment of preservation methods

Storage at 4°C of whole sourdoughs was deemed not suitable for long term preservation as the samples became contaminated with mould only after two months. Storage at -80°C slightly affected the community evenness of the microbiomes extracted from the three sourdough samples (SD_A, SD_B and SD_C), with differences among samples: Shannon diversity decreased in SD_A and SD_B, but increased in SD_C. However, beta diversity analysis revealed that each sourdough sample retained its unique microbial community composition after 12 months, with no substantial large-scale restructuring observed. Taxonomic profiling revealed sample-specific shifts in dominant genera, notably in SD_C, which exhibited reduced *Pediococcus* abundance and increased *Levilactobacillus* and *Fructilactobacillus* (Figure 1). Metabolic analysis (OMNILOG) demonstrated that, despite minor changes in substrate utilisation capabilities, microbiomes retained their phenotypic individuality, suggesting that storage at -80°C with glycerol as the cryoprotectant is an effective method of preserving microbiomes in the long term while maintaining both structural and functional characteristics.

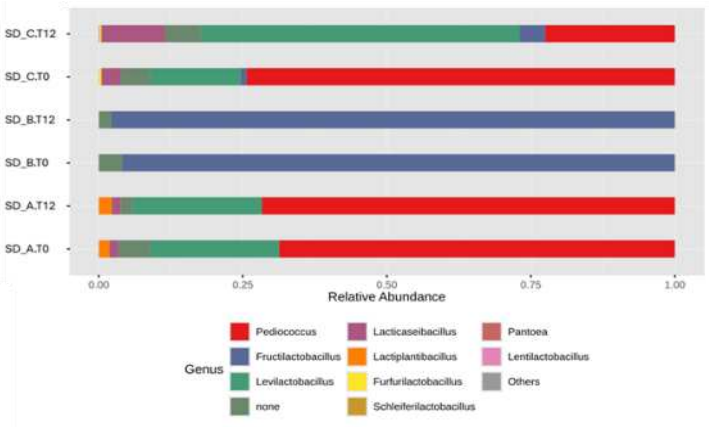


Figure 1 *Relative abundance of the dominant bacterial genera before (T0) and after (T12) preservation of the microbiomes in sourdoughs SD_A, SD_B and SD_C.*

5. Conclusions and Future Perspectives

While the benefits of maintaining sourdough microbial communities are clear, numerous obstacles emerge that demand both technological progress and revised regulatory structures. Initially, there is currently no established methodology for the extended storage of sourdough microbial ecosystems, especially type I varieties, which represent active microbial environments undergoing continuous transformation. Therefore, appropriate conservation techniques must be developed to avoid substantial modifications in the microbial diversity and technological characteristics of these communities. Additionally, moral questions arise concerning the rights and application of preserved sourdough cultures, particularly those holding traditional importance. Recording and protecting the diversity of fermented products and ancestral methods is also crucial for advancing food science and future investigations. Within this framework, dedicated microbial resource facilities are anticipated to become central in creating and executing uniform procedures to catalogue and safeguard the microorganisms of threatened fermented foods, thereby securing availability of these precious materials for coming generations.

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Lactic acid bacteria as a tool to reduce the allergenicity of Arginine Kinase and Tropomyosin from the edible insect *Hermetia illucens*

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This PhD project aims to study and optimize lactic acid bacteria (LAB) fermentation processes to produce or degrade molecules of interest for the food sector. The project is structured into three main phases: *in silico*, *in vitro*, and *in situ* analysis. Each of these phases focused on assessing the proteolytic activity of some LAB strains on Arginine Kinase (AK) and Tropomyosin (TPM) from the edible insect *Hermetia illucens* (black soldier fly, BSF), responsible for IgE cross-reactivity in patients allergic to shrimps and/or mites. Different growth conditions were applied, and immunoblotting assays were performed to verify the reduction of allergenicity.

Utilizzo di batteri lattici come strategia per la riduzione dell'allergenicità di Arginina Chinasi e Tropomiosina di *Hermetia illucens*

Questo progetto di dottorato ha l'obiettivo di studiare e ottimizzare processi di fermentazione con batteri lattici (LAB) in grado di produrre o degradare molecole di interesse per il settore alimentare. Il progetto è suddiviso in tre fasi: analisi *in silico*, *in vitro* e *in situ*. Ognuna di queste fasi è incentrata sulla valutazione dell'attività proteolitica di alcuni ceppi di LAB su Arginina Chinasi (AK) e Tropomiosina (TPM) di *Hermetia illucens* (black soldier fly, BSF), responsabili di cross-reattività verso IgE di soggetti allergici a gamberetti e/o acari. Sono state applicate diverse condizioni di crescita e la riduzione dell'allergenicità è stata verificata tramite immunoblotting.

Key words: allergens, arginine kinase, tropomyosin, lactic acid bacteria, edible insects, epitopes, food safety.

1. Introduction

The growth of the population has created the need for alternative food sources, such as edible insects (Bessa et al., 2020; van Huis, 2022). In this context, the larvae of black soldier fly (*Hermetia illucens*, BSF) represent a promising and sustainable source of nutrients for human consumption (Leni et al., 2020). However, this source may present the risks associated with allergenicity (EFSA, 2015). In arthropods and mollusks, tropomyosin (TPM) and arginine kinase (AK) are the main causes of allergic reactions in the population. Invertebrate AK is an enzyme involved in cellular energy metabolism, but it is also an allergen in crustaceans and mites responsible for IgE cross-reactivity within invertebrate organisms. Recently, AK from BSF was identified, and its IgE reactivity with sera from patients allergic to mites and crustaceans was established (Delfino et al., 2024). To address these allergenic risks, lactic acid bacteria (LAB) fermentation has emerged as a promising solution (El Mecherfi et al., 2020; Licandro et al., 2020; Pi et al., 2021). Indeed, through a proteolytic system composed of cell envelope proteinases (CEP), LAB can degrade proteins into oligopeptides, inactivating IgE-epitopes (Christensen et al., 2022; Kieliszek et al., 2021; Pescuma et al., 2015).

To explore this potential, the present study aimed to investigate the proteolytic activity of LAB strains with sequenced genomes belonging to the University of Parma Culture Collection Official (UPCCO), leading to possible degradation of insect allergens associated to a reduced IgG and IgE-reactivity.

In accordance with the PhD thesis project previously described, this oral communication presents the main results of the following activities:

(A1) *In silico* analysis of LAB genomes to study the distribution of cell-envelope proteases (CEP).
(A2) Test the proteolytic activity *in vitro* by fermentation processes applying different growth conditions (time, temperature, inoculum concentration, etc.) in the presence of allergen, by using LAB strains in different physiological conditions.
(A3) Verify the *in situ* degradation of allergens, such as arginine kinase and tropomyosin, in a food matrix like insect powder as an alternative to crop derived flour.

2. Materials and methods

Five LAB strains belonging to three species (*Lacticaseibacillus rhamnosus* UPCCO 1019 and 1473, *Lacticaseibacillus paracasei* UPCCO 2306 and 2333, and *Lacticaseibacillus casei* UPCCO 4186) were considered for their proteolytic activity through *in silico*, *in vitro,* and *in situ* analyses.

2.1 *In silico* analysis

In RAST software the complete genome of LAB strains was annotated. Bioinformatic analysis was conducted to search for the CEP in LAB, using PrtP, PrtR, and PrtS sequences from NCBI as queries. Alignments between sequences of possible CEP and queries deposited in UniProt were performed by using Clustal Omega service (https://www.ebi.ac.uk/jdispatcher/msa/clustalo) and visualized by GeneDoc.

2.2 *In vitro* analysis

Following the *in silico* analysis, *L. rhamnosus* UPCCO 1473 and *L. paracasei* UPCCO 2333 strains were selected for the *in vitro* assay. Frozen stock cultures were revitalized twice in MRS broth (3% v/v) (Oxoid, Milan, Italy) and incubated aerobically at 37°C for 16h. To avoid interference with LAB activity due to small peptides usually present in complex culture media, 19 MRS-modified mediums have been tested. Based on the evaluation of microbial growth and the response to immunoblotting assays, MRS 7 (5g/L CH_3COONa; 2g/L K_2HPO_4; 0.05g/L $MnSO_4$; 0.2g/L $MgSO_4$; 2g/L $C_6H_{17}N_3O_7$; 20g/L glucose; 5g/L peptone; 5g/L meat extract) was chosen.
The LAB effect on recombinant AK was evaluated considering the cells in different physiological conditions: 1) growing cells (GC) and 2) non-growing cells (NGC).
Condition 1: Briefly, each revitalized culture was inoculated (3% v/v) in MRS 7 broth added with 0.25 µg/µl of AK. The microplates were incubated at 25°C for 16h in Tecan (SPARK, Groding, Austria), recording the OD (600 nm) to monitor microbial growth. Samples were collected every 2h, and plate counts were carried out in triplicate on MRS agar (Oxoid), after aerobic incubation at 37°C for 48h.
Condition 2: Briefly, the strains, at the exponential growth phase, were inoculated (3% v/v) in MRS 7 broth and incubated at 37°C for 16h. The cells were harvested by centrifugation (10,000 rpm for 10 min at 4°C), washed three times in Ringer's solution (Oxoid, Milan, Italy), and resuspended in 1/10 of the initial volume in 100 mM sodium phosphate (pH 7.0) with 5 mM $CaCl_2$. Cell suspensions were kept at 37°C for 30 min for amino acid starvation. AK was diluted in 100 mM sodium phosphate (pH 7.0) with 5 mM $CaCl_2$ at a final concentration of 3 mg/ml. The NGC of LAB strains were incubated with AK in a cell/protein ratio of 2/1 (v/v) for 72h at 37°C. Samples were collected at 6, 20, 32, 48, and 72h (Pescuma et al., 2015).
All samples were centrifuged to separate the supernatant from the pellet and were analyzed by SDS-PAGE. AK immunoreactivity was investigated before and after incubation with LAB strains by IgE immunoblotting assays (Dot-blot assay).

2.3 *In situ* analysis

Hermetia illucens powder (BSF powder) was purchased from Azienda Agricola SAVASTANO (www.progettohermetia.it, Gioia Tauro, RC, Italy).
Two bacterial strains, *Lacticaseibacillus rhamnosus* UPCCO 1473 and *Lacticaseibacillus paracasei* UPCCO 2333, previously selected based on *in vitro* analysis, were used for the fermentation of BSF powder. Before fermentation, the strains were cultivated in MRS broth (3% v/v inoculum) at 37°C for approximately 16 hours under aerobic conditions to obtain a cell concentration of 9 Log CFU/ml. Each bacterial culture grown was then centrifuged (10000 rpm, 10 min, 4°C) and washed three times with Ringer solution. Five g of BSFP were hydrated with 10g of sterile water and separately inoculated (2% v/v) with each bacterial suspension. In parallel, additional samples were prepared by supplementing them with 10g/L of glucose to promote bacterial growth. All samples were incubated under two temperature conditions (37°C and 25°C) in agitation (150 rpm) and collected at 24, 48, and 72 hours. Microbial counts were performed on PCA and MRS agar for all samples.
After fermentation, protein extraction was carried out using a sequential protein extraction method previously developed.
For each sample, 3 g of fermented BSF powder was suspended in 5 ml of Tris Buffer (20 mM Tris, 1 mM Proteases inhibitor cocktail, pH 7,6) and maintained under agitation at 150 rpm for 45 minutes. The mixture was then centrifuged at 15000x g for 30 minutes at 4°C. The resulting supernatant was collected and stored. The remaining pellet was washed with 1 ml of Tris buffer and centrifuged again. Supernatants from both centrifugation steps were pooled to obtain the first extract (Tris extract). The pellet was resuspended in 6 ml of Urea buffer (6 M Urea, 20 mM Tris, 1 mM Proteases inhibitor cocktail, pH 7,6) and incubated overnight at 4°C under continuous agitation. After incubation, the samples were centrifuged and washed with urea buffer as previously described to obtain the second extract (Urea extract). The remaining pellets were resuspended in 4 ml of SDS/DTT buffer (20 mM Tris, 2% SDS, 1% DTT, pH 7,6) and maintained under agitation at 150 rpm for 45 minutes. Then, they were centrifuged at 15000x g for 30 minutes, and the supernatants containing SDS/DTT extract were collected and stored at -20°C (Broekman et al., 2015).
All protein extracts obtained were analyzed by SDS-PAGE. Additionally, the SDS/DTT extracts were analyzed by IgG immunoblotting assay using antibodies against AK and TPM.

3. Results and Discussion

3.1 In silico analysis

Based on the sequence of the predicted epitopes of insect proteins, an *in silico* analysis showed that the cleavage specificity of the CEP PrtP and PrtR could lead to the inactivation of these allergens. The results show that these proteases are diffused in the LAB analyzed and different strains probably possess functional PrtP and PrtR.

3.2 In vitro analysis

3.2.1 Condition 1: Incubation of AK with growing cells (GC)

To verify the proteolytic activity of LAB on AK, SDS-PAGE was carried out first. The stability of AK after incubation for 16h at 37°C in MRS 7 was investigated as a control and no significant modifications of AK bands were observed.
To investigate the effect of LAB fermentation on AK integrity, samples were collected every 2 hours, soluble and insoluble fractions were separated by centrifugation and analyzed by SDS-PAGE.
The electrophoretic profile of the supernatant samples showed a decrease in the intensity of AK band after 6h of incubation and a complete disappearance after 12h. Conversely, insoluble fractions showed an increase in AK band starting from 6 hours of incubation highlighting that LAB fermentation probably induces AK aggregation and insolubilization.
Observing microbial growth, it was interesting to note how the decrease of AK solubility starts at the beginning of the LAB exponential phase and is completed at the end of this phase.

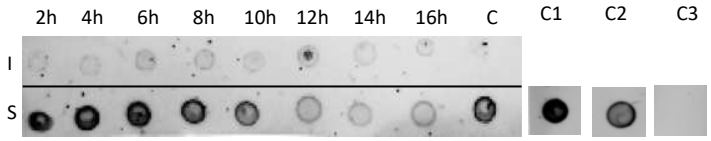


*Figure 1:*IgE-reactivity of AK with GC *L. paracasei* UPCCO 2333. Dot-blot IgE immunoassay of soluble (S) and insoluble (I) fractions of AK collected during incubation carry out by using sera of patient allergic to shrimp and mites.

Dot-Blot assays were carried out to experimentally verify the immunoreactivity of AK after incubation with LAB strains. Sera from patients with evidence of food allergy to crustaceans and/or airway allergy to mites and previously tested for their ability to recognize AK from BSF (Delfino, Prandi, Calcinai, et al., 2024), were used to investigate IgE immunoreactivity of samples. The results showed a decrease in immunoreactivity of AK during the incubation period with LAB for the supernatant samples and no significant reactivity was observed in the pellet samples (Figure 1). So, the results obtained for this condition show how LAB activity induces AK precipitation obtaining aggregates with reduced IgE cross-reactivity.

3.2.2 Condition 2: Incubation of AK with non-growing cells (NGC)

In this case, pellet samples didn't show any bands at approximately 40 kDa, indicating no precipitation effect induced by LAB. However, a decrease of AK band was observed in supernatant samples during the incubation period (6-20-32-48-72 hours), while AK incubated in the absence of LAB cells as a control did not show significant modification on SDS-PAGE. Thus, in this condition, LAB strains did not induce the precipitation of AK but probably lead to its degradation.

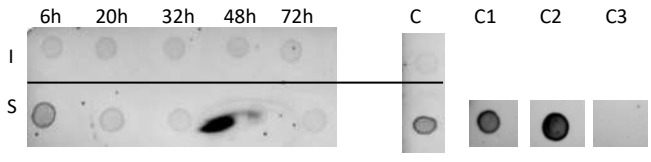


Figure 2: IgE-reactivity of AK with NGC *L. paracasei* UPCCO 2333. Dot-blot IgE immunoassay of soluble (S) and insoluble (I) fractions of AK collected during incubation carry out by using sera of patient allergic to shrimp and mites.

Interestingly, the results of the Dot-Blot experiments show a significant decrease of IgE reactivity for the AK incubated with LAB strains if compared with the control sample (Figure 2).

3.3 In situ analysis

3.3.1 *Hermetia illucens* powder fermentation

The fermentation of BSF powder was carried out by inoculating separately two LAB strains, *L. rhamnosus* UPCCO 1473 and *L. paracasei* UPCCO 2333, at a concentration of 7 Log CFU/g at different temperatures (25-37°C) and in the presence and absence of glucose. After 72h of fermentation at the optimal temperature of 37°C, strains were able to increase in BSF powder with an average of 2 Log CFU/g, while at 25°C, under stress conditions, strains grew more slowly, increasing by 1.6 Log CFU/g.
Significant differences were observed by monitoring pH. The pH of BSF powder has remained the same after 72h of incubation without strain at 37°C and 25°C, with a value equal to 6. While in the fermented BSF powder at 37°C it was observed a pH change. In fact, after 24 hours, the pH decreased in the glucose-supplemented samples, reaching a value of 5.3, while in the other samples the pH was around 5.7 after 72 hours. In the samples incubated under stress conditions (25°C), the pH remained unchanged in those without added glucose, while in the samples fermented with *L. rhamnosus* UPCCO 1473 and *L. paracasei* UPCCO 2333 decreased to 5.5 and 5.3, respectively.

3.3.2 Electrophoretic profiles of protein extracts (SDS-PAGE analysis)

To investigate the potential structural modification of allergens in BSF powder and their associated IgG-immunoreactivity after fermentation with *L. rhamnosus* UPCCO 1473 and *L. paracasei* UPCCO 2333, proteins were extracted from all samples collected at different time points (24, 48, and 72h) and were analyzed by SDS-PAGE. As shown in Broekman et al., (2015), the protein profiles significantly changed between the different extract phases (Tris, Urea, and SDS/DTT extract), and these changes in protein profiles were the result of changes in solubility. In case of the SDS/DTT extracts, protein bands with a MW ± 40 kDa were more pronounced than in the other extract phases, but no significant differences were observed among the fermented samples. All SDS/DTT extracts were analyzed by immunoblotting assays to evaluate the IgG-reactivity of the protein extracted.

3.3.3 IgG immunoreactivity of AK and TPM in BSF powder after LAB fermentation

Western blot assays were carried out to experimentally verify the IgG-immunoreactivity of AK after fermentation with LAB strains by using antibodies that detect Arginine Kinase. As controls, the stability of AK in the absence of strains was also verified, and results showed a similar signal at different time points (24, 48, and 72h) with the BSF powder at T0 for both temperatures.
The results obtained by western blot assays for the samples fermented at 37°C show differences during the fermentation. First, a similar pattern was observed for the samples fermented with both strains without added glucose. In contrast, samples fermented in the presence of glucose showed a decrease in signal intensity, corresponding with the reduction in quantity of AK in the fermented BSF powder. After 48 hours of incubation, the intensity of the signal significantly decreased in all samples, especially those with added glucose. At the final time point (T72), all fermented samples show a marked decrease in signal intensity (Figure 3a), indicating that fermentation led to the degradation of the AK protein by LAB. It's important to highlight that in the sample fermented with the strain *L. paracasei* UPCCO 2333 with glucose (FDT72), no bands were detected, suggesting complete degradation of the AK allergen.

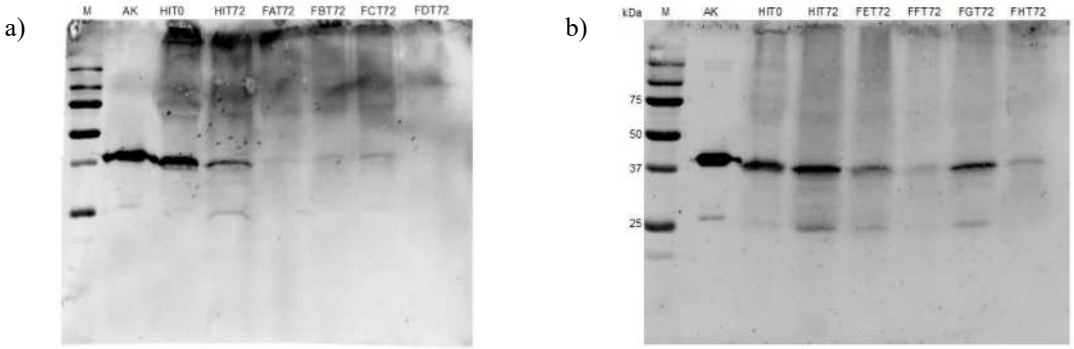


Figure 3: IgG-reactivity of AK in fermented BSF powder. Western blot IgG immunoassay of SDS/DTT extracts of fermented BSF powder by *L. rhamnosus* UPCCO 1473 and *L. paracasei* UPCCO 2333 after 72h of incubation at a) optimal temperature (37°C) and in b) stress temperature (25°C) conditions.

A similar trend was observed in the samples fermented at 25°C. In general, it was possible to note that under these temperature conditions, the strains need more time to degrade the allergen. The IgG-reactivity of AK showed a significant decrease only after 48h of incubation with both strains with added glucose. After 72 hours, these samples showed a marked degradation of AK protein (Figure 3b); however, complete disappearance of the signal was not observed, indicating that the protein was not fully degraded.
In conclusion, the highest level of AK allergen degradation was observed in the samples fermented with *L. paracasei* UPCCO 2333 for 72 hours in the presence of glucose. In particular, the sample fermented at 37°C

showed complete degradation of the AK allergen, suggesting a significant reduction in allergenicity under these conditions.

The effect of LAB strains on the allergenicity in the BSF powder was also investigated on the allergen TPM. Western blot assays were carried out by using an anti-tropomyosin polyclonal antibody produced in rabbit on samples fermented with *L. paracasei* UPCCO 2333 with/without added glucose at 25 and 37°C at the final time point (T72). The results obtained showed different patterns of bands between the samples, probably caused by the low stability of TPM at different temperatures and pH ((Delfino, Prandi, Ridolo, et al., 2024). Also in this case, when the BSF powder was fermented with this strain in presence of glucose at 37°C, the results showed a complete degradation of the allergen after 72 hours of incubation.

6. Conclusions and Future Perspectives

Overall, the results obtained by the investigation through different approaches, *in silico*, *in vitro*, and *in situ* analysis, highlight the potential of LAB fermentation processes to reduce the allergenic risk associated with the consumption of edible insects. In particular, the results indicate that LAB could represent a valid tool against allergens, such as arginine kinase and tropomyosin. In future, it would be of interest to develop a food product based on fermented BSF powder, assess its technological properties, and study the mitigation of IgE-reactivity of allergens.

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Genomic and functional characterization of a novel BSH-positive *Lacticaseibacillus rhamnosus* probiotic candidate with potential hypocholesterolemic effect

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The aim of this PhD thesis is to select a novel probiotic candidate exerting bile salt deconjugation activity, explicated by the microbial enzyme Bile Salt Hydrolase (BSH), strictly correlated to the regulation of blood cholesterol levels. According to that, two *Lacticaseibacillus rhamnosus* strains, were identified as the most promising BSH candidates following an *in vitro* pre-screening. Subsequently, genomic and *in silico* analysis confirmed the presence of *bsh* gene along with other key probiotic traits, further supported by biochemical assays and gene expression study. The top-performing bile acid deconjugating strain was then evaluated for its ability to survive under gastrointestinal conditions using a dynamic *in vitro* gut model. Ongoing studies are focused on relationship between BSH activity and its potential cholesterol-lowering effect.

Caratterizzazione genomica e funzionale di un nuovo ceppo probiotico di *Lacticaseibacillus rhamnosus* BSH-positivo, con potenziale effetto ipocolesterolemizzante

Lo scopo di questa tesi di dottorato è quello di selezionare un nuovo candidato probiotico in grado di esercitare l'idrolisi dei sali biliari, mediante l'enzima batterico Bile Salt Hydrolase (BSH), strettamente correlato alla regolazione dei livelli di colesterolo ematico. In merito a questo, due ceppi di *Lacticaseibacillus rhamnosus* sono stati considerati tra i più promettenti candidati BSH in seguito ad un preliminare screening *in vitro*. Successivamente, analisi genomiche e *in silico* hanno confermato la presenza di geni *bsh* e altre caratteristiche probiotiche importanti, supportati da saggi biochimici e studi di espressione genica. Il ceppo più efficace nella deconiugazione dei sali biliari è stato successivamente valutato per la sua capacità di sopravvivere al transito gastrointestinale mediante un modello dinamico *in vitro*. Studi in corso sono mirati a confermare la correlazione tra attività BSH ed effetto ipocolesterolemizzante.

Keywords: bile salt hydrolase; bile acids; probiotic; lactobacilli; probiogenomic; SHIME model.

1. Introduction

In the context of the PhD project, this oral communication presents the main findings from the BSH characterization of *Lacticaseibacillus rhamnosus* VB4 and VB1 strains, previously isolated from vaginal swabs of healthy donors. The activities performed are summarized as follows:

A1) Whole-genome characterization of *L. rhamnosus* strains to support their probiotic potential and QPS status;
A2) *In silico* identification of the *bsh* genes, primer design and validation;
A3) Assessment of strains survivability, deconjugation ability and *bsh* gene expression under bile acid (BA) exposure;
A4) Monitoring of *L. rhamnosus* VB4 viability using a dynamic gut model;
A5) Evaluation of BA profile modification across the different simulated gut compartments after VB4 administration.

2. Bile Salt Hydrolase (BSH)

The Bile Salt Hydrolase (BSH) is a microbial enzyme that catalyzes the initial deconjugation of bile acids (BAs), a key step required for their subsequent conversion into secondary BAs. The BSH (EC 3.5.1.24) was classified as choloylglycine hydrolase and belongs to the N-terminal (Ntn) hydrolase superfamily, characterized by a N-terminal group which cleave the amide bond of host-derived conjugated BAs (Bourgin et al., 2021; Agolino et al., 2024). Once secreted into the small intestine, gut microbes expressed BSH and initiate the deconjugation of primary BAs, and this reaction release free glycine and taurine amino acid, yielding unconjugated BAs (Joyce et al., 2014). BSH is widespread distributed within the gut microbiota and is more commonly found in Gram-positive bacteria compared with Gram-negative species (Begley et al., 2006). Notably, BSH-positive bacteria differ in their substrate specificity, showing preferential activity toward either glycine-or taurine-conjugated BAs (Foley et al., 2021). These functional differences contribute to distinct impacts on host physiology, including lipid metabolism, gut barrier integrity, immune modulation and bacterial survivability (Chand et al., 2017). The BSH activity plays a pivotal role on maintaining the physiological blood cholesterol levels, although the mechanism of action is not completely clear (Dong et al., 2025). An imbalance of gut bacteria which expresses the BSH enzyme could be

crucial in host health, reducing BAs deconjugation, can lead to decreased fecal BAs loss and reduced the hepatic turnover. Dysregulation of BAs pool increased BAs toxicity into the gut, even favoring serum cholesterol accumulation following the onset of the hypercholesterolemia (Vourakis et al., 2021). Considering the key role of gut bacteria in BAs metabolism, it is considered important to comprehensively identify the presence of *bsh* genes and to characterize their distribution and functional activity within the intestinal ecosystem (Song et al., 2019).

3. BSH as probiotic properties in lactobacilli

BSH activity is well documented among established probiotic genera, with the highest prevalence observed in *Lactobacillus* genera. Lactobacilli are classified as QPS microorganisms and are known to confer various beneficial effects to the human host. Notably, BSH activity is considered a key probiotic trait, contributing to cholesterol-lowering effects and enhancing gut bacterial survivability (Patel et al., 2010), which is supported by different *in vitro* and *in vivo* evidence (Agolino et al., 2024). Although BSH has been extensively studied in lactobacilli, recent finding shown that *bsh* genes are unevenly distributed inside the genus, revealing distinct taxonomic phylotypes. The presence and the genomic distribution of *bsh* genes in probiotic *Lactobacillus* strains are highly variable. Some species harbor a single *bsh* gene, while others existing in multiple copies, revealing different physiological function of the BSH variants (Pavlović et al., 2012). These phylogenetic findings also reflect the evolutionary pressures that shaped their adaptation to other ecological environments beyond the gastrointestinal tract (O'Flaherty et al., 2018).

4. Experimental Procedure

This PhD project aimed to investigate and validate a novel BSH-positive probiotic candidate selected from non-gastrointestinal sources. The workflow began with a preliminary screening of several isolates from human and food matrices, which led to the identification of strains VB4 and VB1 as the most active in BSH, followed by a seven-steps experimental strategy. As follow i) a comprehensive probiogenomic analysis was performed to assess the safety of the candidate strains through genome-based identification of virulence factors, antibiotic resistance genes, mobile genetic elements, and other probiotic-relevant traits; ii) phylogenetic and *in silico* analysis to confirm the presence of the *bsh* gene, iii) set up of growth curves under bile acid (BA) stimulation to study strains survivability; iv) biochemical assay to reveal BAs deconjugation, v) qPCR analysis of *bsh* gene in normal growth condition and under BAs exposure, vi) set up of the SHIME® dynamic gut model, and vii) evaluation of BA profile modification across the different simulated gut compartments after VB4 administration. This strategy allows for the integration of genomic, functional, and ecological data to assess the probiotic potential and bile acid-modulating capacity of a non-conventional, BSH-positive strain.

5. Materials and Methods

The selected *L. rhamnosus* VB4 and VB1 strains were studied first at genome level. Whole genome analysis of both strains was conducted, and the graphical maps of the genome sequence were customized by Proksee Server. Genome quality and annotation were assessed using BUSCO, BAKTA, and Proksee, while functional and comparative analyses employed BLASTKOALA and PanExplorer, respectively. Safety assessment included prophage and virulence were respectively identified using Phigaro and VirulenceFinder. Antimicrobial resistance (AMR) genes were searched through CARD and ResFinder tools. Putative plasmids were identified using the PlasmidFinder database according to probiotic screening criteria. Candidate *bsh* genes were identified via BLASTp tool, aligning amino acid sequence annotated as BSH or putative BSH proteins from lactobacilli with COBALT. Phylogenetic relationships of *bsh* genes were analyzed using Fast Minimum Evolution trees. Most representative *bsh*-like sequence was used as queries for primer study design.

The VB4 and VB1 strains were cultured in MRS (Oxoid) medium broth supplemented with 1.0% of bile salt mixture (Oxoid), and growth curves were modelled according to growth kinetics parameters using R-Grofit package (Kahm et al., 2010). Cells from late stationary phase growth in MRS and MRS supplemented with bile salt, were centrifuged and the supernatant was analyzed by UHPLC/HR-MS in order to quantify BSH potential, expressed as percentage of residual conjugated compounds, while RNA from bacterial cells was extracted and retro-transcript. cDNA samples were subjected to qPCR analysis to evaluate *bsh* gene expression under bile salt exposure.

An *in vitro* dynamic gut model was subsequently set up to study the survivability and the BAs-profile of the VB4 strains. The SHIME system simulates gastric, small intestine and colonic fractions under FED condition. To recreate a representative intestinal microbiota, the colonic compartment was inoculated with a fecal sample obtained from a healthy donor. After strain supplementation, samples were collected at the end of stomach, duodenum, jejunum, ileum phases, and colon after 16, 24 and 48 hours. Strain survivability was assessed by culture method, in stomach/small intestine (St/SI) reactors. In addition, UHPLC/HR-MS was performed to reveal the change in BAs profile after strain administration compared with uninoculated reactors.

5. Results and Discussion

5.1 Probiogenomic analysis

Strains VB4 and VB1 were subjected to whole-genome sequencing and assembly, resulting in 70 contigs (2,926,936 bp) for VB4 and 19 contigs (2,917,389 bp) for VB1, with GC contents of 46.63% and 46.65%, respectively (Table 1). KEGG annotation showed that VB4-specific genes were mostly involved in carbohydrate and amino acid metabolism and cell envelope biogenesis, while VB1-specific genes are mainly related to transcription and DNA repair. These findings align with clustering and ANI results, further supporting that VB4 and VB1 represent functionally divergent strains, adapted to different host environments. Genome safety assessment according to EFSA guidelines (EFSA, 2018) revealed no AMR genes in either strain. No virulence genes were detected showed very low pathogenicity scores (~0.097), in line with QPS status of *L. rhamnosus*. Additionally, both strains lacked plasmids, further supporting their genetic stability. In VB4 and VB1 were identified three and two prophages regions respectively, they may contribute to genome plasticity and functional diversification, offering survival advantages in changing environments. Overall, both VB4 and VB1 are genetically safe. VB4 appears to have more diverse accessory genomes, enriched in functions relevant to probiotic performance and environmental adaptation.

Table 1 *General features of Lacticaseibacillus rhamnosus VB4 and VB1 genome assemblies.*

Features	VB4	VB1
Size (bp)	2,9266,936	2,917,389
Contigs	70	19
GC (%)	46.63	46.65
N50	111,222	406,064
Largest contig (bp)	222,067	1,025,915
CDS	2,769	2704
Gene average length (bp)	903	921
tRNA	45	46
rRNA	4	2
tmRNA (ssrA)	1	1
ncRNA	4	7
oriC	1	2

5.2 *In silico* analysis of *bsh* gene candidates

The presence of *bsh* genes in VB4 and VB1 genomes was confirmed using BLASTp with reference BSH sequences. A single *bsh* gene (1,017 bp) was found on contigs 37 (VB4) and 2 (VB1), encoding 338 amino acids differing by two substitutions. Both proteins belong to the choloylglycine hydrolase family (Pfam PF02275) and contain conserved active site residues, essential for BSH enzymatic function (Figure 1). Phylogenetic analysis of 88 BSH proteins from 18 lactobacilli species, aligned with COBALT, revealed that VB4 and VB1 BSHs clustered with those *L. rhamnosus* and related *Lacticaseibacillus* species, as well as *L. acidophilus*, *L. plantarum*, *L. reuteri* and *L. paragasseri*. These proteins formed three distinct species of subclusters. Interestingly, they also showed relatedness to PVA (penicillin V acylase) proteins from *Lactococcus lactis* and *Lysinibacillus sphaericus*.

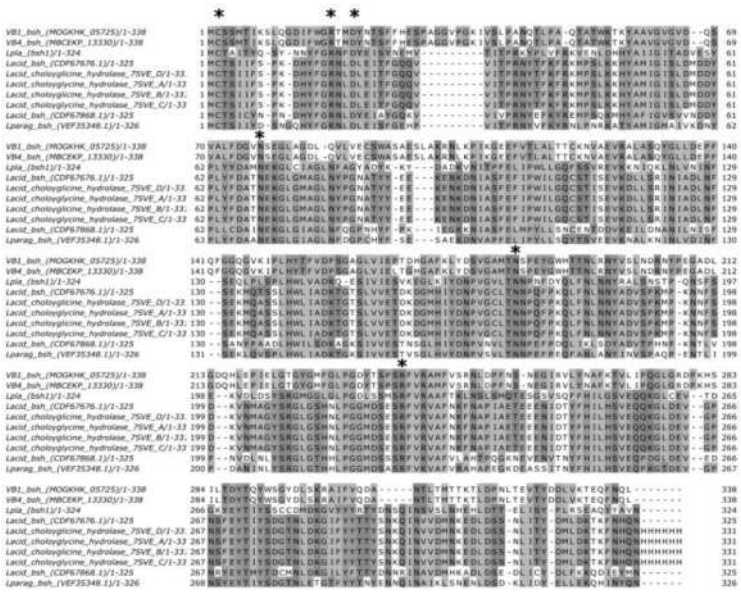


Figure 1 *Multiple amino acid sequence alignment of 10 BSHs proteins, using different reference strains putative BSH sequence. Asterisks indicate the active sites.*

5.3 Growth kinetic curves, deconjugation ability and *bsh* gene expression under BAs exposure

The growth curve analysis, performed at different times, showed that VB1 had a longer lag phase (h) in control condition (MRS), which significantly decreased in the presence of BAs mixture ($p < 0.05$). In contrast, BAs mixture did not affect the lag phase of VB4 ($p > 0.05$). As expected, both strains significantly reduced growth rate (h^{-1}) and maximum absorbance (A) values in presence of BAs mixture compared to control condition (Figure 2). UHPLC/HR-MS analysis revealed that both strains deconjugated BAs (Figure 3A), but with distinct substrate preferences. VB1 was more efficient at deconjugating glyco-conjugated BAs like GDCA and GCDCA, while VB4 exhibited broader activity, including significant deconjugation of tauro-conjugated BAs such as TCA, TDCA, and TCDCA. Despite their nearly identical BSH protein sequences (differing by only two amino acids), the strains displayed different enzymatic activities and regulations. VB4 showed higher overall BSH activity but did not regulate *bsh* gene expression in response to BAs, indicating constitutive expression. In contrast, VB1 significantly upregulated *bsh* gene transcription when exposed to BAs (Figure 3B). VB4 showed broader BSH activity, especially toward tauro-conjugated bile acids than VB1; this could contribute to better regulation of the BAs pool, and influence how gut microbes interact with the host. Despite similar *bsh* gene sequences, gene expression did not completely match enzyme activity, suggesting that other cellular mechanisms, such as membrane changes and detoxification systems, may play a role in this difference.

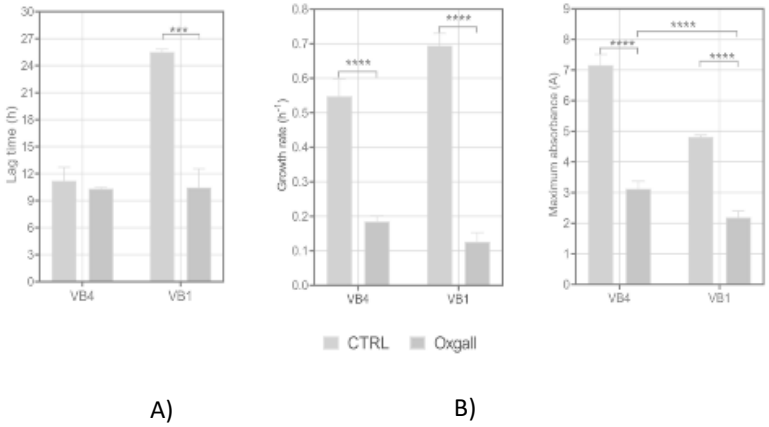


Figure 2 *Growth ability of VB4 and VB1 strains in presence of the BAs mixture (Oxgall). Kinetic parameters, lag phase (λ, expressed in h), max growth rate (μ, expressed as h^{-1}), and maximum cell density (A, expressed as OD_{600nm}). Significant differences were calculated with two-way ANOVA and indicated with asterisks, as follows: ***, $p \leq 0.001$; ****, $p \leq 0.0001$ (two-way ANOVA). Plotted with GraphPad Prism software.*

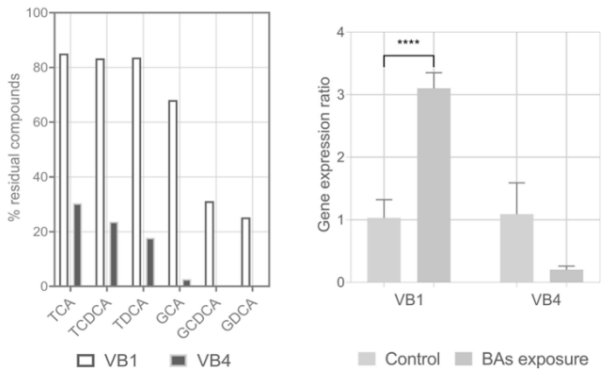


Figure 3 *Deconjugation ability of VB4 and VB1 strains in presence of BAs mixture (Oxgall). Residual unconjugated percentages (%) of GCA, GDCA, GCDCA, TCA, TDCA, and TCDCA were estimated in supernatants collected during late stationary phase (**A**). Significant differences were calculated with two-way ANOVA and indicated with asterisks, as follows: ***, $p \leq 0.001$; ****, $p \leq 0.0001$ (two-way ANOVA). Expression profile of bsh genes of VB4 and VB1 in MRS (control condition) and MRS supplemented with 1% (w/v) BAs mixture (**B**). Significant differences were calculated with a student's t-test and indicated with asterisks, as follow: ****, $p \leq 0.0001$. Plotted with GraphPad Prism software.*

5.4 Study of *L. rhamnosus* VB4 viability in SHIME model

The VB4 strain was administered in the SHIME model, simulating upper GIT conditions.to better understand its BSH ability. Starting from ~9 log CFU/mL, cell counts slightly dropped to 8.49 in the stomach, then decreased during small intestine transit (8.29, 7.68, and 8.14 log CFU/mL in duodenum, jejunum, and ileum, respectively) but remained relatively stable.

5.5 Semi-quantitative analysis of BAs pool in simulated small intestine and colon

The UHPLC/HR-MS revealed that VB4 supplementation affected the profiles of BAs during simulated digestion. In VB4-treated samples, tauro-conjugated BA decreased significantly from duodenum to jejunum, while glyco-conjugated BA remained stable. Compared with controls, VB4 significantly decreased several conjugated BAs in the Jejunum and ileum, with 20% lower levels in the ileum. Unconjugated BA remained stable in the VB4 samples, except for cholic acid, which decreased in the ileum. In the T_{16h} colon, conjugated BA highly decreased in VB4 samples (≥ 60%), while unconjugated cholic and deoxycholic acids increased. At T_{24h}-T_{48h}, conjugated BAs increased in VB4 samples, while cholic acid decreased only in VB4-treated samples.

6. Conclusions and Future Perspectives

Probiotic strains may represent a promising therapeutic approach by modulating the host's BAs composition. The present study provided a comprehensive genomic and functional characterization within the *Lacticaseibacillus rhamnosus* genus focusing on BSH activity from non-gastrointestinal tract isolated strains. Although the VB4 and VB1 strains exhibited high similarity in their BSH protein sequence, they diverged in enzymatic activity and gene expression profile, suggesting that additional cellular mechanisms could modulate BSH functionality. By integrating genomic, biochemical and physiological approaches, the VB4 strain demonstrated wide *in vitro* BSH ability, and strong tolerance to simulated gastric condition. The administration of the SHIME model further corroborates the probiotic potential of VB4, confirming the strong deconjugation in both glyco- and tauro-conjugated BAs during small intestine transit, releasing unconjugated BAs for downstream microbial transformation. Overall, the multi-level characterization supports *L. rhamnosus* VB4 as a promising probiotic candidate with relevant BSH activity for gut health. To further validate the probiotic capacity of VB4 strain, ongoing research is focused on evaluating the actual cholesterol-lowering effect related to deconjugation ability. Moreover, a deeper investigation into the molecular mechanisms regulating *bsh* gene and eactivityactivity, should be improved the knowledge about the strain-specific differences. Additionally, it will be essential to investigate how probiotic supplementation influences the structure of the gut microbiota and modulate host lipid metabolism. All these efforts could support the development of new target probiotics aimed at preventing or managing mild hypercholesterolemia and associated metabolic disease.

7. Nomenclature

AMR , Antimicrobial Resistance; ANI**,** Average Nucleotide Identity; BA, bile acid; BSH, bile salt hydrolase; *bsh***,** gene encoding bile salt hydrolase; ; CDS, Coding Sequences; CFU, Colony Forming Units; EC, Enzyme Commission number; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA**,** glycodeoxycholic acid; KEGG, Kyoto Encyclopedia of Genes and Genomes; MRS, de Man, Rogosa and Sharpe medium; NCBI, National Center for Biotechnology Information; OD, optical density; Pfam, Protein Families Database; PVA, Penicillin V Acylase; QPS, Qualified Presumption of Safety; qPCR , Quantitative Polymerase Chain Reaction; rRNA, ribosomal RNA; SHIME, Simulator of the Human Intestinal Microbial Ecosystem; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; tmRNA, transfer-messenger RNA; tRNA, transfer RNA; UHPLC/HR-MS, Ultra-High Performance Liquid Chromatography / High-Resolution-Mass Spectrometry.

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Selection of lactic acid bacteria to improve the technological, nutritional and functional value of products and by-products in the agri-food chain

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In the present PhD thesis, different species of wild LAB strains isolated from dairy matrices were used as starter for plant-based fermentation. The main aim was to assess if their use LAB in different plant-based sources may affect their microbiological safety, techno-functional properties, as well as nutritional and chemical profile.

Selezione dei batteri lattici per il miglioramento delle caratteristiche tecnologiche, nutrizionali e funzionali di prodotti e sottoprodotti nella filiera agroalimentare.

Nella presente tesi di dottorato, diverse specie di ceppi di batteri lattici (LAB) isolati da matrici lattiero-casearie sono state utilizzate come starter per la fermentazione a base vegetale. L'obiettivo principale è stato valutare se l'utilizzo dei LAB in diverse fonti vegetali possa influenzare la sicurezza microbiologica, le proprietà tecno-funzionali, nonché il profilo nutrizionale e chimico.

Key words: LAB; lactic acid bacteria; plant-based; fermentation; biopreservation

1. Intoduction

The global population is projected to reach over 9.7 billion people by 2050 (United Nations, 2022), and the current food chain is not sustainable in the long term to guarantee healthy, nutritious, and sustainable food for everyone. In this context, meat production is often unsustainable due to its high demands on land, water, and its contribution to greenhouse gas emissions, prompting a global shift toward more sustainable protein sources (Chiodetti et al., 2025). Among these alternatives, plant-based proteins, or rich plant-based sources, like legumes, could represent a valid option. However, high proteins plant-based products often differ in quality compared to the non-plant version. The lack of certain essential amino acids (Mariotti & Gardner, 2019), generally lower digestibility, bioavailability, and strong off-flavors make these proteins challenging when aiming to achieve a balanced diet. The strong off flavour makes difficult to use these kinds of matrices as ingredient to fortify and increase the protein content in food preparation. In this optic, fermentation is a traditional process known to enhance the technological, sensory, nutritional and safety qualities of food products. Indeed, through fermentation, food matrix constituents are modified, new compounds are synthesized, and modification of food texture and flavor occurs (Chiodetti et al., 2025). Among the different fermentation strategy, the use of Lactic acid bacteria (LAB) could offer a valid and sustainable tool to enhance the chemical, physical, nutritional, and organoleptic characteristics of fermented proteins (Du et al., 2024; Yang et al., 2024). In this optic, the aim of this PhD thesis was to screen different lactic acid bacteria strains isolated from dairy matrices and to test their known phenotypic characteristics in different plant-based matrices. The goal was to assess whether the strains were able to adapt to this new environment and positively influence the organoleptic, chemical, nutritional, and techno-functional properties of the fermented product.

2. Materials and methods

2.1. Lactic acid bacteria

26 different lactic acid bacteria strains (metti la collezione UPPCO) isolated from dairy environments, was used for all the analysis. 6 different species of LAB were considered: 6 *Lactobacillus delbrueckii,* 6 *Lactiplantibacillus plantarum,* 5 *Lactococcus lactis,* 1 *Lacticaseibacillus casei,* 4 *Leuconostoc spp.,* 4 *Streptococcus thermophilus*

2.2. Fermentations monitoring

Different techniques were used to monitor fermentation. They differentiate in end point measure and in continuous. The pH was always measured before and after the fermentations to ensure the correct developments of the LAB

and to ensure the reaching of the safety limits. It helps to evaluate the biopreservation effect that the LAB have on the matrices. To ensure the effectiveness of the different fermentation the plate count was performed. These techniques were used on the

N°	Title	Aim	Matrices	Strains	Main findings
I	Lactic acid fermentation of non-conventional plant-based protein extract (PBPE)	Evaluate the feasibility of applying biopreservation to different types of unconventional dehydrated plant-based proteins by leveraging the metabolic potential of lactic acid bacteria	plant-based protein extracts: pea, faba, soy, potato, pumpkin, hazelnuts, rice, and hemp	12 different lactic acid bacteria strains from four species: *Lactobacillus delbrueckii, Lactiplantibacillus plantarum, Leuconostoc spp., Streptococcus thermophilus*	Strongly adaptability and biopreservation effects were observed in LAB strains, especially *L. delbrueckii subsp. bulgaricus* and *S. thermophilus*, and *L.plantarum* across the tested matrices. Substantial transformation and changes in the aroma profile, with reduction in both hexanal and hexanoic acid levels in fermented PBPE samples, enhancing the final product's organoleptic properties
II	Tailoring the physio-chemical properties and VOCs of pea-based fermented beverages through *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* fermentation	Investigate the fermentative potentialities in a pea emulsion beverage of twenty-six LAB strains isolated from dairy sources.	Pea Protein Emulsion: pea protein isolate 3.5%, sucrose 5%, 1.5% sunflower oil, water	26 different lactic acid bacteria strains from 4 species: *Lactobacillus delbrueckii, Lactiplantibacillus plantarum, Lactococcus lactis, Streptococcus thermophilus*	*St* 5149, *Ldb* 2214 and the blend of the two strains were able to grow in the pea emulsion beverage, improving the physical characteristics and volatile organic compounds profile of the beverage. In particular, the LAB blend demonstrated the best fermentative performances
III	Lactic acid fermentation of Chickpeas (*Cicer arietinum L.*) flour, a multidisciplinary approach to evaluate this as an ingredient in gluten-free bread formulation	A multidisciplinary approach to assess the effect of fermentation by wild Lactic Acid Bacteria on the technological, chemical, nutritional, and organoleptic characteristics of chickpea flour to be used as an ingredient in gluten-free bread formulation.	chickpea flour	*Lactiplantibacillus plantarum* 4199, *Lactobacillus delbrueckii subsp. Bulgaricus* 1932, *Leuconostoc spp*. 4454, *Lacticaseibacillus casei* 4339	Variable biopreservation effect depending on the strain. *Lbd* 1932 and *L* 4454 were the two most effective in limiting the growth of Enterobacteriaceae, while for coliforms, all strains led to a reduction except for strain *Lp* 4199 Concentration of hexanal and hexanol, which are associated with green and legums aromatic notes, tends to decrease or remain similar when fermentation is carried out using strains *Lc* 4339, *Lp* 4199 and *Ldb* 1932
IV	Lactic acid fermentation of Soy (*Glycine max*): development and charaterization of a new dry ingredient.	Develop a new fermented plant-based ingredient investigating the potential of LAB to increment the aromatic and nutritional properties of Soybean	Soybean	22 different lactic acid bacteria strains from 6 species: *Lactobacillus delbrueckii, Lactiplantibacillus plantarum, Lactococcus lactis, Lacticaseibacillus casei, Leuconostoc spp., Streptococcus thermophilus*	The safety of the strains was evaluated with the MIC method (ISO 20776-1:2019) and 3 strains were selected from 3 different species (*Lbd* 2214, *St* 5149, *Lc* 4339). Only *Lbd* 2214 was able to grow and outcompete the contaminants of soy

raw matrices before fermentation to evaluate the contaminants using generic, selective and elective media, and after the fermentation to evaluate the growth of the inoculum and its bio preservation activity against the contaminants. Fermentation, driven by LAB, was monitored in real time using the BacTrac 4300 Microbiological Analyser System® (Sylab, Austria). This system enables real-time detection of bacterial activity by measuring changes in impedance within an alternating current (AC) field. As viable bacterial cells replicate, they metabolize

sugars in the medium into smaller molecules, leading to increased conductivity and a corresponding decrease in resistance and impedance. These changes are recorded by the instrument and expressed as an M% value over time (Monica et al., 2025).

2.3. Chemical and aromatic analysis

The volatile molecule profiles of the samples were detected with GC/MS/SPME technique, as described by Gottardi et al. (2021). Two methods were used to evaluate protein quality. The first method was the Kjeldahl (VELP SCIENTIFICA, Usmate Velate, Italy), following the AOAC International Official Methods of Analysis 2002 (AOAC, 2002), to determine protein content (%), combined with sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) to assess the protein profile of the different batters. The second method was the Lowry protocol (Lowry et al., 1951) to quantify protein content, combined with peptide purification using Vivaspin20® (Sartorius, Hannover, Germany) ultrafiltration (10KDa >> 3KDa) to separate and quantify peptides during fermentation.

2.4. Rheological analysis

The rheological properties of the pea protein emulsions during fermentation were analysed using a controlled stress rheometer (MCR 102, Anton Paar GmbH, Graz, Austria) equipped with a concentric cylinder measuring system (C-CC27-T200/SS, Anton Paar). Fermentation was performed directly in the rheometer cell by performing a time sweep test to measure the structural development of the emulsions during the process.

3. Results

3.1. Fermentation

The first challenge that the selected LAB had to overcome was the difficulty to grow in a plant-based substrate, totally different from the dairy one, in these different studies was also evaluated the ability of LAB to adapt and develop in such challenging matrices. Common findings were that LAB can generally adapt and grow in plant-based matrices, where *Lactobacillus delbrueckii* specie resulting the best-adapting species. In the fermentation of plant-based protein extracts, “I” experiment, it was observed that all inoculated strains remain viable, showing no variation compared to the initial inoculation concentration of 7 Log CFU/g, while others display variable growth capacity across the different matrices, with increases in final concentrations up to 2.9 Log CFU/g. In pumpkin seeds, hemp, and faba proteins (PBPE), an average increase of 1.5 Log CFU/g was observed. Meanwhile in rice and potato (PBPE), where only some of the strains were able to grow. Interestingly, no single strain or species emerged as the best performer across all substrates in the protein extract; rather, a different strain stood out under each specific condition. Among all the protein extracts, best performance overall was showed by *L. delbrueckii subsp. bulgaricus* (strains 1932 and 2214), one *S. thermophilus* (strain 410), and one *L. plantarum* (strain 6215). In the“II” work, in the production of a fermented pea beverage, most of the LAB had good ability to decrease the pH with values reaching a pH below 4.5 within 12 h. In particular, among the 26 strains tested, two strains of *Lactobacillus delbrueckii subsp. bulgaricus* (strains 1932 and 2214) and two strains of *Streptococcus thermophilus* (strains 410 and 5149) exhibited the highest acidifying capacity, with pH values ranging between 3.99 and 4.25. In the “III”, were 4 LAB strains were used to ferment chickpea powder, the results showed that all the strains were able to grow, and the *Ld.b* 1932 showed the best and fastest acidifying ability. In the last work, “IV”, soybean was fermented, and among the three strains selected, *Ld.b* 2214, *S.t* 5149, *L.c* 4339, just *Ld.b* 2214 were able to grow well and outcompete the original contaminant microflora of the soy. Summarizing the results of all the four projects considered in this abstract, cne thing that clearly is visible among this different kind of fermentation was the adaptability of the strain *Ld.b* 2214, that was one of the best strains to ferment all the different matrices.

3.2. Biopreservation

The biopreservation effect on the different protein matrices results to be particularly strain and matrix dependent, this feature was clearly observed in “I” project, were a best candidate was not found for all the protein powders. What we found was that two strains of *S. thermophilus* (strains 4760 and 5149), which showed a good growth in hemp plant-based protein extract (PBPE), did not show any biopreservation effects. In contrast, the three strains

of *L. delbrueckii subsp. bulgaricus*, which duplicated well in hemp PBPE, exhibited also an effective biopreservation, although this was strain-dependent. Interestingly, *L. plantarum* strain 5776, which showed good growth in faba PBPE, was the only strain capable of preventing the proliferation of spore-forming bacteria showing the best biopreservation effect. A similar biopreservative effect was found for the strain *S. thermophilus 5149* in Pumkin PBPE where any growth of spore forming was detected after fermentation. In the pea yogurt-like production ("II"), the results showed that after fermentation, in all the media used, the contaminating bacteria concentration was below the detection limit of 10 CFU/mL, indicating that the fermentation process, driven by *Ldb* 2214, *S.t* 5149 and the blend (*Ldb* 2214 plus *S.t* 5149), led to a decrease in the initial contamination of the pea emulsion. This can be due to the biopreservative effect of LAB, which, through the decrease of pH, or the production of toxins or bacteriocins that may inhibit the growth of competing bacteria (Barcenilla, Ducic, L´ opez, Prieto, & ´ Alvarez-Ord´ o˜ nez, 2022). The results showed that the LAB blend kept the spore-forming bacteria concentration at 3.17 ± 0.28 Log CFU/mL which is comparable with the concentration found in the pea emulsion before fermentation (3.11 ± 0.21 Log CFU/mL.). The variability in the biopreservation effect could be observed also in the fermented chickpea flour, "III" paper, where the effect on the contaminants depends on the strain: *Ldb* 1932 and *L* 4454 were the two most effective in limiting the growth of Enterobacteriaceae, as they maintain the concentration 6 Log lower compared to the Control. An effect can also be observed for *L.p* 4199 and *L.c* 4339, although it is substantially lower. In the soy fermentation performed in the "IV" experiment, only *Ld.b* 2214 was able to outcompete the original contaminants already present in the soy, inhibiting the growth of that population. This is a confirmation of the great adaptability and biopreservation effect that such strain could achieve in plant-based products.

3.3. Rheological properties

Similarly to impedometric measurements or to other parameters that are related to the microbial growth (i.e. pH, titrable acidity, optical density, etc.), rheological measurements can be used to estimate the effects of fermentation on the physicalproperties of liquid or semi-liquid matrices (Sfakianakis, Topakas, & Tzia, 2015). In the Fermented pea beverage (II) the storage modulus (G') exhibited a plateau with values <1 Pa at the beginning of the fermentation. It can be also observed that in this plateau, G' showed lower values compared to the loss modulus (G"), indicating a predominantly liquid-like behaviour. During the fermentation, G' progressively increased up to a maximum value ranging between 700 and 900 Pa, followed by an upper plateau for samples St 5149 and the blend. The results suggest that the network formation and structural properties of the acid induced protein gels are primarily mainly influenced by the microbial growth and the rate of acidification of the medium. Also, since the LAB species used in the present experiments were also reported to produce exopolysaccharides (EPS) during fermentation in different matrices, (Bancalari et al., 2022), it can be hypothesized that the production of these metabolites could contribute to the rheological development of the pea-emulsion gels during fermentation. Regarding the chickpea flour fermentation ("III"), a similar result was observed, during fermentation G' increased progressively, followed by an upper plateau at the end of the process, with *Ld.b* 1932 reported the lowest values of G' at the end of fermentation, while *L.p* 4199 the highest.

3.4. Aroma

Among all the experiments a positive effect on the aromatic profile of the fermented matrices was observed. Focusing on the plant-based protein extracts "I", the fermentation process led to a positive differentiation of the final product compared to the initial one, with an increase in molecules considered to have a positive organoleptic impact and a decrease in hexanal which is associated with green, and legumes note. The same modifications were found in fermented pea proteins (II), were not only was a decrease in the unwanted aromatic compounds, but also the formation of new compounds (i.e. acetoin) typical of dairy products was observed. Moving to a less processed matrix, the chickpea flour of the "III" project, there is not a decrease on the typical legume's aroma, but, on the other hand, the production, in particular for *L* 4454, of hexanol, hexanal and acetic acid was observed. This helped to mask the unpleasant original beany aroma.

3.5. Protein quality

In the chickpea flour fermentation, "III", was measured the activity that LAB has on the protein content and quality of the final products. After 20hrs fermentation, was not find any difference between the final product and the control. This could be due to the short fermentation time that does not permit the LAB to activate secondary metabolism. On the other hand, regarding the soy fermentation in the "IV" project, preliminary results showed an increase in the concentration of peptides with dimension between 10 and 3KDa.

4. Conclusion

The aim of this PhD thesis was to evaluate how LAB isolated from dairy sources perform when they are put outside of their typical environment. The results show strong adaptability and biopreservation effects, especially within *L. delbrueckii subsp. bulgaricus* specie. In particular, the strain *Ldb* 2214, stood out for its ability to grow, acidify, and outcompete contaminant microflora across various plant-based substrates (pumpkin, hemp, faba bean, soy proteins, and pea beverages). Its consistent performance highlights its strong potential for industrial applications in plant-based fermented products. This study highlights the strain- and matrix-dependent nature of biopreservation effects in plant-based fermentation. Fermentation was shown to enhance the rheological properties of plant-based substrates by promoting the formation of protein gels, a process closely associated with microbial growth dynamics, acidification rates, and the synthesis of exopolysaccharides. Furthermore, fermentation positively modulated the aromatic profiles of the products, decreasing undesirable off-flavors and generating new dairy-like aroma compounds that effectively masked typical legume-related odors. The observed reduction in undesirable volatile compounds underscores the importance and potentiality of LAB fermentation to improve both organoleptic properties of plant-based products. Overall, these results demonstrate the promising potential of selected LAB strains, particularly *Lbd* 2214, for developing high-quality, safe, and organoleptically improved fermented plant-based products.

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Exploitation of the functional potential of autochthonous microorganisms from fermented foods

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This PhD project aims to isolate new strains with physiological traits for preventing non-communicable diseases (NCDs) to introduce them in functional foods. Autochthonous lactic acid bacteria (LAB) from dairy products and fermented vegetables were screened for their ability to produce bioactive peptides (BPs) using unconventional protein sources. Fermentates were assessed for functional activities, as well as for their effect on the gut microbiota after *in vitro* digestion. One promising strain underwent proteomic analysis to explore its adaptation mechanisms to different protein sources and proteolytic system. This study provided a comprehensive view of LAB behavior from fermentation to gastrointestinal digestion, up to the impact on the gut microbiota.

Valorizzazione del potenziale funzionale di microrganismi autoctoni da alimenti a fermentazione naturale

Questo progetto di dottorato mira ad isolare nuovi microrganismi con tratti utili alla prevenzione delle malattie non trasmissibili da introdurre in alimenti funzionali. Batteri lattici autoctoni provenienti da prodotti lattiero-caseari e vegetali fermentati sono stati selezionati per la capacità di produrre peptidi bioattivi da fonti proteiche non convenzionali. I fermentati sono stati valutati per le attività funzionali, nonché per l'effetto sul microbiota intestinale dopo digestione *in vitro*. Un ceppo promettente è stato analizzato tramite proteomica per studiarne i meccanismi di adattamento a diverse fonti proteiche e il sistema proteolitico. Lo studio ha offerto una visione completa del comportamento dei batteri lattici, dalla fermentazione alla digestione gastrointestinale, fino all'impatto sul microbiota intestinale.

Keywords: LAB, whey protein, pea protein, bioactive peptides, proteomics

1. Introduction

Following the PhD thesis project, this oral communication presents the main results related to the ability of autochthonous LAB strains to release BPs from whey and pea protein. The most promising strain was cultivated in three different protein-based media: whey protein with lactose, pea protein with glucose, and a mixture of both. The resulting fermentates were analyzed for their microbiological and metabolite profiling, with particular focus on the functional activities. In parallel, an in-depth proteomic analysis was conducted to study how the strain's protein expression profile varied across different growth phases and media. A special focus was placed on the modulation of its proteolytic system and its involvement in the release of BPs.

2. State of the Art

Fermentation has long been used for food preservation, and currently, fermented foods are gaining interest as functional foods because they provide several health benefits to consumers. A central process occurring during fermentation is proteolysis, the breakdown of proteins into smaller peptide fragments. Some of these, called BPs, typically fewer than 50 amino acids in length, and especially those under 3 kDa, can exert beneficial biological activities, including antioxidants, antimicrobial, antidiabetic, anti-inflammatory, and ACE-inhibitory effects (Guo et al., 2023). LAB are dependent on proteolysis to obtain amino acids since they cannot synthesize all of them *de novo*. As a result, fermentation carried out by LAB is considered a sustainable, safe, and cost-effective way to produce health-promoting BPs in a variety of fermented food products (Pescuma et al., 2013). The proteolytic system of LAB comprises (i) cell-envelope proteinases (CEPs) that hydrolyze extracellular proteins into oligopeptides (4–30 amino acids), (ii) specific peptide transporters that import these peptides into the cell, (iii) intracellular peptidases that further degrade them into di- and tripeptides or free amino acids for metabolic use, and (iv) a peptide secretion system that may export some small peptides. Peptides cleaved by CEPs and not taken up, and those hydrolyzed by intracellular peptidases and secreted by the protein export system or released during cell lysis may accumulate in the extracellular medium, acting as potential BPs (Savijoki et al., 2006). In this context, a protein-rich substrate is crucial. Cheese whey, despite being a dairy by-product, offers high protein and nutrients like lactose, making it valuable in food applications. It serves as a base for high-value products (e.g., whey protein concentrates (WPC), processed through physicochemical treatments, while biological uses are common in non-food sectors. Fermentation to valorize this by-product for BPs production remains underexplored and has primarily focused on the use of highly proteolytic LAB starters, such as *Lactococcus lactis (*Pescuma et

al., 2008). However, there is limited research on the potential of less proteolytic species, such as *Lb. paracasei*. Also, the growing demand for plant-based alternatives, due to ethical and environmental concerns, is driving interest in alternative protein sources. Pea is rich in protein; however, its potential for BPs production and functional improvement are still little studied (Demarinis et al., 2022). LAB dynamically adapts to different protein sources, and the composition of the growth medium can significantly influence their metabolic and functional behavior. In this context, metaproteomics represents an advanced tool for understanding microbial responses to different substrates.

3. Materials and Methods

3.1 Isolation and screening of LAB strains

Autochthonous LAB strains were isolated from both dairy (natural milk cultures, cheese-whey, semi-hard cheese, and washed rind cheese) and plant-based (fermented *Brassica rapa*, sauerkraut, sourdough, and water kefir) products and identified through 16S rRNA sequencing. Proteolytic activity was tested with the o-phthaldialdehyde (OPA) method as described by Church et al. (1985). The strains were also screened for their ability to grow in a modified MRS medium with whey or pea protein as a sole nitrogen source by turbidimetric growth curves.

3.2 Peptide release from whey or pea protein

Whey and pea protein fermentation was carried out by inoculating the selected strains in whey medium (W; 15 g/L WPC80 and 50 g/L lactose) or pea medium (P; 15 g/L PPC80 and 50 g/L glucose). The cell-free supernatant was used to evaluate peptide production through SE-HPLC (Innocente et al., 2023). After peak integration, the percentage of peptide release (PP%) was calculated:

$$PP\% = \frac{C_Psample}{C_0} x100 \qquad \text{(eq.1)}$$

where *C_Psample* indicates the area of the hydrolyzed protein fractions with MW<3,185 Da, while C_0 indicates the area of the non-hydrolyzed protein fractions.

3.3 Assessment of functional activities

2,2-difenil-1-picril-idrazile (DPPH) radical scavenging activity was assessed by following the method described by Cui et al. (2022). The method described by Benzie and Strain (1996) was used to assess the ferric-reducing antioxidant power (FRAP) of the cell-free supernatants. The antimicrobial activity was tested against pools of *Listeria monocytogenes* (strains ScottA, Di4A_284, DSM 7644), *Staphylococcus aureus* (Di4A_226, DSM 2569, Di4A_CAR1-N), *Escherichia coli* (Di4A_8048, Di4A_TO, Di4A_UD), *Salmonella enterica* (DSM 9386, Di4A_#40, Di4A_#52), and *Enterococcus feacium* (Di4A_2146, Di4A_173, Di4A_TOEf). The effect of the fermentates on the growth parameters of these microorganisms was assessed according to Innocente et al. (2023). Angiotensin-converting enzyme (ACE) inhibitory activity was tested according to Cushman and Cheung (1971). *In vitro digestion* was performed according to Brodkorb et al. (2019), and *in vitro* fecal fermentation according to Perez-Burillo et al. (2019). Metagenomic analysis (Rossi et al., 2024) and HPLC-PDA (Ashaolu et al., 2019) were used to evaluate the contribution of the fermentates to gut microbiota and to short-chain fatty acids (SCFA).

3.4 Microbial proteome and metabolite profiling of *Lb. paracasei* AF43 cultivated in different media

Lacticaseibacillus paracasei AF43 was inoculated (about 10^6 UFC/mL) into W, P, and a 1:1 mixture of both (WP) media and incubated at 30 °C. During growth, pH was measured and bacterial counts determined using the drop-plate method on MRS agar plates incubated at 30 °C in anaerobic conditions. After centrifugation, cell pellets were washed twice with Ringer's solution for proteomic analysis. Protein extraction and purification, as well as LC-MS/MS metaproteomics and data analysis, were performed according to Porcellato et al. (2024). Supernatants were collected for metabolite profiling and functional activities evaluation. Sugars and organic acids were quantified following the method proposed by Rossi et al. (2024), amino acids were determined as described by Gao et al. (2023), and peptide release and the functional activities of the fermentates were evaluated as described previously (paragraphs 3.2 and 3.3).

4. Results and Discussion

148 LAB strains, each displaying a distinct phenotypic and molecular fingerprint, were isolated, including 119 from dairy and 29 from plant-based sources. Among these, 63 LAB strains demonstrated growth and proteolytic activity on whey and pea proteins as detected by OPA assay. 19 strains were further identified as capable of peptide release through SE-HPLC. PP% values ranged from 4.97 ± 0.02 % to 11.62 ± 2.54 % for whey protein with *Lb. delbrueckii* subsp. *delbrueckii* LbL2 as the best peptide-releaser. As for pea protein, higher levels of peptide release were detected, with *Leuconostoc pseudomesenteroides* I3 showing a PP% value of 20.29 ± 4.58 %. Cell-free fermentates were subsequently evaluated for their antimicrobial, antioxidant, and ACE-inhibitory activities, with all samples exhibiting at least one functional property. As for DPPH, all fermented samples showed increased

activity compared to the unfermented W, with a trend similar to *Levilactobacillus brevis* C31 in Figure 1a. In the case of the FRAP assay, only two fermentates for W and three for P showed enhanced antioxidant activity, with the best results for *Streptococcus macedonicus* LbL43 cultivated in W (Figure 1b). All fermented samples also demonstrated improved ACE-inhibitory activity compared to the unfermented media, with an effect comparable to that observed after fermentation with *Lactiplantibacillus plantarum* M2 (Figure 1c). Regarding antimicrobial activity, various target microorganisms were tested, and all fermentates exhibited antimicrobial activity against at least one of the targets. Interestingly, the most affected pathogen was *L. monocytogenes* (Figure 1d). The sample - obtained following the fermentation of W with *Lb. paracasei* AF43 - reduced the growth of this pathogen, unlike the unfermented W, which even promoted its growth if compared with *L. monocytogenes* traditional culture media (CTRL). The fermentates were also tested for their effect on the gut microbiota after *in vitro* digestion. Seven strains demonstrated the ability to survive gastrointestinal transit in concentrations that may guarantee the probiotic effect. Moreover, some of the fermentates were also capable of positively modulating the gut microbiota, as well as the profile of SCFA that are linked to the eubiosis of the gut environment.

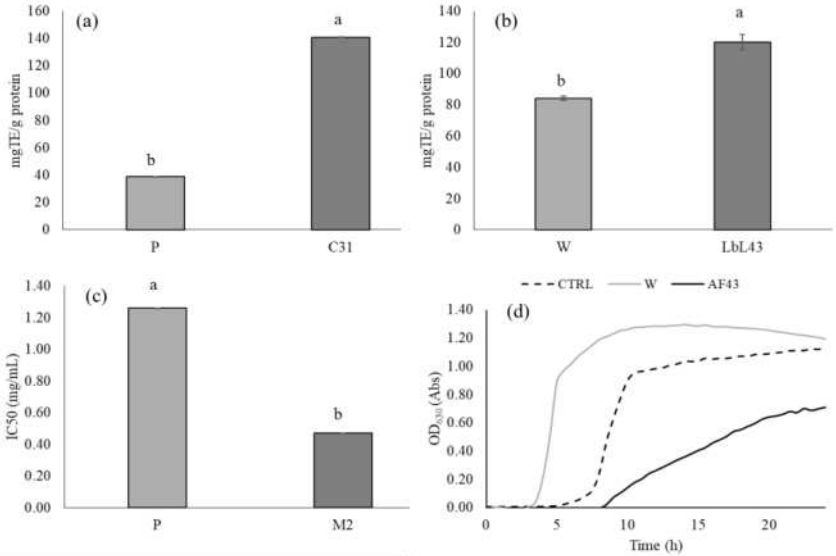


Figure 1 *Antioxidant activity (mg_{TE}/g protein) determined by DPPH (a) and FRAP (b) assays, ACE-inhibitory activity expressed as IC50 (mg/mL) (c), and antimicrobial activity against L. monocytogenes of fermentates and the corresponding unfermented sample (P or W). CTRL: pathogen grown in BHI. Bars labeled with different letters are significantly different ($p < 0.05$).*

Given the importance of the valorization of industrial by-products for sustainable development and the current protein transition, *Lb. paracasei* AF43 - showing proteolytic activity across both protein sources, along with promising functional properties - was selected to further evaluate its role in the valorization of both animal- and plant-based proteins. Since the growth medium can influence the strain's metabolic activity and, consequently, its health-promoting properties, LC-MS/MS proteomics was used to investigate the changes occurring in its proteome during fermentation. Samples were collected at both mid-exponential (mid-exp) and stationary (stat) growth phases, determined via turbidimetric growth curves in W, P, and WP. Strain AF43 reached mid-exp at 33 h in W and 11 h in P and WP, with the stat observed at 48 h in W and 14 h in the other media (Figure 2). Initial cell concentrations were almost 6 log CFU/mL (pH 6.5–6.6), while bacterial loads during mid-exp and stat reached 7–8 log CFU/mL for all media. Acidification patterns varied among media, with the most rapid pH decrease observed in P and WP. However, P led to the lowest final pH, while whey protein resulted in a slower acidification, with pH values of almost 5.5 and 4.8 at mid-exp and stat, respectively.

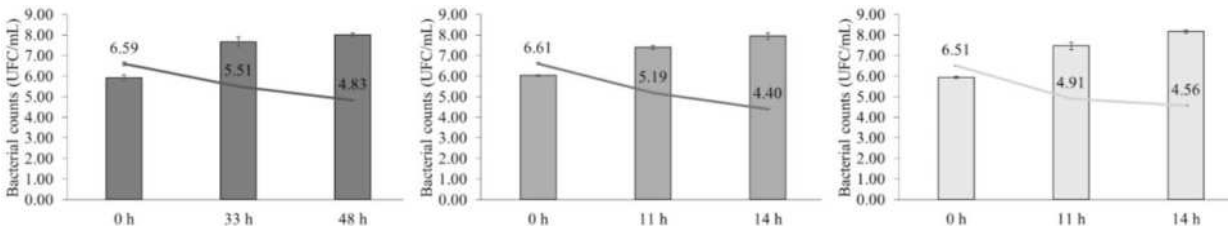


Figure 2 *Fermentation progression of Lb. paracasei AF43 in W, P, and WP media. Data are reported for each sampling point in terms of cell viability (log CFU/mL; bars) and pH (lines).*

Proteome comparisons among the media were conducted on data from stat, since comparative analysis between

the two phases revealed minimal differences. In this phase, 1,006 protein groups were detected across the three media. KEGG annotation linked these protein groups to 94 metabolic pathways. Of these, 23 pathways significantly differed among media, particularly those belonging to ribosome, carbohydrate, and amino acid metabolisms.

Ribosome metabolism is a marker of microbial growth and stress status, and it was significantly downregulated in W, indicating possible stress despite its nutrient richness. On the contrary, it was upregulated in P and WP, likely due to higher glucose availability, which supported active growth. These trends align with what was observed by growth kinetics. Consistently, glycolysis was upregulated in P, with elevated expression of key glycolytic protein groups. In contrast, W showed suppressed expression of this metabolism, suggesting adaptation to complex sugars like lactose. Galactose metabolism was upregulated in W due to lactose and galactose presence, and unexpectedly in P, where only two lactose-related protein groups were overexpressed, possibly playing a role in functions outside lactose metabolism. This highlights the flexibility of *Lb. paracasei* AF43 in adapting to different carbon sources, such as starch, sucrose, or pentoses, whose pathways were upregulated in P. Over 30 proteins linked to pyruvate metabolism were detected, mainly related to lactic acid production as confirmed by organic acids results, but no significant differences were observed across conditions.

Amino acid metabolisms include both protein hydrolysis and *de novo* biosynthesis, ways through which LAB supply amino acid needs. Overall, the metabolic pathway related to biosynthesis of amino acids was upregulated in P and downregulated in W ($p<0.05$), which might reflect the faster growth of *Lb. paracasei* AF43 in P. These results are consistent with Wang et al. (2013) when culturing a strain of *Lb. casei* in milk and soy milk. As for WP, the amino acid biosynthesis showed intermediate expression between W and P, suggesting that the mixture provided a better supply of the amino acids needed by the microorganism.

However, LAB rely mainly on their proteolytic system to obtain amino acids, since they have numerous amino acid auxotrophies. All the components of the proteolytic system of *Lb. paracasei* AF43 were found in its proteome, including 17 proteases and peptidases able to cut dietary proteins. The only protease found is PrtP, the main CEP in *Lb. paracasei*, without differences in expression between the media. *Lb. paracasei* is known to express low to no CEPs, often depending on other cultures for obtaining oligopeptides. However, they compensate by a wide activation of intracellular peptidases and transporters, resulting in the generation of shorter peptide sequences (Savijoki et al., 2006). This trait makes *Lb. paracasei* a suitable species for producing BPs, since the most effective BPs are the shortest ones. In this study, 16 peptidases with different specificities were found. Among these, PepO, PepE, and PepN exhibited different expressions across media ($p<0.05$). Specifically, these protein groups were downregulated in W and upregulated in WP. Since peptide bioactivity is influenced not only by peptide length but mostly by its amino acid sequence, these specificities can shape the profile of bioactive peptides released during protein hydrolysis, as demonstrated by Chen et al. (2024), who linked changes in proteases expression to ACE-inhibitory activity. Additionally, by calculating the cumulative expression of peptidases and proteases by the sum of log_2LFQ intensities (Figure 3a), it was evident that the proteolytic activity was significantly higher in WP than in W. This intracellular proteolysis is closely linked to oligopeptide import, via the oligopeptide permease (Opp) system, which all components were detected. Cumulative log_2LFQ intensities of Opp Transporters (Figure 3b) were significantly higher in W compared to P, suggesting a more active oligopeptide import in the first medium, while it was intermediate in WP. Peptides secretion may occur through the peptide export system which has shown the same trend as the Opp system (Figure 3c), but mostly during cell lysis, leading to the release of BPs available to exert functional properties in the extracellular environment.

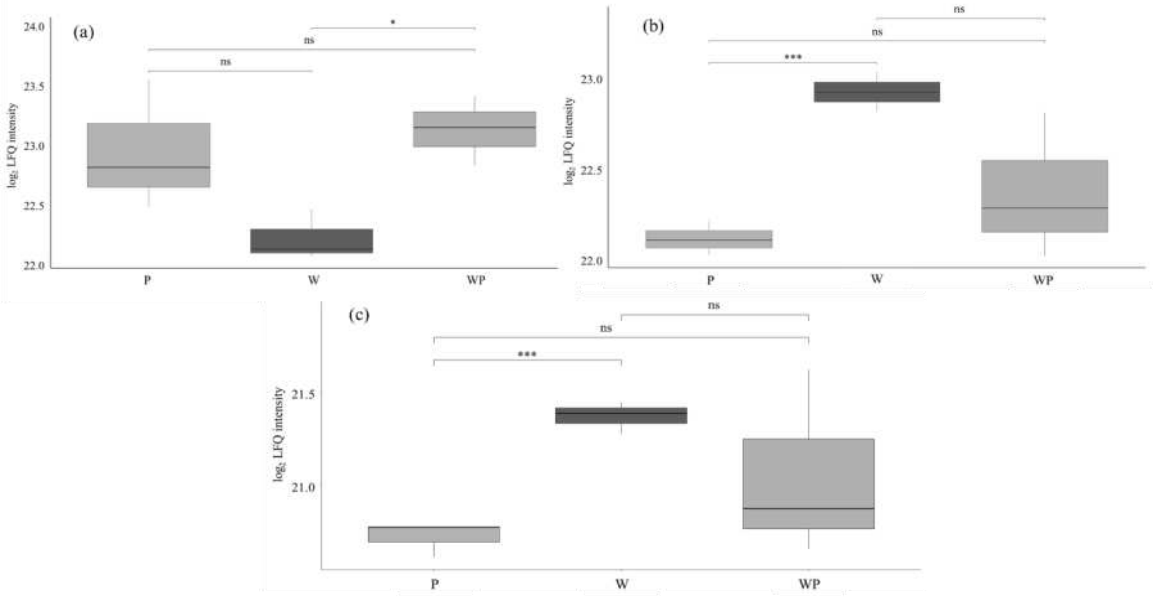


Figure 3 *Cumulative expression levels of (a) peptidases and proteases, (b) Opp transporters, and (c) protein export system in Lb. paracasei AF43 cultured in P, W, and WP media. Expression is represented as the sum of log_2 LFQ intensities of all protein groups assigned to each functional category. Statistical significance: (*) $p < 0.05$, (***) $p < 0.001$, ns = not significant.*

5. Conclusions and Future Perspectives

These findings highlight how substrate composition drives bacterial metabolism and the resulting functionalities. In particular, the components of the proteolytic system of the strain, modulated by growth conditions, can be improved to enhance the functional properties of fermented products, providing the basis for designing health-promoting, protein-based fermentates. In particular, *Lb. paracasei* AF43 cultivated in the protein mix showed enhanced activation of the proteolytic system, with increased expression of peptidases and sustained expression levels of transporters compared to the other media. This result highlights the potential of hybrid animal- and plant-based protein sources within a sustainable approach. However, this potential still needs to be confirmed through functional activity assays, which are currently under investigation. A key next step includes the application of peptidomics to identify the peptides generated during fermentation and to correlate them with known bioactivities. Also, the *in vitro* findings should be complemented by *in vivo* studies to validate the physiological effects of the BPs and the overall health-promoting properties of the beverage. Furthermore, sensory and technological assessments of the fermentates will be essential to ensure consumer acceptability, stability of the product, and feasibility of the process. In the end, the scaling up of the production for commercial applications is crucial.

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Oral presentation sessions
PhD students – 3rd year
Abstracts

PARALLEL SESSION 10
Emerging Analytical Tools for Food Quality and Safety

Combining Instrumental and Sensory Methods to Assess the Quality and Authenticity of Foods of Animal Origin

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The PhD project aims to explore the effect of farming system and origin on the quality of foods of animal origin, adopting a multidisciplinary approach relying on the use of sensory, microbiological, and instrumental data for providing a comprehensive quality assessment of the selected products. The last activities of the PhD project are reported. Firstly, a characterization of the sensory profile of beef meat belonging to different European countries was performed. Secondly, a sensory evaluation of UHT whole milk samples obtained from dairy cows kept according to different farming systems and collected during different seasons (i.e., winter and spring times) was carried out.

Metodi strumentali e sensoriali congiunti per la valutazione della qualità e dell'autenticità di alimenti di origine animale

Il progetto di dottorato si propone di esplorare l'effetto del sistema di allevamento e dell'origine sulla qualità degli alimenti di origine animale, adottando un approccio multidisciplinare basato sull'utilizzo di dati sensoriali, microbiologici e strumentali per fornire una valutazione completa della qualità dei prodotti selezionati. In questo contributo, sono riportate le attività del progetto di tesi di dottorato relative all'ultimo anno di attività. Una parte dell'attività ha riguardato la caratterizzazione del profilo sensoriale di carni bovine provenienti da diversi Paesi europei. In un secondo studio, è stata effettuata una valutazione sensoriale di campioni di latte intero UHT ottenuti da diversi sistemi di allevamento e raccolti durante stagioni differenti (inverno e primavera).

Key words: Sensory evaluation; technological properties; quality; milk; beef; consumer test.

1. Introduction

In accordance with the PhD project previously described, this oral communication summarizes the main results of the last activities devoted to:
A1) Characterization of beef meat samples;
A2) Characterization of UHT whole milk.

2. Instrumental Analytic Techniques Applied in Foods of Animal Origin

Human sensory perception can be complemented by "artificial senses," which rely on the use of instrumental technologies applied in various domains such as quality control, shelf-life assessment, and authentication of food products. These tools offer several advantages being rapid, efficient, non-destructive, and environmentally sustainable (Mahanti *et al*., 2024). Among the artificial sensing technologies, electronic noses (e-noses) and electronic tongues (e-tongues) based on electronic or hybrid sensors are of particular interest. The e-nose system consists of an array of sensors designed to interact with compounds in food, mimicking the way the human olfactory system perceives different odors (Wang & Chen, 2024). This technology has been utilized, for example, to evaluate the quality of beef fillet (Sun *et al*., 2024), monitor chicken meat freshness (Chotimah *et al*., 2024), authenticate Parmigiano-Reggiano cheese (Becchi *et al*., 2024), and identify the adulteration of honey (Gonçalves *et al*., 2023). Another fast, non-destructive method is the computer vision system (CVS), also known as electronic eye. Comprising an illumination unit, a high-resolution camera, and a computer, CVS enables the classification of food products by grade, detection of visual defects, and estimation of characteristics such as color, shape, size, and surface defects. Applications include the estimation of color, marbling level, quality prediction of beef meat (Munekata *et al*., 2023), chicken meat (Ismail *et al*., 2024) and characterization and discrimination of honey having different botanical and geographical origins (Wójcik *et al.,* 2023). Integrating instrumental and microbiological methods with sensory analysis allows to investigate how farming systems could influence the quality of foods of animal origin (Kasapidou *et al*., 2023).

3. Experimental Procedure

As part of this PhD project, a five-steps experimental design was set up using instrumental and sensory analyses to assess the quality of foods of animal origin, such as poultry meat, beef meat, dairy products and honey. The results of the instrumental and sensory analyses were then processed jointly using multidisciplinary approach. This work is part of a European project entitled INTAQT — INnovative Tools for Assessment and Authentication of

chicken meat, beef and dairy products' QualiTies, whose main objective is to investigate how husbandry practices and production systems affect the intrinsic quality of animal products.

4. Materials and Methods

Experimental procedures for the activities A1 and A2 are reported as follows.
A1) Quality attributes were evaluated using both sensory and instrumental methods on a total of 187 meat samples (*Longissimus dorsi*, 9th rib, weighing approximately 200 grams) which were collected from 5 countries: IT (n=31; indoor system with high feed input, from Italy), ES (n=18; indoor system with high input feed, from Spain), CH (n=71; indoor system with medium input feed or grass-based system with low input feed, from Switzerland), UK (n=31; grass-based system with high input feed, from the United Kingdom), and FR (n=36; indoor system with medium input feed or grass-based system with high input feed, from France). Samples were used for assessing color (L*a*b*; n=5 replicates/sample), shear force on both raw and cooked meat (Warner Bratzler shear test; n=5 replicates/sample) and lipid oxidation (TBARS). As for sensory evaluation, 72 samples were selected for the consumer test (120 assessors). Each taster evaluated seven samples, that were scored for tenderness and juiciness on verbal scales, along with olfactory, gustatory, overall liking and meat quality. The evaluation concluded with a brief section gathering sociodemographic and personal data. Instrumental and sensory data were analyzed using One-way ANOVA by testing the effect of the country of origin. When significant, means were separated by Tukey-HSD test ($p<0.05$).
A2) Sensory analyses were conducted on UHT whole milk samples (n=30, provided by a French company) obtained from different farming systems (i.e., pasture-based, semi-mountain, and intensive) and collected during different seasons (i.e., winter and spring). In Italy, sensory profiling was performed using a rapid descriptive test known as Flash Profiling. Experienced assessors evaluated six samples per session, developed individual lexicons, selected 3 to 5 key attributes, and ranked samples based on these descriptors using an ordinal scale. Each session lasted approximately one hour. Data were analyzed using Generalized Procrustes Analysis (GPA), which revealed that attributes such as "cooked milk," "cream/butter," and "barn/hay" were the most distinctive. A hedonic assessment with Italian consumers was also conducted. Participants evaluated six randomly selected milk samples for attributes such as appearance, smell, taste, texture, and overall liking. They also completed a Check-All-That-Apply (CATA) test, and JAR scale, using descriptors based on the Flash Profiling results and literature review. A brief questionnaire collected sociodemographic data, UHT milk consumption frequency, and purchasing habits. To test the significance of attributes the data were analyzed using Cochran's Q test.

5. Results and Discussion

5.1.1 Effect of country of origin on technological properties

As for color, significant differences were observed (Table 1). In detail, samples from Italy (IT), Switzerland (CH) and Spain (ES) were lighter if compared with the others. In addition, samples from the UK showed the highest redness ($p < 0.001$). As for shear force, the samples from the UK exhibited higher shear force in raw meat ($p < 0.001$). A different trend was observed when this parameter was measured in cooked meat: the samples from Switzerland were found to be tougher compared to the other samples considered ($p<0.05$). This difference can be attributed to collagen content and cross-linking along with the remarkable changes occurring during the cooking process, such as juice losses. Regarding the evaluation of the lipid oxidative profile, the analyses are currently in progress.

	Country of origin						
	UK	CH	ES	FR	IT	SEM	*P-value*
Lightness - L*	35.6b	38.6a	38.5a	33.2c	40.0a	0.2	***
Redness - a*	20.6a	18.4b	18.9ab	18.3bc	16.8c	0.2	***
Yellowness - b*	10.7a	9.4b	10.3ab	9.4b	9.5b	0.1	***
Shear force –Raw meat (N)	25.2a	21.2ab	16.1bc	14.7c	19.3bc	0.6	***
Shear force – Cooked meat (N)	37.9ab	39.9a	30.1c	34.0bc	37.5ab	0.9	*

Table 1 Effect of the country of origin on the technological properties of beef meat. *UK: United Kingdom, CH: Switzerland, ES: Spain, FR: France, IT: Italy*; *SEM= standard error mean****= P<0.001; *=P<0.05; a- c= mean values followed by different letters significantly differ according to the country of origin.*

5.1.2 Effect of country of origin on sensory traits

As for the results obtained from the sensory analysis conducted in Italy, the samples that showed greater tenderness were those from Spain (Table 2). In contrast, the samples from Italy were less appreciated in terms of flavor and overall liking. Regarding the meat quality traits, the samples from Switzerland obtained the highest scores. The high variability of the results observed could be attributed to the different production systems adopted, particularly

the different farming and feeding systems, and not to the country of origin itself. It is important to highlight that these results are part of the consumer test conducted in Italy, and it will be interesting to combine them with the results obtained by the other project partners involved.

Table 2 Effect of the country of origin on technological properties of beef meat. *UK: United Kingdom, CH: Switzerland, ES: Spain, FR: France, IT: Italy*; *SEM= standard error mean* ***= P<0.001; ns= not significant; a- c= mean values followed by different letters significantly differ according to the country of origin.*

	Country of origin						
	UK	CH	ES	FR	IT	SEM	*P-value*
Tenderness (0-100)	46.1bc	52.9ab	60.2a	47.2bc	42.3c	0.9	***
Juiciness (0-100)	50.4	49.9	48.1	48.8	49.2	0.9	ns
Flavour (0-100)	58.7a	56.9a	53.6a	54.5a	42.3b	0.8	***
Overall liking (0-100)	55.7a	55.7a	55.3a	50.4a	41.6b	0.8	***
Meat quality (0-5)	3.4ab	3.5a	3.3ab	3.2b	2.8c	0.1	***

5.2 Sensory characterization of UHT whole milk using rapid descriptive analysis and consumer test

From the preliminary results of the sensory analysis, it can be observed that the attributes most able to discriminate between the milk samples are “cooked milk” and “cream/butter”, while “barn/hay” characterizes those collected during the spring season. The “barn/hay” attribute, for example, may be linked to the farming system of dairy cows raised on pasture and in semi-mountain areas, whereas “cream/butter” may be more noticeable in milk obtained from cows raised in intensive systems (Figure 1).

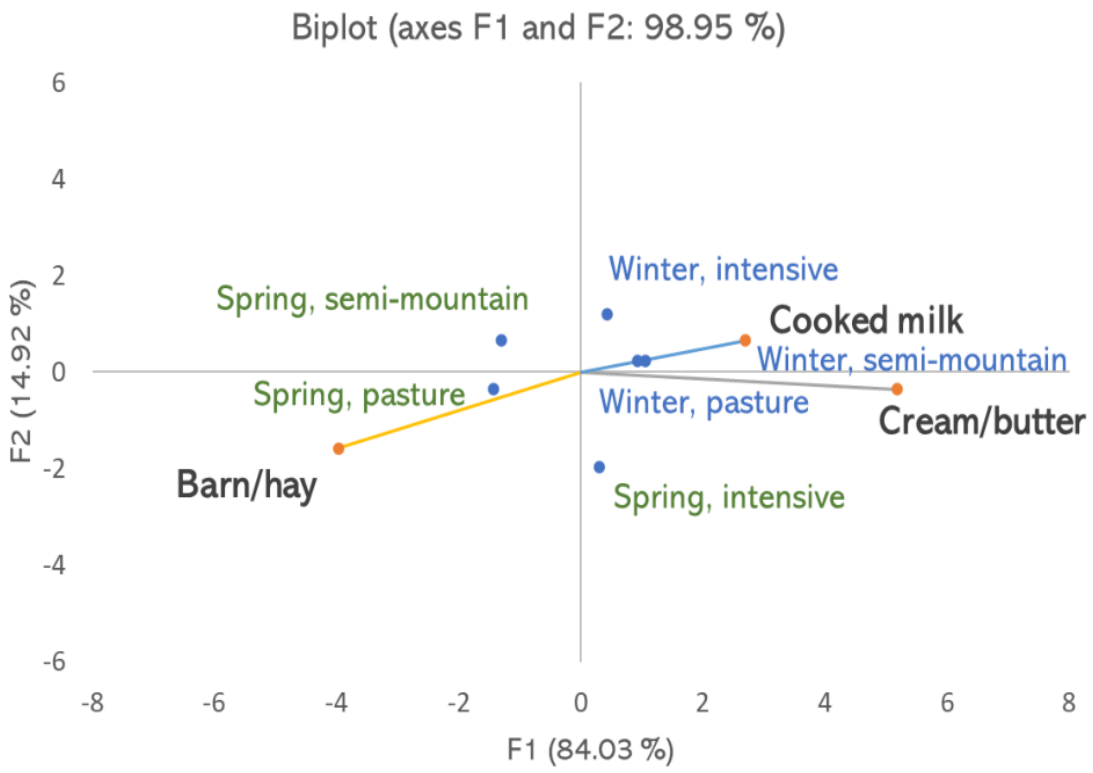


Figure 1 Biplot of Generalized Procrustes Analysis (GPA) of UHT milk sample.

From the sensory analysis, conducted through consumer tests, the preliminary results indicate that overall aroma liking is strongly associated with two main attributes: “caramel-like” and “popcorn-like” notes, while taste liking tends to show an inverse relationship with bitterness and sourness (Figure 2). Finally, when looking at the liking scores across sensory phases, a few clear patterns emerge. Milk from semi-mountain and pasture arming systems obtained during winter a received the highest liking scores. On the other hand, the milk from cows farmed on pasture and collected during spring showed the lowest liking scores. Notably, the latter was also associated with

the "stable/hay" descriptor in the descriptive analysis (Figure 3).

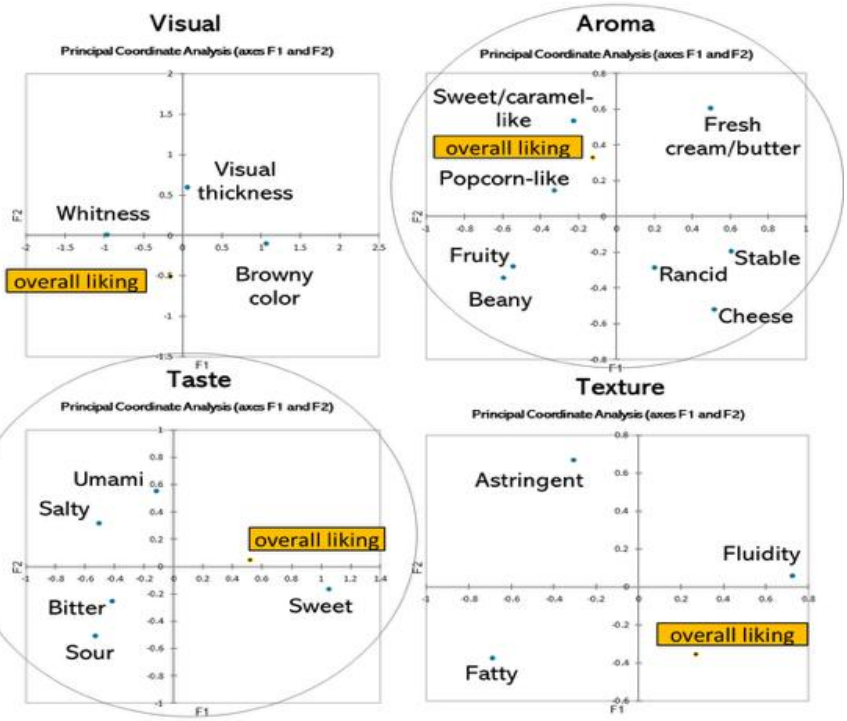


Figure 2 Principal Components Analysis of CATA test results for visual, aroma, taste and texture attributes.

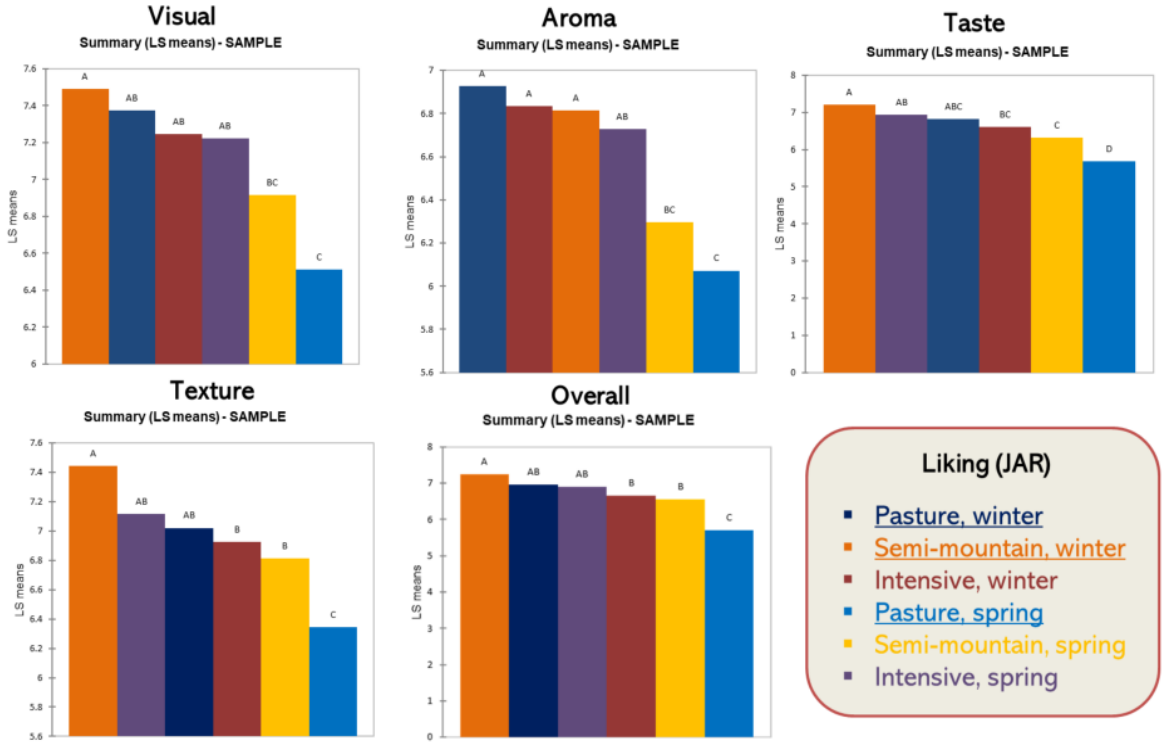


Figure 3 Liking results with JAR scale.

6. Conclusions and Future Perspectives

The differences may be explained by considering that the farming systems adopted in each country may have resulted in specific characteristics in the quality traits of the resulting animal products. The next steps in the proposed case studies will focus on the joint analysis of the sensory data collected in Italy together with that obtained by the other partners involved in the project to gain a deeper understanding of the quality profile of the meat and milk samples.

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Advanced chemical characterization and functional assessment of hemp flour-enriched bread: opportunity and challenges in valorizing hemp chain by-products

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The objective of the PhD thesis was to use hemp seeds for the fortification of a widely consumed food product, wheat bread, and to evaluate the chemical and sensory effects at four different supplementation levels (0%, 10%, 15%, 25%). Compositional parameters such as protein and lipid content, and dry matter were determined. Furthermore, the impact of fortification on the amino acid profile, fatty acid composition, as well as the analysis of volatile and phenolic compounds, was investigated using chromatographic and spectrophotometric techniques. To provide a more comprehensive assessment of nutritional quality, an *in vitro* simulated gastrointestinal digestion was performed to study the bioaccessibility of phenolic compounds extracted from the fortified products.

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Caratterizzazione chimica avanzata e valutazione funzionale del pane arricchito con farina di canapa

L'obiettivo del presente lavoro di tesi è stato l'impiego di semi di canapa per la fortificazione di un alimento di largo consumo quale il pane, valutandone gli effetti chimico e sensoriali su quattro differenti livelli di aggiunta (0%, 10%, 15%, 25%). Sono stati determinati parametri composizionali quali il contenuto proteico e lipidico e la sostanza secca. Inoltre, è stata approfondita l'influenza della fortificazione sul profilo amminoacidico, sulla composizione degli acidi grassi, nonché sulla caratterizzazione dei composti volatili e fenolici mediante tecniche cromatografiche e spettrofotometriche. Per una valutazione più completa della qualità nutrizionale, è stata condotta una simulazione *in vitro* della digestione gastrointestinale, finalizzata a studiare la bioaccessibilità dei composti fenolici estratti dai prodotti fortificati.

1. Introduction

Hemp (*Cannabis sativa* L.) is a worldwide crop able to generate several by-products useful for different purposes (food, pharmaceutical, biomass) as reported in **Figure 1** (European Parliament and Council, 2013, 2021). Hemp seed represented the edible part of the plant characterized by interesting chemical and nutritional properties (Rizzo et al., 2023) used mainly for oil extraction. After this technological process, the hemp seed residues are used to produce hemp flour (HF). HF retains all the chemical properties of the seed from which it derives as high protein levels (20-30%) with an interesting amount of essential amino acids, fats (25-35%) mainly composed from polyunsaturated fatty acids, carbohydrates (20–30%), mostly in the form of insoluble dietary fiber (Siano et al., 2019) and a plethora of phenolic compounds with antioxidant activity [4]. Given the interesting chemical characteristics and compliance with the EU legislation (THC <0.3% on plant), HF can be considered as an excellent ingredient in the food sector. Several studies available in the literature explore the use of HF in bread-making from different hemp varieties or mix, but none of them have comprehensively investigated its impact combining the chemical composition, *in vitro* digestion and sensory evaluation of the final bread samples. Therefore, the present study aims to provide an in-depth characterization of bread made with three different substitution levels of HF (mix of Felina 32 and Futura 75, 10%, 15% and 25%) on wheat flour. This work will provide a deep understanding of the potentiality of hemp in delivering high-value composite bread samples to increase and improve the use of this plant in the food chain.



Figure 1 Hemp (*Cannabis sativa* L.) uses

2. Materials and Methods

2.1 Samples

HF was purchased from Canapuglia (Conversano, Italy), composed as mix of Felina 32 and Futura 75 varieties. While wheat flour was kindly provided by Agugiaro & Figna s.p.a. (Collecchio, Italia). Bread samples have been produced applying the same protocol and conditions described by (Del Vecchio et al., 2025). The bread samples obtained were coded as (HB0%, HB10%, HB15%, HB25%). Two independent batches represented biological replicates were produced for each bread formulation. Each batch was subjected to duplicate analytical measurements (technical replicates) across all the next parameters reported, yielding four data points per formulation.

2.2. Proximate composition, fatty acids and amino acids profile, polyphenolic and antioxidant quantification, volatile organic compounds.

Proteins, fats and dry matter were determined using standard procedures by AOAC, 2023(Dr. Latimer, 2023). For fatty acid profile, lipids were extracted from HF and bread samples using a slightly modified version of the Folch method procedure and the fatty acids methyl ester (FAME) were obtained by the acid-catalysed transmethylation of the extract (Annaratone et al., 2009) and mixed with C:23 internal standard before injection on a GC-MS. The identification was confirmed by analysing the Supelco®37 Component FAME Mix. For the determination of amino acids profile, bread and HF were submitted to a hydrolysis step in acid conditions according to (Caligiani et al., 2007). The amino acid composition was analyzed using a GC-MS with previous derivatization. A proper identification and quantification of all the amino acids characteristic of the considered samples was performed on the basis of their registered mass spectra, as by analysing an amino acids standard solution adding N-leucine, in order to calculate the response factors. Soluble polyphenols were extracted from 500 mg both HF and bread samples using a method reported by (Dall'Asta et al., 2013). Folin-Ciocalteu's phenol reagent was used to determine the total phenolic content (TPC), following protocol proposed by (Martelli et al., 2020). To assess the antioxidant activity of HF and bread samples, the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was conducted according to the protocol proposed by (Kedare & Singh, 2011).
Similarly, the ABTS test was performed using the radical 2,2′-azinobis (3-ethylbenzothia-zoline-6-sulfonic acid) reagent (ABTS), according to (Nenadis et al., 2004). The characterization of the volatile fraction was performed according to the protocol of (Cirlini et al., 2012), using the solid phase microextraction technique (HS-SPME) coupled to GC-MS using an internal standard (toluene) for performing a semi-quantification.

2.3 Untargeted metabolomic profile of cooked and digested bread samples: free and bound phenolic compounds

Free and bound phenolics were extracted from both cooked and digested bread samples and for HF (Zhang et al., 2023). The fingerprinting of cooked and digested hemp-enriched bread samples was conducted using high-resolution mass spectrometry (HRMS) based on a Q-ExactiveTM Focus Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific, Waltham, MA, USA) coupled to a Vanquish ultra-high-pressure liquid chromatography (UHPLC) pump and equipped with heated electrospray ionization (HESI)-II probe (Thermo Scientific, USA). Additionally, the annotation of phenolic compounds was carried out with Phenol-Explorer database (version 3.60) and their semi-quantification was performed by classifying all phenolic compounds into different subfamilies, for instance: anthocyanins, flavanols, phenolic acids, flavones and related flavonoids, tyrosols and simple phenolic compounds, and flavonols. For each subfamily, standard calibration curves were built utilizing authentic standard compounds, representative of each class (i.e., cyanidin, catechin, ferulic acid, luteolin, oleuropein and quercetin, respectively). The content of each phenolic subclass was finally expressed as mg Equivalents (Eq)/ 100 g.

2.4 Sensory evaluation

Sensory evaluation of bread was carried out through an acceptability test. Bread samples were produced a few hours before the analysis. Each crumb sample of 3x2x2 cm3 size was extracted from the loaf and identified with three-digit random number code and simultaneously presented on a plate in randomized order. Consumers were asked to taste and evaluate one sample at a time. Between each sample tasting, water was served to cleanse the palate. The sensorial study received approval from Research Ethics Board of the University of Parma (Prot0069359, 28-02-2025). Samples were presented for their evaluation to 60 untrained judges (recruited from the University of Parma among students and staff: 35 males and 25 females, age between 18 and 55. They were asked to evaluate odour, taste, and overall acceptability of bread samples using a nine-point hedonic scale where 1 = extremely dislike, 2 = dislike very much, 3 = dislike, 4 = slightly dislike, 5 = neither like nor dislike, 6 = slightly like, 7 = like, 8 = like very much and 9 = extremely like.

3. Results and Discussion

3.1 Proximate composition, fatty acids and amino acids profile, polyphenolic and antioxidant quantification, volatile organic compounds.

HF showed a high amount of lipids (10.13 ± 0.12%), proteins (30.68 ± 0.48%) and dry matter (90.20 ± 0.22%). Protein content showed a significant increase in bread samples ($p < 0.05$) in respect to the control (HB0%) at all substitution levels analyzed (HB10%, HB15%, and HB25%). More in detail, the highest substitution level (HB25%) exhibited a significant increase ($p < 0.05$) in protein content of 14.98 ± 0.29%, compared to 10.62 ± 0.09% found in HB0%. However, no significant differences were observed between the HB10% and HB15% enrichment levels. The same behavior was observed for lipids. In fact, the highest lipid value was found in HB25% (5.77 ± 0.18%) while no significant differences were observed between HB10% (4.03 ± 0.02%) and HB15% (4.51 ± 0.17%). HB0% showed the lowest amount of lipids (3.05 ± 0.12%). With the same water content in the formulation, the dry matter content did not differ based on the different samples.
Saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) were identified in the HF and enriched bread samples. the use of HF increased significantly ($p < 0.05$) linolenic acid (C18:3) content from 0.48 ± 0.01 g/100g FA (HB10%) and 0.65 ± 0.06 g/100g FA (HB15%) to 1.04 ± 0.12 g/100g FA (HB25%). The progressive increase of C18:3 ω-3 highlighted the interesting role of HF in enhancing fat quality of bread, adding essential fatty acids, reflecting the high amount of C18:3 (34.17 ± 0.28 g/100g FA) in HF. Several MUFAs such as palmitoleic acid (C16:1), heptadecenoic acid (C17:1), eicosenoic acid (C20:1), and oleic acid (C18:1) were found. Among these components, the most abundant found in HF was C18:1 (16.15 g/100g FA), that presented the higher significant amount ($p < 0.05$) in HB0% (36.86 ± 1.62 g/100g FA) compared to enriched bread samples (HB10% = 33.26 ± 1.66%; HB15% = 33.33 ± 0.04; HB25% = 31.33 ± 1.60%). Among the saturated fatty acids (SFAs) identified, palmitic acid (C16:0) and stearic acid (C18:0) were the most abundant in the control sample (HB0%), with levels significantly higher than those observed in the hemp flour-enriched bread samples. Generally, the total saturated fatty acids (Σ-SFAs) increase as the percentage HF in the formulation rises, ranging from 16.73 ± 0.42% in the control bread (HB0%) to between 17.90 ± 0.45% and 21.01 ± 0.50% in the enriched bread samples. Our data on HF revealed high levels of essential amino acids such as lysine (4.20 ± 0.20 mg/g), leucine (4.80 ± 1.20 mg/g), and phenylalanine (3.70 ± 0.60 mg/g). Additionally, HF was found to be rich in non-essential amino acids, including alanine (5.50 ± 0.20 mg/g), serine (3.60 ± 0.10 mg/g), proline (5.70 ± 0.20 mg/g), glycine (7.40 ± 0.10 mg/g), glutamic acid (6.80 ± 0.70 mg/g) and aspartic acid (4.00 ± 0.20 mg/g). The addition of HF led to an increasing trend of lysine level, although non-significant differences were found among bread samples. Enrichment with ingredients rich in essential amino acids such as HF could be an effective strategy to enhance the quality profile of wheat bread. Regarding the total phenolic content (TPC) and antioxidant activity (AO), HF showed a high TPC of 2.20 ± 0.26 mg GAE/g and AO values of 3.33 ± 0.11 mg GAE/g (DPPH assay) and 8.04 ± 0.15 mg GAE/g (ABTS assay). Indeed, TPC values showed a significant increase in breads where HF was added. In more detail, the lower TPC value was observed in the control samples (HB0%=0,82 ± 0,01 mg GAE/g), while statistically higher values were reported in bread with 10 (1.08 ± 0.05 mg GAE/g), 15 (1.18 ± 0.02 mg GAE/g) and 25% (1.28 ± 0.05 mg GAE/g) of HF. To better characterise bread sample with HF, their antioxidant properties were also evaluated. To achieve this, two distinct but complementary methods, DPPH and ABTS assays—were employed. The DPPH value increased indeed from 0.13 mg TEAC/g of the control bread (HB0%) to 0.63 mg TEAC/g of bread with 25% HF (HB25%). Similarly, the ABTS value increased from 0.18 mg TEAC/g (HB0%) to 1.37 mg TEAC/g (HB25%). It is important to underline that these results are based on spectrophotometric tests, which are valuable for sample screening, but do not give information concerning bioaccessibility of compounds during or after consumption. The main representative volatile compounds (VOCs) of bread enriched with hemp flour are 1-pentanol (pungent, fermented, bready notes), 1-octen-3-ol (earthy, herbal notes), 2-octen-1-ol (green and vegetable notes), 2-nonenal (fatty, green, waxy notes), 2-octenal (fatty, green, herbal notes), humulene (woody notes), citral (citrus notes), and hexanoic acid (fatty, sour note), belonging to alcohol, aldehyde, terpene, and acid categories, respectively. Hemp has a distinctive aroma, due to the presence of terpenes (Pellati et al., 2018). The HF is rich in terpenes such as humulene, limonene, β-myrcene, and caryophyllene with characteristic aroma and potential health benefit. Different terpenic compounds were indeed detected in HF, while only humulene (HB10% = 0.004 ± 0.002 μg/g; HB15% = 0.006 ± 0.001 μg/g; HB25% = 0.01 ± 0.004 μg/g) and citral (HB10% = 0.006 ± 0.002 μg/g; HB15% = 0.009 ± 0.001 μg/g; HB25% = 0.014 ± 0.004 μg/g), were found in bread samples. The HF led to some changes in terms of VOCs of bread, giving rise to a particular volatile profile characterized by compounds deriving from HF such as humulene, citral and hexanoic acid, especially when the enrichment was high (25%).

3.2 Untargeted metabolomics profile of digested bread samples: the fate of free and bound phenolic compounds.

UHPLC-HRMS analysis was performed to characterize both control and HF enriched bread samples following an *in vitro* gastrointestinal digestion, without including HF as sample in the investigation. This allowed to annotate 4,374 metabolites. Figure 2 revealed a consistent separation between HB0% and enriched bread samples. More in detail, the results were reported using a HCA (Figure 2A) and PCA (Figure 2B). These findings suggested that the

chemical profile of bread formulations was significantly influenced by the digestion process, with polyphenols being particularly susceptible to degradation.

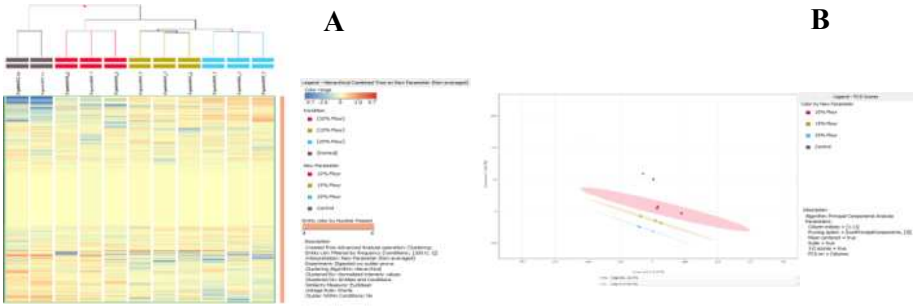


Figure 2 Unsupervised multivariate statistical analysis on the chemical profile of *in vitro* digested enriched bread samples.

Following feature annotation, a semi-quantitative approach was performed to determine the content of free, bound and digested phenolic subclasses, evaluating the impact of HF inclusion in bread samples. In general, phenolic acids were the most abundant compounds, showing a significant difference between the free and bound fractions. The free fraction ranged from 4.20 mg/100g (at 10% HF) to 5.30 mg/100g (at 15% HF), whereas the bound fraction from 0.40 mg/100g (at 25% HF) to 0.47 mg/100g (at 15% HF). Anthocyanins represented the second most abundant class, with no statistically significant differences observed between free and bound fractions. The digestion process did not appear to significantly affect the bioaccessibility of these compounds. The content of the other four phenolic subclasses in free and bound forms - flavanols, flavonols, other flavonoids, and low molecular weight and other phenolic classes (LMW), respectively - was low and negligible compared to the previous two classes. An interesting outcome was the amount of low molecular weight and other phenolic classes (LMW) and other flavonoids in the digested fraction, which showed an increase across all HF inclusion levels, with no significant differences. Finally, stilbenes were the least abundant phenolic compounds, with a small increase observed in the free fraction of 25% HF. However, the digestion process negatively influenced their availability, limiting their absorption in the gut. This may be attributed to their chemical structure, as well as the interaction with dietary fiber within food matrix (Lippolis et al., 2023).

3.3 Sensory acceptability evaluation

Concerning the sensory acceptability assessment, untrained panelists evaluated attributes such as odour, taste, and overall acceptability. In detail, the taste scores showed no significant differences ($p<0.05$) among the three different levels of HF enrichment, showing values around 5 for all HF bread samples. However, all the HF bread breads samples obtained values significantly lower than HB0% (6.98 ± 1.51). In the free comments area, the assessors described a lingering bitter aftertaste in enriched bread samples. It is likely that the perceived bitter aftertaste negatively affected the overall flavour appreciation of the bread samples; however, the extent of dislike did not appear to be directly proportional to the level of HF inclusion. Regarding odour, no significant differences ($p<0.05$) were observed between HB0% and hemp-enriched bread samples. All samples scored values around 6 (5.75-6.46), thus indicating slightly positive odour attributes in all samples. The overall acceptability was the only parameter that proportionally significantly decreased with hemp flour increase in bread formulation. The higher score was assigned for HB0% (7.18 ± 1.42), followed by HB10% (5.87 ± 1.60), HB15% (5.68 ± 1.69) and HB25% (5.09 ± 1.94). Despite a decrease in the sensory characteristics in terms of taste and odour of bread added with hemp flour, based on the data obtained for the acceptability (scores close to 6), it can be concluded that products containing 10% and 15% hemp flour can be anyway accepted by consumers.

4. Conclusion and Future Perspectives

Hemp seed and then hemp flour, primarily regarded as a by-product of the hemp fiber supply chain, offers a sustainable and nutritious option for enriching bread. Incorporating varying amounts of HF into bread formulations allows the transfer of several key chemical components, such as essential fatty acids, proteins, phenolic compounds with potential antioxidant properties abundant in hemp seeds, and certain volatile organic compounds, into the final bread product. The inclusion level is pivotal to balance nutritional improvement and sensory effects. Our work allowed us to identify 10 and 15% as the optimal inclusion levels. In addition, in order to deepen the bioaccessibility of the phenolic compounds which HF is rich, the untargeted metabolomic analysis revealed that HF enrichment modifieds the chemical composition of bread, especially affecting polyphenolic compounds. Free phenolic compounds increased in a dose-dependent manner, with flavonoids being the predominant group, while bound phenolic compounds remained consistent across all levels of HF fortification. Furthermore, the *in vitro* digestion process showed a marked decrease in detectable polyphenols, indicating that their bioaccessibility may be limited due to interactions between fiber and polyphenols. Based on these findings, future studies could apply a fermentation step in hemp flour before bread-making to enhance the bioaccessibility of phenolic compounds. Microbial activity could help break down cell wall fiber and release more bound compounds.

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Electrochemical and Machine Learning Approaches for the Classification and Functional Assessment of Oenological Tannins During Wine Ageing

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This PhD thesis investigates the impact of enological tannins on the ageing process of red wines. Linear sweep voltammetry, coupled with multivariate statistical analysis and machine learning techniques, was utilised to predict dynamic changes in key wine parameters during maturation.

Il ruolo dei tannini enologici durante la maturazione del vino e la previsione del loro effetto tramite tecniche elettrochimiche

L'obiettivo della tesi di dottorato è quello di approfondire la conoscenza del ruolo dei tannini enologici sulla matuazione dei vini rossi. In questo senso, è stata utilizzata la voltammetria lineare insieme all'analisi multivariata e modelli di machine learning per prevedere l'evoluzione di diversi parametri del vino.

Keywords: Linear sweep voltammetry; oenological tannins; wine ageing; machine learning

1. Introduction

Wine ageing is a process in which the wine is stored in different containers to stabilise it and improve its characteristics. Traditionally, wine is aged in oak barrels, but this practice has some disadvantages, such as high cost, long storage time, sanitation problems, etc. (Tao et al., 2014). Thus, containers made of alternative materials are used, such as stainless steel, concrete, clay/ceramic, and glass, in the case of bottle ageing.
Regardless of the material, one central aspect of red wine ageing has to do with the degree of oxidation that the wine undergoes during the ageing process. In the case of red wine, oxidation chemistry involves reactions between different wine components, in particular tannins and anthocyanins. To enhance the outcomes of ageing, in particular in relation to colour stabilisation and resistance to oxidation, it is common practice to supplement wine with exogenous tannins, which are commercially available from different botanical sources (eg, oak, chestnut, gall, grape skin and seeds). Among the expected outcomes that are relevant to wine ageing, colour stabilisation, colour enhancement, and antioxidant protection are among the most sought by winemakers (Vignault et al., 2019). However, as the composition of exogenous tannins can vary depending on several factors (wood type and seasoning, grape variety, production method), the outcomes of tannin addition can be quite variable and, to a large extent, unpredictable, which complicates their use in vinification. Consequently, the availability of technologies that can assist winemakers in tailoring tannin use for the actual needs of wine is of great relevance for the wine industry.
The framework of this PhD project refers to the development of rapid analytical approaches that can be used for improved management of wine ageing. In the second year of work, great emphasis has been put on the development of tools related to tannin use in winemaking. As analytical techniques such as UV-Vis spectroscopy and HPLC require important investments in equipment and trained staff, so they remain restricted to laboratories, we concentrated our attention towards Electrochemical methods, which are well adapted to the rapid analysis of phenolic compounds in wine (Kilmartin, 2016). Accordingly, different avenues have been explored in relation to the potential of this technique in the context of tannin analysis, which are outlined below.

2. Materials and Methods

This study focused on commercial oenological tannins derived from three different botanical sources: oak (Quercus), grape seed (Vitis vinifera), and gall. These tannins were added to a red wine made from Corvina grapes at a final concentration of 0.5 g/L. A total of 45 commercial tannin samples were analysed for the classification experiments, with 15 samples from each botanical group. Electrochemical analyses were conducted using linear sweep voltammetry (LSV). The voltammograms obtained from each sample were processed to calculate their first and second derivatives, enhancing subtle differences in the electrochemical profiles associated with each type of tannin. The resulting data underwent multivariate statistical analysis, including principal component analysis

(PCA), to explore the potential for distinguishing among the different botanical origins. Additionally, a machine learning approach was utilised to classify the tannins. Synthetic voltammograms were generated using a Generative Adversarial Network (GAN) specifically trained to mirror the distribution of the real data. These synthetic data were smoothed, their derivatives calculated, and the expanded dataset was used to train several machine learning models, including Support Vector Machine (SVM), Random Forest (RF), Extreme Gradient Boosting (EGB), and Naïve Bayes Classifier (NBC). To examine the impact of exogenous tannins on wine oxidation and color evolution, a dedicated experiment was conducted. Red wine samples were treated with 25 mg/L of free sulfur dioxide (SO_2), 0.5 g/L of tannin, and 7.5 mg/L of oxygen. The samples were placed in 50 mL sealed vials with no headspace to prevent gas exchange. Oxygen consumption was monitored daily using a non-invasive, chemiluminescence-based method. Colour measurements were taken immediately after adding tannin, using a colorimeter operating in the CIELab colour space, and the ΔE parameter was calculated to quantify overall colour changes. Sulfur dioxide concentrations were determined using enzymatic methods. All statistical analyses and machine learning model training were performed using custom scripts in Python and with XLSTAT software (Addinsoft, France).

3. Results

As mentioned above, electrochemical methods have proved very useful for the analysis of phenolic compounds. This is why they were chosen to be used to investigate how the voltammetric profile of a wine change when tannins of different origins are added. Voltammetry offers a novel approach to analyse tannin behaviour by providing insights into their redox properties, making it a valuable tool for understanding enological tannin characteristics. Currently, this represents one of the first applications of voltammetry for studying tannins. Linear voltammograms show oxidation and reduction peaks that vary in height and position depending on the concentration and type of electroactive compounds. Derived voltammograms amplify subtle variations, making it easier to identify and characterize specific compounds. According to the current state of the art voltammetric analysis of wine phenolics shows distinct oxidation areas corresponding to different compounds. Around 300-400 mV, catechins and epicatechins oxidize. At approximately 470 mV, quercetin glycosides oxidize, followed by malvidin anthocyanins around 640 mV, a peak that diminishes in older wines, suggesting anthocyanin degradation. Above 700 mV, isolated phenols like vanillic and cinnamic acids, along with meta-diphenols, undergo oxidation. In Figure 1, in which we find the linear, 1st and 2nd derivative voltammograms of 3 different oenological tannins (oak, grape seed and gall tannins), we can see how, depending on the botanical from which the tannin is extracted, the voltammetric profile of the wine under investigation changes. From this assumption, it was decided to explore the ability of this technique to classify tannins based on their botanical origin.

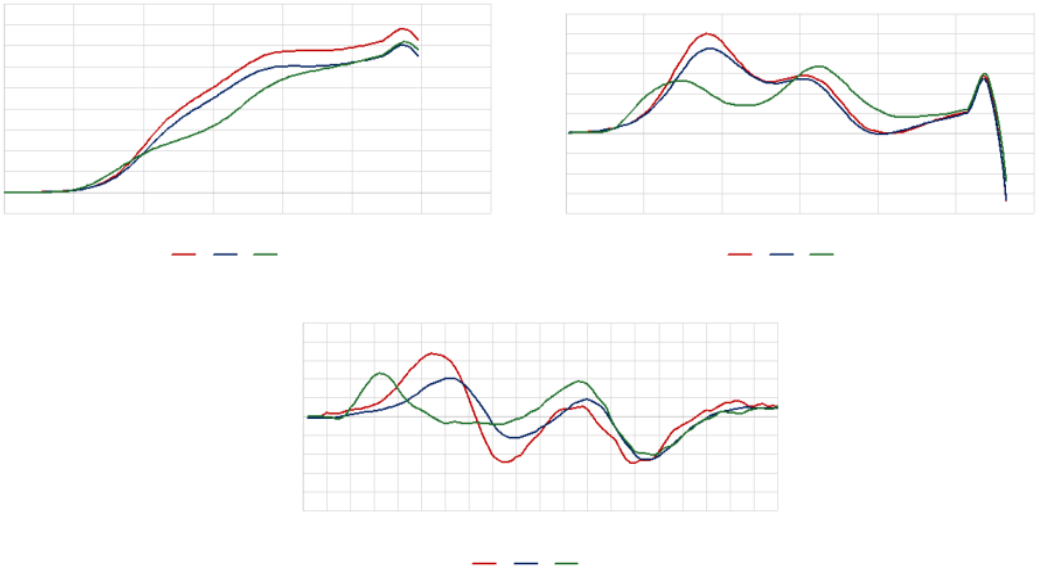


Figure 1. a), b) and c) represent the linear, the 1st derivative, and the 2nd derivative, respectively, voltammetric profile of a red wine to which different types of tannin have been added (Q=Oak; V = Grape seed; G = Gall)

Following these initial observations, a set of 45 samples of commercial tannins belonging to the three main botanical groups of enological relevance (Oak, grape, gall) were submitted to electrochemical analysis.

3.1 Application of linear sweep voltammetry for the botanical classification of oenological tannins

To classify the botanical origin of tannins using linear sweep voltammetry, a machine learning approach was employed. We initially collected voltammograms from 45 different tannins, each added at a concentration of 0.5 g/L into a red wine sample, consisting of 15 samples for each of the three botanicals: oak, grapevine, and gall. To train the machine learning (ML) models, we developed and implemented a Generative Adversarial Network (GAN) using a customized deep learning framework in Python. The GAN was specifically designed to generate synthetic voltammograms that replicate the distribution of the real data in our dataset. Using the GAN, we created 30 'new' voltammograms for each botanical. The synthetic data underwent smoothing, and both the first and second derivatives were calculated, as illustrated in the flowchart in Figure 3. From this enriched dataset, we trained several ML models, including Support Vector Machine (SVM), Classification and Regression Random Forest (RDF), Extreme Gradient Boosting (EGB), and Naïve Bayes Classifier (NBC). Among these models, the SVM exhibited the most impressive performance, achieving an accuracy of 0.992 for the training set and 0.967 for the validation set. Significantly, it also reached 100% accuracy with previously unseen data, demonstrating that this approach could be highly effective for the rapid analysis of the botanical origin of the tannins under investigation.

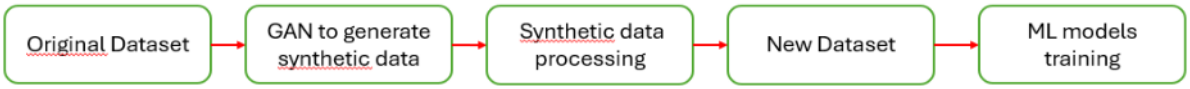


Figure 3. Multivariate statistical approach Flowchart

3.2 Color evolution, oxygen consumption rate (OCR) and sulphur dioxide (SO_2) consumption of wine added with different oenological tannins.

As previously discussed, the ageing process involves a complex network of oxidative reactions that engage both native and exogenous tannins. To assess the capacity of exogenous tannins to modulate these reactions, a dedicated experiment was conducted. A red wine was treated with 25 mg/L of free SO2, 0.5 g/L of tannin, and 7.5 mg/L of oxygen, all placed in sealed 50 mL vials to eliminate any headspace. Wine was allowed to react with oxygen, and several relevant parameters were measured, including the decline of SO2 and colour evolution. Voltammograms recorded at the start of the incubation process (T0) were employed to model the progression of these parameters. Daily monitoring of oxygen levels was performed using a non-invasive method based on chemiluminescence. Colour analysis was carried out using a colourimeter functioning in the CIELab colour space immediately after the addition of each tannin, with the derived parameter ΔE utilised to assess overall colour changes. Sulfur dioxide levels were measured through enzymatic methods.

A Kruskal-Wallis test was performed to compare the average ΔE change immediately after addition among the different tannin groups. The parameter ΔE represents the distance between two colors in the CIELab color space, providing a numerical measure of perceived color difference (with ΔE values interpreted as follows: ≤ 1.0 = not perceptible by human eyes; 1-2 = perceptible through close observation; 2-10 = perceptible at a glance; 11-49 = colors more similar than opposite; 100 = colors exactly opposite). The results showed that oak tannins produced the greatest increase in ΔE, followed by grape seed tannins and then gall tannins. These differences were statistically significant, indicating that the three groups differ from each other.

To elaborate data about OCR, it was decided to use the oxygen half-life (T1/2). This is because this parameter has been found to have a better correlation with voltammograms than mg/L of oxygen consumed per hour. A Kruskal-Wallis test was conducted to compare oxygen consumption among different tannin groups. The results showed that oak tannins consumed oxygen the fastest, followed by grape seed tannins, while gall tannins exhibited the slowest consumption rate. To understand whether the oxygen half-life can be predicted from the wine voltammetric profile, a multivariate approach was used, specifically, using a PLS-R an R^2 of 0.84 was obtained. This result is encouraging as it tells us that voltammetry could be useful in understanding whether a wine will consume oxygen faster or slower. About SO_2 consumption, the ratio of free sulphur dioxide to consumed oxygen was calculated; it was observed that the group of tannins that cause oxidation to lead to lower consumption of this important compound for wine shelf-life, are those from oak, then we find those of grape seed, and finally those of gall. Again, several machine learning algorithms were tested; however, their performance was not satisfactory. In fact, in many cases, there were overfitting problems, suggesting that in the future, the database should be larger.

In conclusion, it has been shown that linear sweep voltammetry approached with machine learning can be used for botanical classification of oenological tannins, avoiding more laborious and costly analyses. Commercial

oenological tannins, depending on their botanical origin, have very different properties in terms of their influence on colour, oxygen consumption and sulfur dioxide consumption.

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Analysis of volatilome of virgin olive oils and flavored oils: quality grade evaluation and study of modification during storage

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This PhD thesis focused on the development of chemometric models for estimating the commercial category of virgin olive oils. The aim was to support sensory analysis and improve the efficiency of quality control in an industrial setting. In parallel, the volatilome of a selected set of virgin olive oil samples was monitored during storage to identify the markers associated with their downgrading. Furthermore, the volatile profiles of flavored oils were characterized to evaluate the specific compounds linked to the different flavoring agents employed.

Analisi del volatiloma di oli vergini di oliva e oli aromatizzati: valutazione della qualità e studio delle modifiche durante la conservazione

La presente tesi di dottorato si è concentrata sullo sviluppo di modelli chemiometrici per la stima della categoria commerciale degli oli vergini di oliva, con l'obiettivo di supportare l'analisi sensoriale e migliorare l'efficienza del controllo qualità in ambito industriale. Parallelamente, è stato monitorato il volatiloma di un set selezionato di campioni durante la conservazione, al fine di individuare i marcatori associati all'eventuale declassamento del prodotto. Inoltre, sono stati caratterizzati i profili in composti volatili di oli aromatizzati, con l'obiettivo di identificare i traccianti specifici legati ai diversi agenti aromatizzanti impiegati.

Key words: volatile compounds, chemometric models, shelf life, flavored oils.

1. Introduction

In accordance with the PhD thesis project previously described, this oral communication reports the main results of the following three activities directed to:

A1) creation of sensory and instrumental datasets for developing chemometric models to predict VOOs commercial category to support sensory assessment and inter-laboratory trial;
A2) evaluation of the sensory characteristics and volatile profiles of selected samples (EV and V) monitored during a storage period of 12 months by SPME-GC-MS;
A3) identification and quantification of volatile profile in flavored oils.

2. Analysis of volatilome

In the European Union, VOOs are classified into 3 different commercial categories; extra virgin (EV), virgin (V), and lampante (LO), based on both physicochemical and sensory parameters (Reg. EU 2022/2104). The official sensory evaluation method (Panel test), although widely used, is time-consuming and may have poor reproducibility if assessors are not adequately trained (Barbieri et al., 2020). Therefore, the identification and quantification of volatile organic compounds (VOCs) in VOOs is of great importance to assess their quality. In fact, some VOCs have been proposed as markers to detect positive (e.g., fruity) and negative sensory attributes (defects) according to their concentrations (Valli et al., 2020) and targeted and untargeted instrumental methods based on the analysis of these molecules can be considered an interesting tool to support the sensory analysis (Panel test) (Quintanilla-Casas et al., 2020). . Techniques such as headspace solid-phase microextraction (SPME) combined with gas chromatography–mass spectrometry (GC-MS) are increasingly used for quality assessment (Aparicio-Ruiz et al., 2022). Additionally, rapid instrumental techniques like Headspace Gas Chromatography–Ion Mobility Spectrometry (HS-GC-IMS), Flash Gas Chromatography (FGC) have proven effective for fast screening and pre-classification of olive oil samples, enhancing the efficiency of quality control processes (Barbieri et al., 2020). Shelf-life evaluation is also critically linked to the chemical and sensory evolution of olive oils over time. Oxidative degradation, driven by factors such as oxygen exposure, temperature, light and antioxidant or pro-oxidant content, leads to the formation of neo-formed volatile compounds associated with off-flavours and sensory downgrading (Martin-Torres et al., 2023). Analytical tools such as GC-IMS, FGC, and particularly SPME-GC-MS are widely employed to monitor changes in VOC profiles during storage and to identify specific oxidation markers (Freitas et al., 2024). In the context of flavored olive oils, the same targeted and screening approaches combined to the sensory analyses are also essential to evaluate the embedding of flavoring molecules from different matrices (herbs, spices, fruits, etc.) and to assess product stability over time (Lamas et al., 2022).

3. Experimental Procedure

In this PhD thesis the following experimental procedure was set up. The virgin olive oil (VOO) samples were sensory evaluated by five different panels and robustly classified, applying a decision tree previously set-up and published. In parallel, headspace volatile compounds of VOOs were analysed by the HS-CG-IMS technique in five different labs involved in the trial, using the same type of equipment. The results were collected in a dataset that will be used to develop the predictive models for rapid screening of VOOs, particularly useful in cases of borderline (BL) samples classified between virgin (V) and extra virgin (EV) commercial categories. Finally, the volatilome of selected samples was analyzed by SPME-GC-MS technique to assess potential qualitative and quantitative changes over time. The same techniques were also applied to analyse a specific set of flavored oils by Olitalia s.r.l., (the co-founding company of this project) to identify and quantify unique volatile markers for establishing an internal quality control system.

4. Materials and Methods

The 154 samples collected during the two-years and a half research period were provided by olive oil companies (Federolio, Federazione Nazionale del Commercio Oleario). These VOO samples were from three different olive oil campaigns (from 2021 to 2025) and information related to cultivars, geographical origins, and commercial classifications was collected. The official procedure for sensory analysis (Panel test) was conducted by five different panels involved in the research project: one official and four professional committees. In addition, a decision tree approach based on agreement among the panels on commercial category and main perceived defect, as described by Barbieri et al., in 2020, was applied. For this reason, the sensory results were reported as the means of medians. The headspace volatile analysis of VOOs was conducted using the GC-IMS Flavourspec® and the instrumental setting used was the same as previously described in the work conducted by Valli et al., in 2020 study with some modifications. At the same time, the shelf-life evaluation on 22 VOOs (samples stored in tins at room temperature for 12 months) was carried out by SPME-GC-MS technique, following the preparation step and chromatographic conditions described in Aparicio et al., 2022. The tentative identification of volatile compounds was performed by MS comparing the spectrum fragmentation of each analyte with the reference spectra reported in the NIST library (2008 version). The headspace fraction of six different flavored olive oil samples was studied using both HS-GC-IMS and SPME-GC-MS techniques. Instrumental parameters were consistent with those described above, and data processing followed the methodologies reported by Valli et al., 2020 and Aparicio-Ruiz et al., 2022, with some modifications.

5. Results and Discussion

5.1 Chemometrics models to estimate VOOs commercial categories

The 154 samples were analysed to create a robust sensory and instrumental dataset to build chemometric models. For this purpose, all samples were evaluated by five different sensory panels and classified according to a decision tree, based on the agreement among the panels regarding the commercial category and the main perceived defect (Barbieri et al., 2020). The sensory evaluation resulted in the following classifications: 27 samples as EV, 80 as V, 40 as borderline (BL) (classified between EV and V), and 7 as L. According to the decision tree application, samples classified by the panel as BL, either of the first type (disagreement on the commercial category) or the second type (disagreement on the most perceived defect) were subjected to a blind formative re-assessment. In addition to sensory analysis, the profile in VOCs of the same oils was analysed using HS-GC-IMS technique in five different laboratories equipped with the same type of instrument. Raw data from HS-GC-IMS analysis came from 3D chromatograms (heatmaps) characterized by GC retention time (seconds), IMS drift time (milliseconds), and ion current intensity (millivolt). From the analysis of 15 preselected standard volatile compounds, 3D spectral intensity regions were identified using Vocal software. These volatile molecules are known to be markers of specific sensory attributes of VOOs, both positive (e.g. fruity) and negative, such as the main defects (e.g. fusty-muddy, rancid) used to classify the samples. The intensity values of VOCs, obtained through HS-GC-IMS analysis at the University of Bologna (UniBo) laboratory, were elaborated by four PLS-DA models previously built (EV vs noEV; L vs noL; L vs V; EV vs V), as described by Valli et al. (2020). Through the combination of probability data obtained from the application of the four chemometric models, the 154 analysed samples were estimated. In total, 88 samples were predicted as V, 21 as IN (inconsistent), 41 as EV, and 4 as L. Of particular interest are IN samples, so named because models are unable to assign a unique commercial category based on the simultaneous evaluation of the generated probabilities. Until now, 78% of the analysed samples have been correctly classified according to sensory classification. Moreover, based on the data obtained through HS-GC-IMS analyses conducted by the five laboratories involved in the study, an inter-laboratory study was carried out to assess the reproducibility of the results. Data from the UniBo laboratory were excluded due to noticeable differences compared to the other laboratories. These differences are likely attributable to the use of an older-generation HS-GC-IMS instrument characterized by lower sensitivity than the more advanced version used by the other laboratories. Analysis of monomer and dimer signal intensities showed that one of the four laboratories reported higher values for some

VOCs. This may be due to variations in the purity of the nitrogen used as carrier gas. However, by the analysis of the second principal component (PC2), the samples formed distinct clusters based on the laboratory but followed a similar overall trend. This suggests that, despite instrumental variations, the systems can extract comparable information, demonstrating overall consistency in analytical performance across laboratories. Moreover, to investigate the level of agreement among laboratories, two preliminary PLS-DA models for each laboratory (EV *vs* noEV and V *vs* noV) were developed using the dataset of 154 samples. Model significance was assessed using permutation testing. The EV *vs* noEV models were found to be non-significant, probably due to the low number of EV samples (27) compared to V (80) samples within the overall dataset. This limited EV number affects the significance of all the models, regardless of the laboratory in which the analysis was performed. However, both predictive models showed good agreement among the four laboratories. Considering the dataset built for each laboratory, for the models Ev *vs* noEv 85% of the samples were classified in the same way among 4 laboratories while 97% from at least 3 laboratories. Instead for the models V *vs* noV 84% of the samples were classified in the same way among 4 laboratories while 96% of at least 3 laboratories. An alternative strategy explored was the calibration of PLS-DA models using a combined dataset comprising all laboratories (23 variables × 154 samples × 4 laboratories). In this approach, the models provide an estimate of the commercial category based on the data from a specific laboratory while taking into account the overall dataset. This strategy resulted in statistically significant models, as well as improved agreement percentages among the laboratories. Infact, for the models Ev *vs* noEv 91% of the samples were classified in the same way among 4 laboratories while, 99% from at least 3 laboratories. Instead, for the models V *vs* noV 89% of the samples were classified in the same way among 4 laboratories while 98% of at least 3 laboratories.

5.2 Virgin olive oils shelf-life study

The volatile profile of 22 virgin olive oil samples was monitored over a 12-month storage period using an integrated analytical approach that combined SPME-GC-MS, HS-GC-IMS and sensory evaluation (Panel test). Data obtained from SPME-GC-MS, focusing on 18 selected volatile markers, were analysed with the sensory results using principal component analysis (PCA) to investigate the evolution in volatile profile over time. The PCA biplot shown in Figure 1 reveals a distribution of samples according to their commercial classification, and also a considerable separation between fresh and stored samples along the PC1. The sensory samples classify as EV, highlighted in green, are primarily placed in the area of the plot characterised by negative values for both PC1 and PC2. This area is associated with the positive sensory attribute 'fruity' and with volatile compounds commonly linked to 'freshness' (formed by enzymatic LOX pathway). In contrast, samples classified as V, particularly those analysed at the end of the storage period (T12), clustered in the fourth quadrant of the biplot. This region is associated with the sensory defect 'rancid' and volatile markers resulting from oxidative degradation. Finally, a cluster of V samples is observed in the upper right area of the biplot (positive values for both PC1 and PC2), mainly characterised by the fusty-muddy sediment sensory attribute. This defect is typically associated with anomalous fermentation and degradation processes, as evidenced by the presence of specific volatile compounds (e.g. ethyl acetate, ethanol, 3-methyl-1-butanol). These results were corroborated by PCA performed on HS-GC-IMS data combined with sensory analysis. In addition, predictive chemometric models based on HS-GC-IMS data further supported these results.

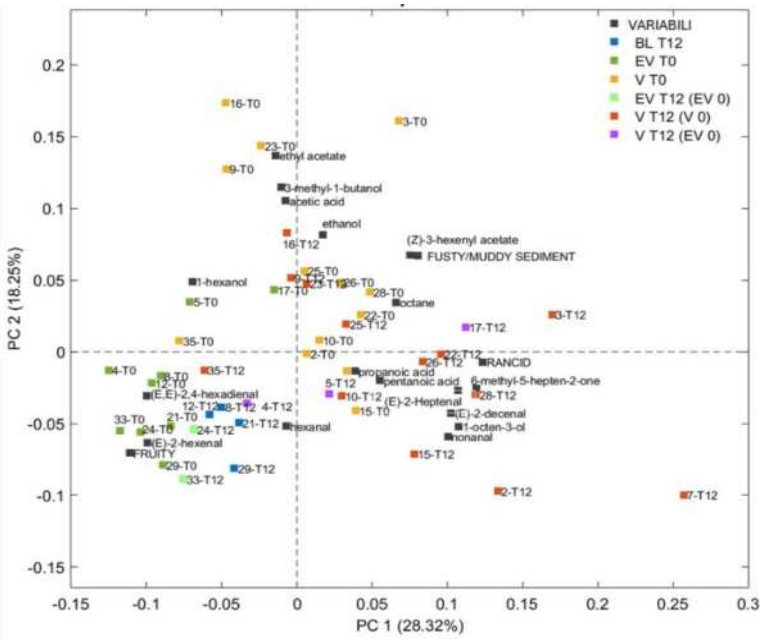


Figure 1 *Score plot resulting from PCA of the concentrations of 18 volatile markers analyzed using SPME-GC-MS and sensory results by Panel test in olive oils stored at zero (T0), and 12 months (T12). The samples are color-coded based on their initial commercial category at time zero and their evolution after twelve months of storage*

5.3 Analysis of Volatile Compounds in Flavored Oils Using SPME-GC-MS and HS-GC-IMS

The analysis of volatile compounds was carried out by SPME-GC-MS and HS-GC-IMS. This study enabled both qualitative and quantitative characterization of the volatile profiles of oils flavored with basil, rosemary, oregano, chili pepper, and truffle, highlighting specific markers and significant differences among the various aromatic matrices.

Specifically, the rosemary flavored oil, was characterized by monoterpenes such as *eucalyptol, fenchene, β-pinene, m-myrcene*, and *α-pinene*, associated with balsamic and resinous notes. Among these, f*enchene* was a marker detected exclusively in the rosemary flavored oil, while *eucalyptol*, also detected in basil and oregano flavored oils, was present at significantly higher levels in the rosemary sample. The chili pepper oil showed a volatile profile influenced by the base oil composition, as well as compounds derived from lipid oxidation. However, basil and oregano oils were distinguished by their higher content of terpenic compounds. The basil flavored oil was characterized by specific compounds such as *anethole* and *linalyl acetate*, both contributing to balsamic notes, as well as *ocimene* and *terpinen-4-ol*, which were unique to this sample. While the *β-Linalool* compound, which was also found in the oregano oil, was present in significantly higher concentrations in the basil oil. The oregano flavored oil showed characteristic markers such as *4,8-dimethyl-nonadiene* and *o-cymene*, while *γ-terpinene*, which is also present in basil oil, was detected in significantly greater amounts in the oregano sample. In contrast to the herb-based oils, the truffle and lemon flavored oils displayed a different volatile profile. The first characterised especially by sulfur compounds such as *2,4-dithiapentane*, *dimethyl disulfide*, and *methyl mercaptan as well as nonanal and 1-octen-3-ol*. These compounds are responsible for sulfurous, earthy, and mushroom aromas, which were not detected in the other samples and are characteristic of truffle flavored oil. In contrast, the volatile profile of lemon flavoured oil is characterized by the presence of compounds typical of citrus fruits, which can be classified into distinct chemical classes. Among these, terpenes (e.g., *DL-limonene, citral*) and esters (e.g., *neryl acetate*) play a central role in providing the fresh, citrus like aroma characteristic of lemon. In parallel, the same samples were also analyzed using HS-GC-IMS to obtain a volatile fingerprint. The resulting Heat maps obtained by HS-GC-IMS revealed characteristic monomer and dimer signals within chromatographic regions between 450 and 1400 seconds. These distinct patterns enabled clear visual differentiation of the flavored oils and identification of key spots associated with each flavoring as shown in Figure 2.

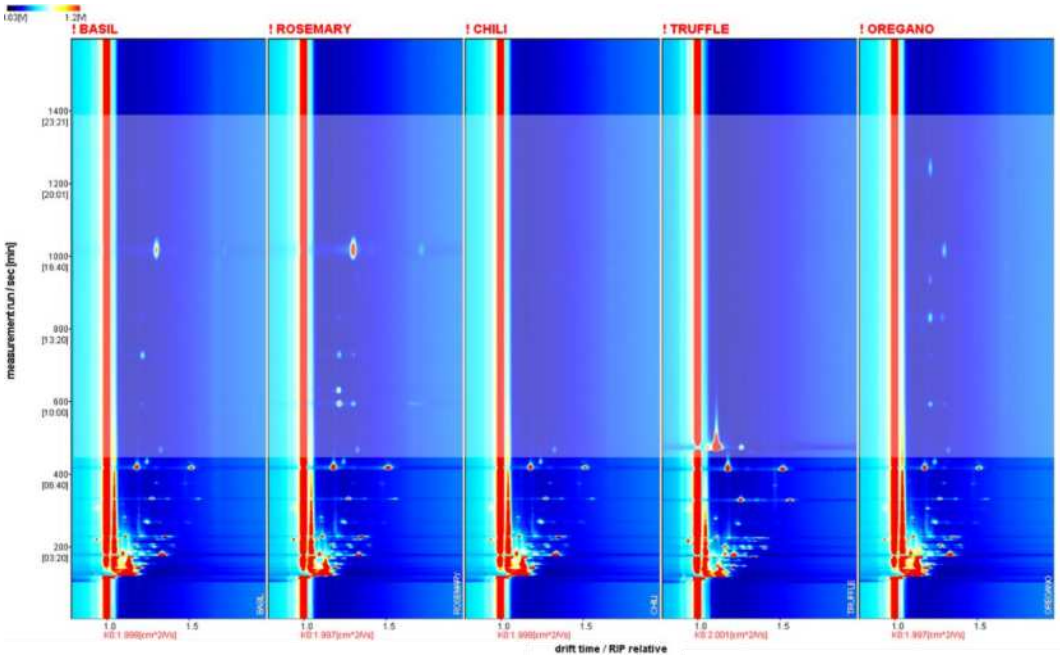


Figure 2 *Heat map comparison of basil, rosemary, chili pepper, truffle, and oregano flavored oils obtained by HS-GC-IMS analysis. The highlighted region indicates the area with the greatest differentiation in volatile compound profiles among the samples.*

6. Conclusions and Future Perspectives

The study on the application of chemometric models showed that this approach is effective to support the Panel Test. In fact, the inter-laboratory analysis revealed a high degree of consistency in the results obtained by the laboratories involved in the study, with a satisfactory level of agreement between the predictive models (EV vs. no EV; V vs. no V), regardless of the development strategy used. These results confirm the potential of HS-GC-

IMS and its associated predictive models in both the industrial sector and for official control bodies. The technique could contribute to increasing the number of samples analysed due to its rapid execution and analytical standardization. Concerning the shelf-life study, multivariate analysis (PCA) applied to data obtained from SPME-GC-MS and HS-GC-IMS enabled the discrimination of samples according to their qualitative evolution over time. Specifically, a progressive reduction in volatile markers associated with fruity notes, and an increase of markers related to defects (e.g. rancid and fusty/muddy) was observed. These results confirm the effectiveness of an integrated approach combining sensory evaluation with targeted and screening analytical techniques for monitoring qualitative changes in olive oil during storage. This approach could be a useful tool for more accurately determining the 'best before' date to be indicated on the label. As a future perspective, the shelf-life study should be expanded to include a larger and more representative number of samples, ensuring a balanced distribution between extra virgin (EV) and virgin (V) olive oil categories. Furthermore, the dataset should be enriched with complementary analyses and additional relevant variables, such as the determination of the phenolic fraction, in order to enable a more comprehensive and accurate assessment of the risk of product downgrading over time. Finally, the study on flavored oils demonstrated that the combined application of targeted and screening analytical approaches is effective in characterizing their volatile profiles, allowing the determination of specific volatile markers associated with each type of flavoring. The results are promising for developing internal quality control systems within production settings.

7. Nomenclature

VOO, Virgin Olive Oil; EV, Extra Virgin; V, Virgin; L, Lampante; BL, Borderline; IN, Inconsistent, EU, European Union; LOX, Lipoxygenases; VOC, Volatile Compound, HS-GC-IMS, Headspace Gas Chromatography–Ion mobility spectrometry; FGC, Flash Gas Chromatography; SPME-GC–MS, Solid-Phase Microextraction-Gas Chromatography–Mass Spectrometry; PCA, Principal Component Analysis; PLS-DA, Partial least squares discriminant analysis.

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Identification and quantification of microplastics in the clam *Chamelea gallina* using different analytical techniques

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This PhD thesis concerned the development of protocols for the extraction and analysis of microplastic content in the clam *Chamelea gallina* by two different types of analytical techniques, Raman microspectroscopy (μRaman) and analytical pyrolysis coupled with gas chromatography/mass spectrometry (Py-GC/MS). The development and characteristics of the two protocols were documented and discussed, and the analytical procedures were applied to the analysis of samples of clams collected in the Adriatic Sea for the characterization of microplastics content in this mollusc.

Identificazione e quantificazione di microplastiche nella vongola *Chamelea gallina* mediante l'utilizzo di diverse tecniche analitiche

Questa tesi di dottorato ha riguardato lo sviluppo di protocolli per l'estrazione e l'analisi del contenuto microplastiche nella vongola *Chamelea gallina* con due diverse tipologie di tecniche analitiche, la microspettroscopia Raman (μRaman) e la pirolisi analitica accoppiata con gascromatografia/spettrometria di massa (Py-GC/MS). Lo sviluppo e le caratteristiche dei due protocolli sono stati documentati e discussi e le procedure analitiche sono state applicate all'analisi di campioni di vongole raccolti nel Mare Adriatico per la caratterizzazione del contenuto di microplastiche in questo mollusco.

Keywords: *Chamelea gallina*, microplastics, μRaman, Py-GC/MS, Adriatic Sea.

1. Introduction

Following the PhD thesis project previously described (Aloia, 2023), here are the main results of the following four activities related to:

(A1) the sampling plan of the *Chamelea gallina* clams on the coast of the Adriatic Sea, with the definition of the collection points and the number of samples to obtain a representative amount of data.
(A2) the development of an analytical protocol for the extraction of MPs from clam *C. gallina* and the optimization of the instrumental conditions for the characterization of polymeric microparticles with μRaman.
(A3) the development of an analytical protocol for the extraction of MPs from clam *C. gallina* and optimization of the instrumental conditions for Py-GC/MS.
(A4) the definition of the level of contamination found in the clam *C. gallina* samples and comparison between the two analytical techniques.

2. Microplastic contamination in Food and Beverages

MPs contamination has become ubiquitous, and these emerging contaminants are becoming increasingly of interest not only for their damaging effects on the ecosystem but also for their potential toxicity to living organisms and humans. One of the main routes of exposure to MPs is through food and drink regularly consumed, which could be potentially contaminated. However, contamination data for a certain variety of foods is still insufficient in literature, and the lack of validated analytical protocols for complex food matrices does not make available a set of data that could be useful for an exposure risk assessment.

Raman and Py-GC/MS are among the most widely used analytical techniques for MPs analysis, capable of providing complementary data. The first one gives information on the polymeric nature as well as the morphology of MPs, whereas the second, being a destructive technique, does not provide physical information, and the characterization is returned in terms of concentration by weight with a higher sensitivity than spectroscopic techniques.

Raman spectroscopy has been used over the last decade to develop protocols for the analysis of MPs in food and beverages with a particular focus on fish and shellfish due to their strong link to the marine ecosystem, one of the most contaminated by plastic waste. The evaluation of data in the literature for this analytical technique has shown that the lack of specific standard protocols on sample preparation and data presentation makes it difficult to compare and correlate the contamination data that are currently available (Aloia et al., in press).

Figure 1 reports the number of articles dedicated to each food category over the last 10 years which have involved applications of Raman spectroscopy in the development of protocols for the extraction and analysis of MPs.

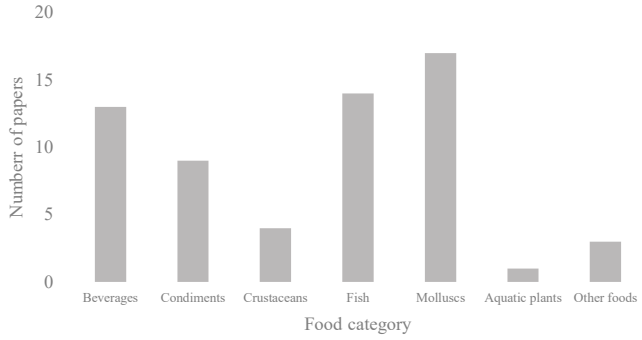


Figure 1 *Number of papers related to MPs occurrence in food and beverages between 2014-2024, employing Raman spectroscopy, divided by food category.*

3. Materials and Methods

3.1 Analytical protocol for the extraction and µRaman analysis of MPs

In this PhD thesis, a protocol for the extraction of MP content in *C. gallina* clam samples was developed for µRaman, adapting protocols already present in the literature for other matrices. The protocol consists of 4 steps: 1) alkaline digestion (KOH 10% w/w) (Dehaut, 2016); 2) gravimetric separation; 3) second separation by addition of a 50% w/w KI solution (Phuong, 2018); 4) filtration on glass fiber filters (Whatman GF/A, 1.6 µm porosity). To verify the level of contamination in the environment and reagents, two blanks (procedural and environmental) were prepared for each sample. In addition, the recovery of the procedure has been verified through recovery tests carried out on the 3 reference materials at different dimensional ranges (6 µm, 160 µm, 3 mm). The extraction protocol is described in detail in Aloia R (2024).

Visual counting and morphological sorting of the microparticles were carried out by Stereomicroscope (Leica M205C) and chemical characterization with µRaman (DXR3 Thermo Fisher Scientific), equipped with a 532 nm laser and 3 objectives (10x, 20x, 50x). The filters were scanned with a magnification of 10x and 20x, while the spectra were acquired with a magnification of 50x and in the range between 50-4000 cm^{-1}. The chemical attributions were carried out by comparing the spectrum obtained with instrumental libraries and with the available literature.

3.2. Protocol development for the analysis with analytical pyrolysis (Py-GC/MS)

A second protocol has been developed for the extraction and analysis of MPs with Py-GC/MS. A chemical/enzymatic clean-up procedure was chosen, based on the protocols found in the literature for other types of matrices. The enzymatic approach is performed according to Brits et al. (2024), with some modifications. 10 g of clams are treated with a mixture of TRIS buffer/SDS and incubated for 1 hour at 60°C in a water bath under stirring. The sample is cooled down and Proteinase-K with 50 mM $CaCl_2$ solution is added. After incubation at 50°C for two days, a Kreon™ capsule containing a mixture of amylase/lipase/protease is added to the sample and incubated overnight. The sample is filtered on a stainless-steel filter with a porosity of 10 µm. The filter is treated with Fenton reagent, and the solution obtained is filtered through a 15 mm diameter glass fiber filter of 1 µm porosity, then washed with petroleum ether before being placed in a petri dish. The filter obtained is placed in a cup for pyrolysis, and a solution of TMAH is added before analysis.

4. Results and Discussion

4.1 Optimization of the protocol for µRaman analysis

The adaptation of the protocol for µRaman analysis required the selection of the type of filter with the lowest background Raman signal and the best readability. The filter evaluation methodology and related score system is illustrated in Table 1. A full description of this methodology is reported in Aloia (2024). The filter with the best rating is the glass fiber (GF), then used for the preparation of 34 samples of clams.

4.2 Instrumental strategy for µRaman analysis of MPs

The analysis of 34 samples of clams was carried out with µRaman using two different strategies:

A) "Combined" approach: The sample filters were first read at the Stereomicroscope to detect suspicious microparticles, considering the contamination in both procedural and environmental blanks. These suspected MPs were characterized by µRaman to confirm or not their polymeric nature.

B) "µRaman only" approach: The filters related to the samples were fully read by µRaman, and all suspicious particles were characterized for MPs. The two blanks were read in the same way and the contamination of the procedural blank (related to the contamination of the reagents and the laboratory) was used as a threshold value to determine the positivity of a sample, for polymer (PE, PS, etc.) and dimensional range (10-19 µm, 20-49 µm, etc.)

Table 1 *Filters and respective scores for each parameter considered in the optimization of the analytical protocol for µRaman*

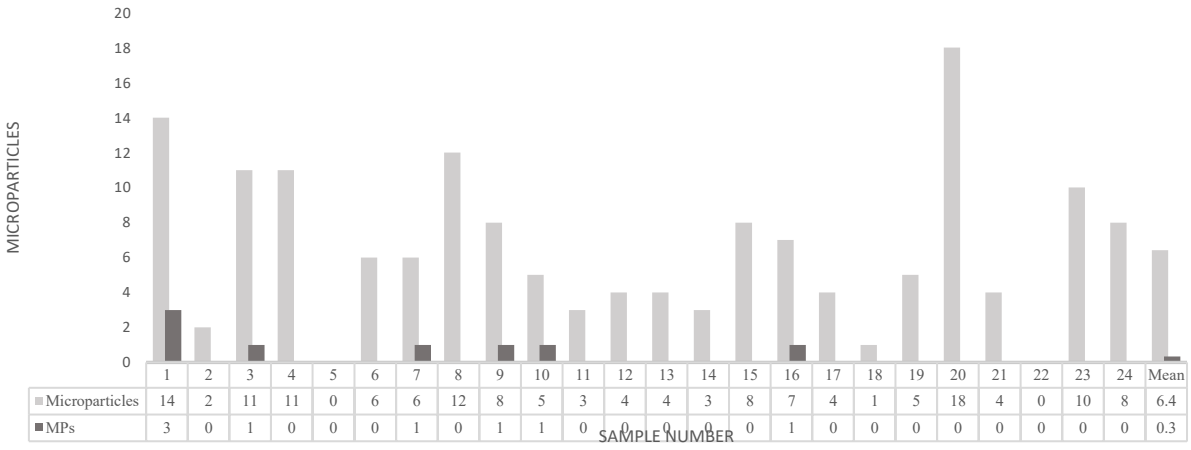
Parameters/Filters	Polycarbonate membrane (PC)	Silicon microperforated membrane (Si)	Glass fiber filter (GF)
Number of filters for each sample	►	▼	▲
Size of filters	►	▲	►
Readability	▲	►	►
Interfering Raman signal	▼	▲	▲
Cost of filters	▲	▼	▲
Recovery rates of standard materials*	▲	▼	►

Score legend: Optimal (▲); Acceptable (►); Problematic (▼).
*The recovery was evaluated from the stereomicroscope reading

Although the B approach allows to characterize all the microparticles identified as suspects, with a greater probability of detecting MPs, it is extremely time-consuming, unlike the A approach which is based on a preselection of the particles to be characterized. The two methods gave different results compared to the number of MPs found on average in the samples.

4.3 MPs content in clam *C. gallina* obtained from the application of µRaman

The first 24 samples were analysed using the combined approach. Figure 2 shows the number of microparticles selected for each sample from the stereomicroscope reading and the MPs found. A total of 154 microparticles and 9 MPs were analysed including 3 PP, 2 PS, 2 PET and 2 PE with an average of 0.3 MPs per sample. As a whole, 47% of the microparticles analysed were constituted by organic matter or laser-burned, and therefore not polymeric. In addition, 14% of the microparticles characterized showed no activity to µRaman while 13% is attributable to a mineral nature with quartz and calcium carbonate detected in most cases.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Mean
Microparticles	14	2	11	11	0	6	6	12	8	5	3	4	4	3	8	7	4	1	5	18	4	0	10	8	6.4
MPs	3	0	1	0	0	0	1	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0.3

Figure 2 *Microparticles characterized and content of MPs for the first 24 samples of clam* C. gallina.

The last 10 samples were analysed with the "µRaman only" approach. A total of 121 MPs were found, considering the cut-off values given by the average of procedural blanks. Among the types of polymers, PE and PS were found to be the most frequently encountered for a total of 71 and 35 particles, respectively, and accounting for 88% of all MPs found in the total of samples (Figure 3a, refer to the Nomenclature section for indications of polymer acronyms). Figure 3b shows the content of MPs found for each sample, with an average of 12.1 MPs per sample. This approach was found to be more effective in the search for MPs, if we compare the average value obtained for the two sets of samples (0.3 against 12.1 MPs on average per sample) but is particularly time consuming and therefore applied only to a small fraction of the total samples.
Both approaches allow detection of MPs and are mentioned in the literature depending on the expected particle content, selecting a portion of the filter to be analysed to reduce reading times, although the choice of test portions

may also be affected by the uneven distribution of particles on the filter surface. Finally, the results obtained in this project show how the estimation of the content of MPs is more reliable using only µRaman for microparticle selection.
Gedik and Gozler (2022) analysed the same clam species collected in the Black Sea. Both the average size and the type of polymers found are different, with the prevalence of PET (34%), PE (31%) and PP (20%). This can be explained by the different geographical points of mollusc collection.

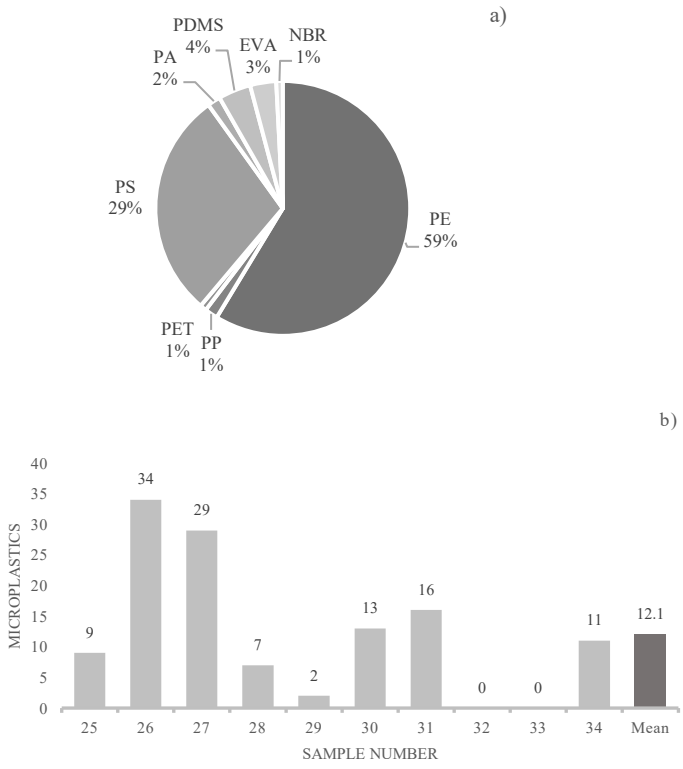


Figure 3 *Typology of polymers found in the last 10 samples of clam* C. gallina *(a) and content of MPs found in each sample (b).*

4.4 Application of analytical pyrolysis to MPs analysis

The protocol developed for analytical pyrolysis made it possible to analyse the content of MPs with dimensions greater than 1 µm present in the *C. gallina* clam. The weight of 10 g has been maintained, as preliminary work on protocol development has shown that some polymers are close to the lower limit of the calibration curve. The matrix is first treated by digestion using several enzymes, while the residual material is further treated with the Fenton reagent, an advanced chemical treatment suitable for destroying residual organic material. In addition, filter washing with different aliquots of petroleum is necessary to reduce the content of lipids and eliminate paraffins that can undermine the identification of polymers, and may overlap PE marker signals (Halbach et al., 2022). Polymers were identified by defining the specific markers for each polymer cluster in the presence of the TMAH derivative agent (Fischer and Scholz-Böttcher, 2017).

5. Conclusions and Future Perspectives

This PhD thesis has allowed us to know the levels of contamination MPs for the clam *C. gallina* collected in the Adriatic Sea and the development of protocols with two different analytical techniques and for which up to date there was no available data in the literature. From the methodological point of view, the results obtained by µRaman using two different instrumental approaches highlighted the significant impact of the analytical strategy adopted on the estimation of MPs content. An additional protocol consisting of a clean-up procedure with several steps has been developed for the Py-GC/MS analysis. The developed protocol that includes a chemical/enzymatic type treatment has been determined as the most suitable for dealing with interferences for analytical pyrolysis. In the future, the analysis of 34 samples of *C. gallina* clam will be performed using the protocol developed for analytical pyrolysis, to obtain two sets of data to be compared.

6. Nomenclature

MPs: microplastics; µRaman: Raman microspectroscopy; Py-GC/MS: analytical pyrolysis coupled with gas chromatography/mass spectrometry; KOH: potassium hydroxide; KI: potassium iodide; TRIS buffer: tris(hydroxymethyl)aminomethane; SDS: sodium dodecyl sulfate; $CaCl_2$: Calcium chloride; TMAH: Tetramethylammonium hydroxide; PE: polyethylene; PS: polystyrene; PP: polypropylene; PET: polyethylene terephthalate; PDMS: Polydimethylsiloxane; PA: polyamide; EVA: Ethylene-vinyl acetate; NBR: acrylonitrile-butadiene rubber.

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Designing advanced materials and self-contained platforms for analytical applications in food analysis

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This PhD thesis focuses on developing smart (bio)analytical devices using plastics and cellulosic materials, combined with eco-friendly benchtop fabrication techniques and 3D printing. Functional nanomaterials will be integrated to enhance device performance and ensure effective interaction with biological components. These portable, miniaturized platforms will enable on-site detection of molecules of nutritional and biological interest. The approach aims to support advancements in diagnostics and bioresearch.

Key words: Sensors; biosensors; electrochemistry; analytical device; food analysis.

1. Introduction

Recently, the demand for smart analytical (bio)devices has dramatically increased; notably, electrochemical sensors equipped with functional nanomaterials (NMs) offer the possibility to produce miniaturized and portable analytical tools, suitable for on-site analysis/diagnostics and bioresearch. In this project, flexible plastics and cellulosic substrates will be used for (bio)analytical device development. Emerging low-environmental impact benchtop technologies for the development of lab-made integrated (bio) devices as xurography, wax-printing, laser-molding, thermal-assembling and stencil-printing, will be employed. Moreover, additive manufacturing (3D-printing) approaches will be also used for integrated devices manufacturing. Different NMs will be employed to improve the performance and ensure communication with the biological elements. Benchtop technologies will be employed to integrate the more appropriate NM in the all-in-one devices (lab-on-strip/paper). The nano-platforms will be used for the determination of molecules of agrifood, nutritional and biological interest.

2. Experimental works

In accordance with the PhD thesis project previously described (Paolini, 2025), this oral communication reports the main results of the following activities:

A1) Silveri, F., Paolini (co-first author), D., Della Pelle, F., Bollella, P., Scroccarello, A., Suzuki, Y., & Compagnone, D. (2023). Lab-made flexible third-generation fructose biosensors based on 0D-nanostructured transducers. *Biosensors and Bioelectronics, 237*, 115450.

A2) Paolini, D., Della Pelle, F., Scroccarello, A., Silveri, F., Bollella, P., Ferraro, G., Fukawa, E., Suzuki, Y., Sowa, K., Torsi, L., & Compagnone, D. (2024). Enhanced electron transfer efficiency of fructose dehydrogenase onto roll-to-roll thermal stamped laser-patterned reduced graphene oxide films. *ACS Applied Materials & Interfaces*, *16*(17), 22443-22454.

A3) Paolini, D., Della Pelle, F., Scroccarello, A., Di Cristoforo, I. V., Ferraro, G., & Compagnone, D. (2025). Eco-innovative papers integrating nanostructured graphenic films. Toward sustainable multifunctional integrated sensors. ***Under Review***.

A4) Paolini, D., Di Cristoforo, I. V., Silveri, F., Flavio Della Pelle, F., Scroccarello, A., Bollella, P., Keisei, S. & Compagnone, D. (2025). Bamboo-paper integrating laser-induced graphene biosensor for the determination of inulin in biological fluids. ***Under Review***.

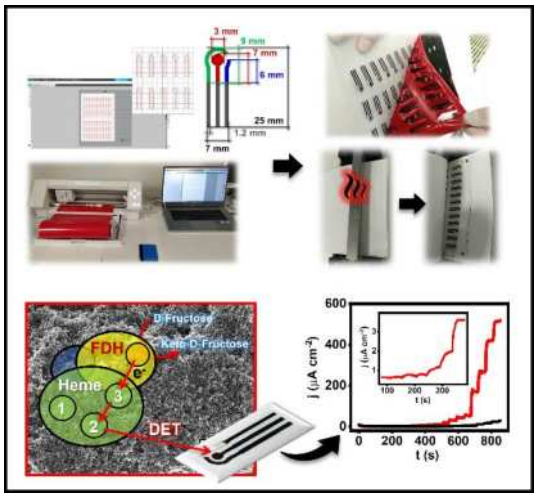
A5) Paolini, D., Dortez, S., Pacheco, J. M., Compagnone, D., Della Pelle, F. & Escarpa, A. (2025**).** 3D-printed integrated electroanalytical device for ischemic stroke *point-of-care testing*. ***Under Review***.

A1) LAB-MADE FLEXIBLE THIRD-GENERATION FRUCTOSE BIOSENSORS BASED ON 0D-NANOSTRUCTURED TRANSDUCERS

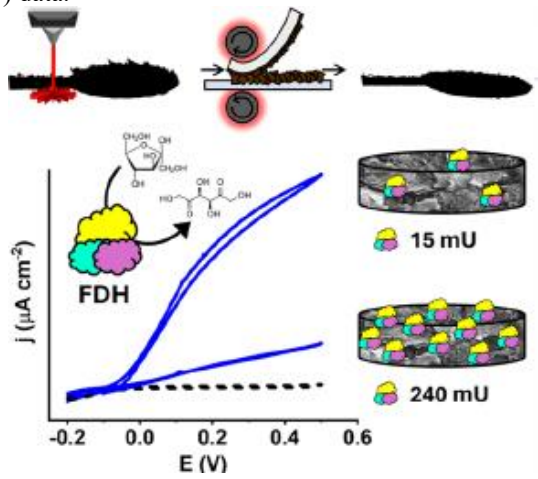
Herein, we report a scalable benchtop electrode fabrication method to produce highly sensitive and flexible third-generation fructose dehydrogenase amperometric biosensors based on water-dispersed 0D-nanomaterials. The electrochemical platform was fabricated via Stencil-Printing (StPE) and insulated via xurography. Carbon black (CB) and mesoporous carbon (MS) were employed as 0D-nanomaterials promoting an efficient direct electron transfer (DET) between fructose dehydrogenase (FDH) and the transducer. Both nanomaterials were prepared in water-phase via a sonochemical approach. The nano-StPE exhibited enhanced electrocatalytic currents compared to conventional commercial electrodes.

The enzymatic sensors were exploited for the determination of D-fructose in model solutions and various food and biological samples. StPE-CB and StPE-MS integrated biosensors showed appreciable sensitivity (~150 μA cm^{-2} mM^{-1}) with μmolar limit of detection (0.35 and 0.16 μM, respectively) and extended linear range (2–500 and 1–

250 µM, respectively); the selectivity of the biosensors, ensured by the low working overpotential (+0.15 V), has been also demonstrated. Good accuracy (recoveries between 95 and 116%) and reproducibility (RSD ≤8.6%) were achieved for food and urine samples.
The proposed approach because of manufacturing versatility and the electro-catalytic features of the waternanostructured 0D-NMs opens new paths for affordable and customizable FDH-based bioelectronics.

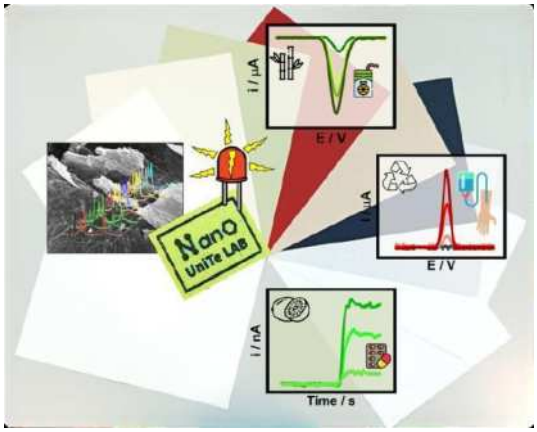


A2) ENHANCED ELECTRON TRANSFER EFFICIENCY OF FRUCTOSE DEHYDROGENASE ONTO ROLL-TO-ROLL THERMAL STAMPED LASER-PATTERNED REDUCED GRAPHENE OXIDE FILMS
Herein, a strategy to stamp laser-produced reduced graphene oxide (rGO) onto flexible polymers using only office-grade tools, namely, roll-to-roll thermal stamping, is proposed, proving for the first time its effectiveness for direct bioelectrocatalysis. This straightforward, scalable, and low-cost approach allows us to overcome the limits of the integration of laser-induced rGO-films in bioanalytical devices. Laser-produced rGO has been thermally stamped (TS) onto different polymeric substrates (PET, PVC, and EVA) using a simple roll-laminator; the obtained TS-rGO films have been compared with the native rGO (untransferred) via morphochemical and electrochemical characterization. Particularly, the direct electron transfer (DET) reaction between fructose dehydrogenase (FDH) and TS-rGO transducers has been investigated, with respect to the influence of the amount of enzyme on the catalytic process. Remarkable differences have been observed among TS-rGO transducers; PET proved to be the elective substrate to support the transfer of the laser-induced rGO, allowing the preservation of the morphochemical features of the native material and returning a reduced capacitive current. Noteworthily, TS-rGOs ensure superior electrocatalysis using a very low amount of FDH units (15 mU). Eventually, TS-rGO-based thirdgeneration complete enzymatic biosensors were fabricated via low-cost benchtop technologies. TS-rGOPET exhibited bioanalytical performances superior to the native rGO, allowing a sensitive (0.0289 μA cm^{-2} μM^{-1}) and reproducible (RSD = 3%, n = 3) D-fructose determination at the nanomolar level (LOD = 0.2 μM). TS-rGO exploitability as a point-of-need device was proved via the monitoring of D-fructose during banana (*Musa acuminata*) postharvest ripening, returning accurate (recoveries 110-90%; relative error −13/+1%) and reproducible (RSD ≤ 7%; n = 3) data.



A3) ECO-INNOVATIVE PAPERS INTEGRATING NANOSTRUCTURED GRAPHENIC FILMS. TOWARD SUSTAINABLE MULTIFUNCTIONAL INTEGRATED SENSORS

Laser-induced conductive nanofilm (LIF) electronics have gained increasing attention for their versatile pattern design. The majority of the devices are based on plastic/polymeric substrates; thus, the manufacturing of sustainable LIF-based paper devices suitable for real applications is still an open issue. In this study laser-induced graphene oxide (rGO) was integrated into different cellulosic substrates, to fabricate complete nanostructured paper-based sensors able to respond to different analytical needs. Eco-innovative cellulosic substrates were investigated, including recycled papers and papers from textile and agro-industrial wastes, and manufactured with fibres alternatives to trees. Paper-sensors have been in series manufactured via an accessible stencil printing approach and rGO-film was easily integrated by pressure. The paper/rGO morphological, structural/chemical, and electrical/electrochemical features were deeply investigated. In brief, each paper accommodates the rGO in a different way, leading to peculiar graphene film formation and chemical rearrangements affecting electrochemistry. The latter appears mainly influenced by the paper's ability to preserve the native rGO-film exfoliated nature dominated by sp2 carbon domains. Paper-rGO sensors' multifunctional ability has been proven in different analytical applications, working at cathodic and anodic potential with different electrochemical techniques. Reproducible data (RSD ≤ %7; n=3), nano/micromolar limits of detections, and satisfactory recoveries (91-108 %) were obtained working with agri-food, biological, and pharmaceutical samples, proving paper-rGO exploitability and competitiveness. Noteworthy, for each application a dedicated paper sensor among the produced sensors was used, demonstrating that the cellulosic substrate affects the electrosensing ability vs. different compounds.



A4) BAMBOO PAPER INTEGRATING LASER-INDUCED GRAPHENE BIOSENSOR FOR THE DETERMINATION OF INULIN IN BIOLOGICAL FLUIDS

3rd generation enzymatic sensors are based on the direct electron transfer (DET) phenomenon. Nevertheless, the use of paper substrates for DET-based electro-analytical devices is still underexplored.

Herein, a bamboo-derived paper was exploited to host laser-induced graphene (LIG) obtained from polyimide and accommodate Fructose dehydrogenase (FDH), with the final aim to fabricate biosensors for the determination of inulin, a natural fructose polymer used as an exogenous marker for glomerular filtration rate (GFR) estimation for kidney functionality evaluation.

At first, different kinds of papers, including office papers, nitrocellulose, and bamboo paper, were compared, studying their LIG and FDH hosting ability, deepening crude electron-transfer features, and electro-catalysis towards DET reaction, revealing the active role of the substrate. Bamboo paper resulted in the most performing one, revealing biocatalytic reactions superior also to untransferred-LIG, thus it was selected to determine inulin in human urine and serum. Artificial biofluids were used to build-up in-matrix calibration curves, obtaining satisfactory linearity and sensitivity (urine: 1.6-22.7 ppm, LOD = 0.3 ppm; serum 4.6-11.4 ppm, LOD = 1.0 ppm), along with remarkable reproducibility (RSD ≤ 4.9%, n = 3). Eventually, the bamboo-paper biosensors were employed to determine inulin in real urine and serum samples at clinically relevant levels, obtaining satisfactory recoveries (90 – 111%; RSD ≤ 7.9%, n = 3).

Summing up, a sustainable substrate allowed the construction of a paper-based third-generation biosensor, shedding light on LIG on paper's potential for bioelectrocatalysis and realizing a smart tool for inulin determination in biological samples.

A5) 3D-PRINTED INTEGRATED ELECTROANALYTICAL DEVICE FOR ISCHEMIC STROKE *POINT-OF-CARE TESTING*

Ischemic stroke represents one of the main causes of death and disability, affecting approximately 15 million people every year. Numerous clinical studies have revealed a strong correlation between brain damage caused by ischemia and elevated iron levels in the affected tissues. In this context, transferrin (Tf), a key protein involved in iron transport and metabolism, has emerged as biomarker. In particular, the transferrin saturation (TSAT) ratio, i.e., the proportion of transferrin-bound iron (Tf-bound iron) to the total iron-binding capacity (TIBC), has emerged as a critical parameter in the diagnosis and monitoring of ischemia.

Recent advancements in micro- and nanotechnologies have facilitated the development of smart biosensors and advanced analytical tools. Among these, additive manufacturing, particularly 3D printing, has gained significant traction due to its affordability, versatility, and customizability. Indeed, electrochemical microfluidic devices produced using 3D printing emerge as promising tools for the development of the next generation of *point-of-care* testing (POCT) devices.

Herein, we report on the development of a 3D-printed electrochemical microfluidic device to assess TSAT. The device has been designed and manufactured to integrate the immunoassay step, a crucial component in isolating transferrin from serum samples. This innovation addresses a key challenge in this field, which has limited the development of smart analytical devices. Indeed, the 3D device incorporates the immunoassay procedure, enabling the simultaneous electrochemical detection of Tf-bound iron and TIBC. This approach allowed for accurate TSAT assessment in serum samples obtained from patients diagnosed with ischemic stroke.

The device proposed combining cost-effectiveness, integration of complex analytical steps, and ease of use, contributing to the advancement of decentralized medicine and personalized healthcare, in the context of ischemic stroke management.

3. Conclusions and Future Perspectives

Summing up, this PhD presents a comprehensive and innovative approach concerning the development of eco-sustainable, flexible, and miniaturized (bio)analytical devices for food and clinical analysis. By integrating low-impact benchtop fabrication techniques and 3D printing with functional nanomaterials, the research has demonstrated how customized platforms can be engineered for efficient, portable, and cost-effective sensing. Across the five experimental activities, the common thread lies in the strategic combination of material science, particularly the use of nanostructured carbon-based materials like carbon black, mesoporous carbon, and laser-induced graphene, with enzyme-based bioelectrocatalysis to achieve direct electron transfer and enhanced analytical performance. The use of unconventional substrates such as cellulosic materials - especially those derived from recycled or tree-free sources - and flexible plastics underlines the project's commitment to sustainability without compromising functionality. These substrates not only act as support structures but actively influence the electrochemical behavior of the final devices, demonstrating a synergistic effect between material and sensor response. Furthermore, the transition from simple benchtop sensors to complex, integrated systems such as microfluidic devices for point-of-care testing confirms the adaptability of the approach to both food diagnostics and clinical contexts. Overall, the research highlights how accessible fabrication technologies, smart material integration, and a sustainability-oriented mindset can converge to realize next-generation (bio)sensing platforms, capable of meeting real-world analytical needs in a decentralized and personalized manner.

Looking ahead, future developments will focus on expanding the range of detectable analytes and further integrating devices with different components. Moreover, efforts will be directed toward scaling up and amplifying fabrication processes to enable real-world deployment in food safety monitoring and decentralized healthcare diagnostics.

Acknowledgements:

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Oral presentation sessions
PhD students – 3rd year
Abstracts

PARALLEL SESSION 11
Genomics and Microbiomes for Food Systems and Environment

Development of a comprehensive cheese metagenome catalogue reveals potential markers of origin and quality

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Tutor: Prof. Francesca De Filippis

In the present Ph.D. research project, a comprehensive cheese microbiome catalogue by merging and analyzing 1,593 metagenomic samples from diverse cheeses was developed. The results revealed distinct microbiome diversity patterns and functional genes associated with cheese origin, flavor, quality, and health benefits, suggesting new microbial biomarkers and expanding the known cheese microbiome repository by ~60%.

Sviluppo di un catalogo completo di metagenomi del formaggio rivela potenziali marcatori di origine e qualità

Nel presente progetto di ricerca di dottorato, è stato sviluppato un catalogo completo del microbioma del formaggio unendo e analizzando 1,593 campioni metagenomici provenienti da diversi formaggi. I risultati hanno rivelato gruppi distinti in termini di biodiversità del microbioma e geni funzionali associati all'origine, al sapore, alla qualità e ai benefici per la salute del formaggio, suggerendo nuovi marcatori microbici e ampliando di circa il 60% i dati pubblici disponibili in riferimento al microbioma del formaggio.

Key words: fermented foods; flavors; health–related genes; probiotics.

1. Introduction

In line with the Ph.D. research project previously outlined, this oral communication summarizes the main outcomes from four primary research activities focused on characterizing and cataloguing cheese microbiomes through metagenomics and machine learning. Specifically, these activities aimed to:

1) compile, harmonize, and curate cheese–associated metadata from diverse sources, facilitating standardized comparative analyses of cheese microbiomes;
2) integrate and analyse high–throughput shotgun metagenomic sequencing data from public databases, curated repositories, and newly sequenced Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) cheese samples;
3) evaluate the microbial diversity, taxonomic composition, and key functional genes associated with flavor, quality, and health–promoting properties;
4) employ advanced machine learning and functional phylogenomic approaches to identify microbial biomarkers linked to cheese *terroir*, strain–level diversity, and functional potentials relevant for food safety, quality, and sustainable innovation.

2. Shotgun metagenomic sequencing

Shotgun metagenomics is an untargeted sequencing method designed to capture and analyze the entire genomic content of microbial communities directly from environmental samples. Unlike targeted methods, such as 16S rRNA sequencing, shotgun metagenomics offers the advantage of obtaining comprehensive genomic data, facilitating simultaneous taxonomic and functional profiling, as well as the reconstruction of whole microbial genomes (Quince *et al.*, 2017). In recent years, this methodology has rapidly emerged as a leading approach within the field of fermented foods microbiology, aimed at characterizing complex microbial consortia. By leveraging shotgun metagenomics, researchers can now uncover microbial diversity in fermented products with unprecedented resolution, enabling detailed insights into microbial contributions to flavor, quality, and health–related biomarker identification (Valentino *et al.*, 2024). Moreover, shotgun metagenomics significantly impacts the precision fermentation sector, facilitating targeted selection and optimization of beneficial microbial strains, thereby improving product consistency, enhancing nutritional profiles, and reducing waste (Chai *et al.*, 2022). Consequently, this technology holds substantial promise for fostering more sustainable and efficient food production processes.

3. Experimental design

Firstly, meticulous metadata curation and harmonization were conducted, standardizing 18 distinct metadata variables associated with cheese production practices, including milk source, processing techniques, microbial starter cultures, ripening period, and geographical origin. This comprehensive metadata curation was critical to subsequent comparative analyses, facilitating the correlation of microbial community structure with specific

cheese production parameters. Secondly, raw sequence data were obtained from three different sources: curatedFoodMetagenomicData (cFMD) (n = 1,044) (Carlino *et al.*, 2024), publicly available datasets (n = 233), and newly sequenced samples of PDO and PGI cheeses (n = 316). This integrative approach provided a robust sample size and ensured a representative range of cheese microbiomes. All samples underwent rigorous quality control procedures, including the removal of sequencing adapters, trimming of low–quality bases, and elimination of human or contaminant DNA sequences. After preprocessing, high–quality reads were subjected to two main workflows:

a) assembly–free approach, in which the microbial profiles were computed using MetaPhlAn4 version October 2022 (Blanco–Míguez *et al.*, 2023). The outputs were used to compute statistical analyses, such as α– and β–diversity assessments, community composition evaluations, and classification supervised machine learning models;
b) assembly–based approach, in which the high–quality reads were assembled into metagenome–assembled genomes (MAGs), allowing the identification of individual microbial genomes within complex communities. Deeply, a taxonomic labelling of each species–level genome bins (SGBs) was performed by considering a previously adopted strategy by Pasolli *et al.* (2019), where a SGB associated with a reference genome found in the GTDB–Tk database (Parks *et al.*, 2022) was labelled as known SGB (kSGB), whereas uSGB was used to label unknown SGBs since they did not include any reference genome. Moreover, the outputs were then annotated by aligning sequences to established functional databases, providing insights into genes associated with cheese flavor, antimicrobial resistance, virulence factors, and health–promoting molecules.

4. Materials and Methods

High–throughput shotgun metagenomic sequencing raw reads from cFMD (n = 1044), publicly available dataset (n = 233), and newly sequenced PDO and PGI cheeses (n = 316) were merged and analyzed. Furthermore, a metadata collection and standardization ($N_{metadata}$ = 18) allowed the harmonization of diverse information about cheeses. Preprocessed high–quality reads were analyzed through two parallel strategies: a) an assembly–free approach using MetaPhlAn4 for microbial profiling and diversity analysis; b) and an assembly–based approach to reconstruct MAGs and identify SGBs. These SGBs were taxonomically labelled using GTDB–Tk and functionally annotated to investigate genes linked to flavor, antimicrobial resistance, virulence, and health–promoting properties. Classification supervised machine learning models were also employed to classify the cheese microbiomes among metadata. Moreover, phylogenetic analyses were employed to investigate potential novel species, while functional approaches to link microbial taxa to functional cheese patterns.

5. Results and Discussion

5.1 Microbial species diversity is correlated to the considered metadata

We first assessed the microbial diversity across different cheese compartments, namely the core, core plus rind, and rind (Fig. 1). Alpha diversity, measured using Pielou's evenness index, revealed significant differences across compartments (Fig. 1A). The core plus rind group exhibited the highest median evenness value (0.21), followed by rind (0.17), and core (0.07). Pairwise statistical comparisons indicated highly significant difference only between core versus rind (p–value < 0.05). To evaluate overall community dissimilarity β–diversity was computed between compartments, we employed the Aitchison distance on centered log–ratio (CLR)–transformed microbial abundance data. Permutational Multivariate Analysis of Variance (PERMANOVA) confirmed that the compartment significantly contributed to community structure (p < 0.001). Using t–distributed stochastic neighbor embedding (t–SNE) a separation was effectively captured in terms of compartment–specific clustering (Fig. 1B).

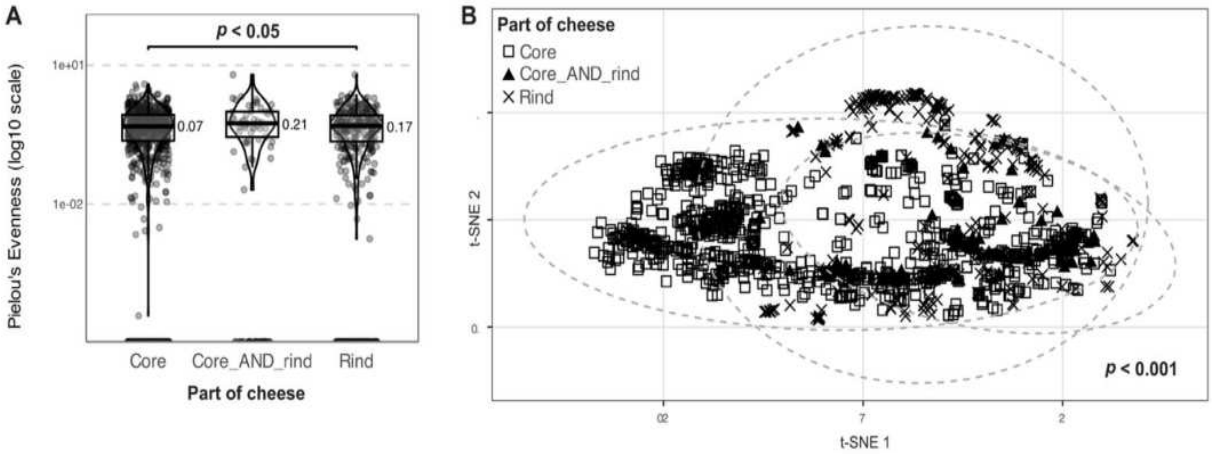


Figure 1 **A**) *α–diversity was measured using Pielou's evenness index. Boxplots display interquartile ranges and medians,*

with violin plots showing the full distribution. Differences were assessed with Wilcoxon–Mann–Whitney tests. Medians and significant p–values are reported ($p < 0.05$). **B)** *β–diversity was evaluated using Aitchison distances (CLR–transformed data) and illustrated employing t–SNE projection to highlight community dissimilarities across cheese compartments. Each symbol represents a cheese compartment: squares = core, triangles = core plus rind, crosses = rind. Dashed ellipses highlight the clustering patterns of each compartment. PERMANOVA ($p < 0.001$) result indicates the variance explained by clustering of cheese compartments in two dimensions.*

5.2 Different microbial patterns shaped by the cheese compartment

The microbial taxonomic profiles of cheese compartments (core, core plus rind, and rind) exhibited distinct community structures, as illustrated in Figure 2. Indeed, by applying supervised machine learning, specifically a Random Forest model, samples were robustly classified based on their microbial profiles. Quantitatively, one–vs–rest receiver operating characteristic curves demonstrated strong predictive performances across compartments, achieving an overall bootstrap–derived area under the curve of 0.82, with a 95% confidence interval in a range of 0.77–0.87 (Figure 2A). To further explore the composition of cheese compartments microbiomes, a natural logarithmic scaled (log1p) heatmap was plotted (Figure2B), highlighting several taxa clearly patterning the compartments. Species such as *Debaryomyces hansenii*, *Lactococcus lactis*, and *Lactobacillus delbrueckii* exhibited marked differences in abundance between core, core plus rind, and rind samples, suggesting their potential roles as biomarkers of cheese compartment.

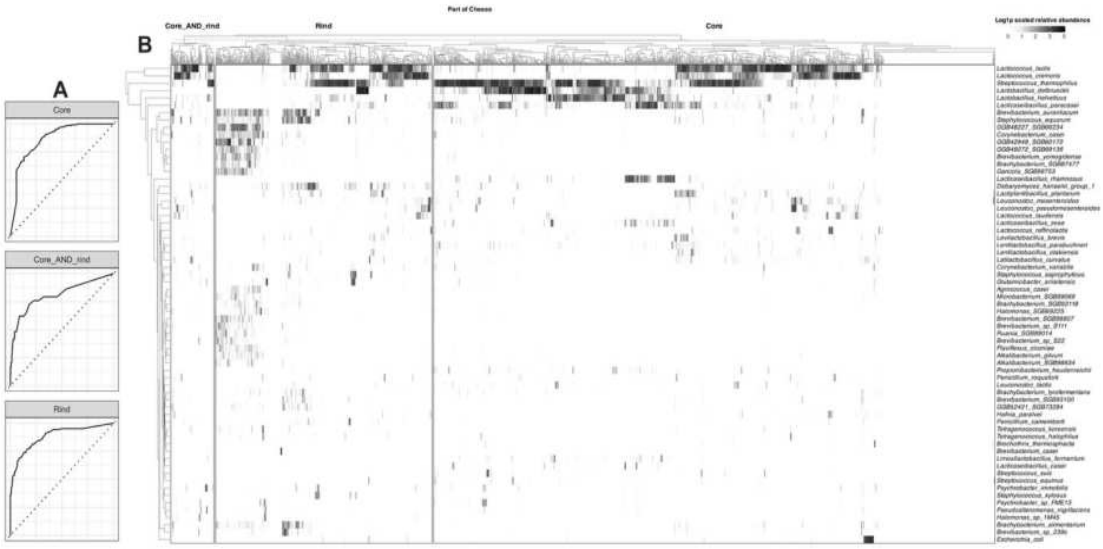


Figure 2 **A)** *One–vs–Rest receiver operating characteristic curves illustrating classification performance for each cheese compartment individually against all others, computed using a Random Forest model. The plots illustrate sensitivity (y–axis) versus specificity (x–axis) for each category.* **B)** *Heatmap showing the log_{10}–scaled relative abundance of microbial species across cheese compartments. The heatmap is vertically split by compartment (core plus rind, rind, core), and hierarchical clustering was applied to both rows (species) and columns (samples) to identify consistent microbial patterns. Darker shades correspond to higher relative abundances, with lighter shades indicating lower abundance or absence.*

5.3 Broadening the phylogenetic diversity of representative bacterial genomes

The phylogenetic analysis of SGBs unveiled extensive microbial diversity (Figure 3). The circular phylogenetic tree constructed using PhyloPhlAn (Asnicar *et al.*, 2020) identified numerous uSGBs, pointing toward previously undescribed microbial taxa that could influence cheese functionality, quality, and safety.



Figure 3 *Circular phylogenetic tree constructed using PhyloPhlAn, displaying metagenome–assembled genomes (MAGs) derived from the cheese microbiome dataset. The tree is rooted on the Archaea clade, which is indicated centrally to provide evolutionary context. Outer rings mark the status of each species–level genome bin (SGB), distinguishing known SGBs (kSGBs) and unknown SGBs (uSGBs); the latter are highlighted with solid black bars to reflect the unexplored diversity revealed through metagenomic reconstruction.*

5.4 SGBs functional patterns in vitamin biosynthesis

The presence/absence heatmap of vitamin biosynthetic genes across MAGs (Figure 4) revealed distinct patterns of functional potential among SGBs. The clustering analysis highlighted major functional groups of genes related to vitamins production such as riboflavin and folate biosynthesis, and distinct clusters of specific functional niches within the cheese microbiomes.

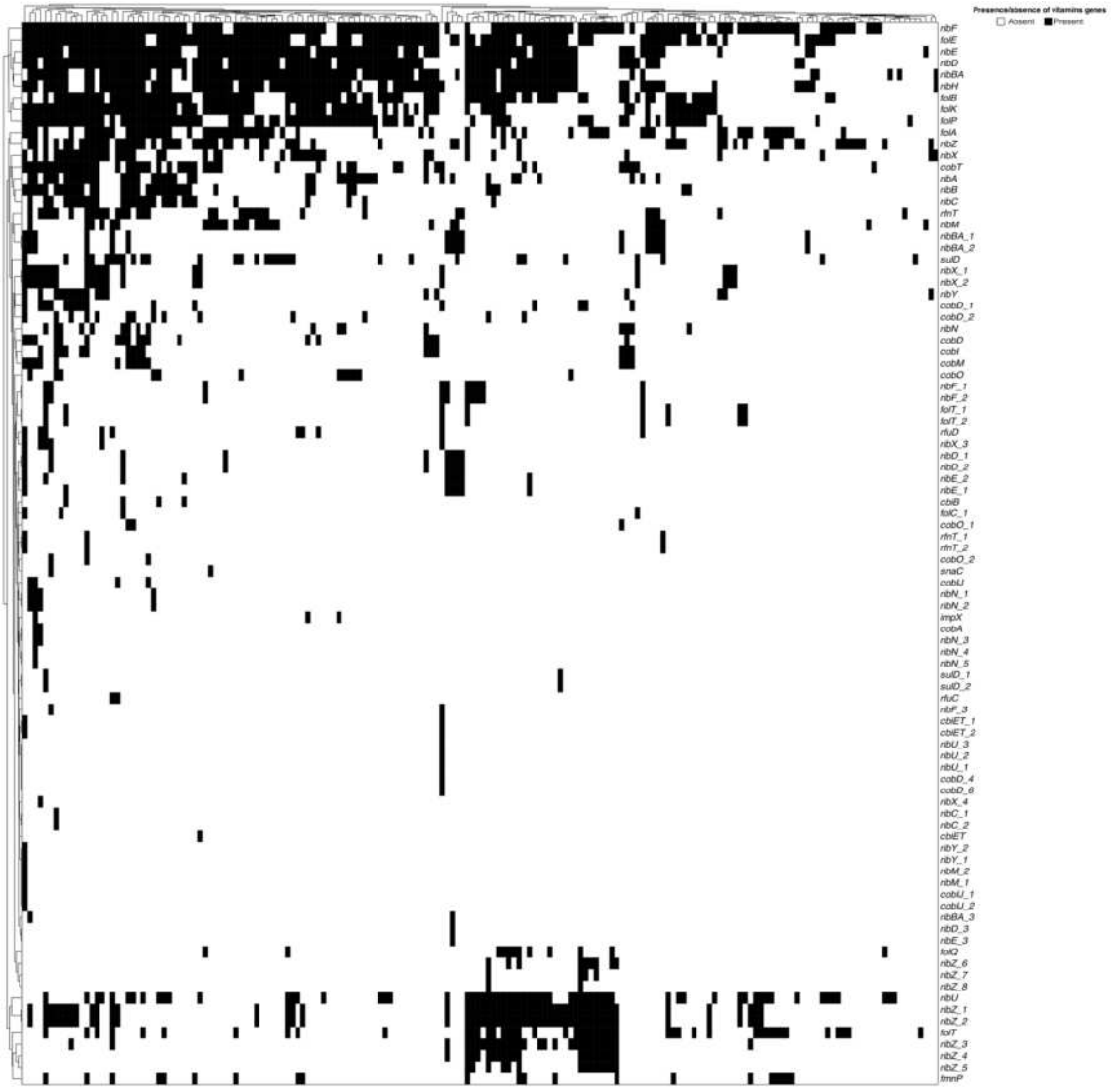


Figure 4 *Presence/absence heatmap of genes involved in vitamin biosynthesis across metagenome–assembled genomes*

(MAGs). Rows represent individual vitamin–related genes, while columns correspond to MAGs. Gene presence is indicated in black and absence in white. Hierarchical clustering was applied to both axes to highlight co–occurrence patterns and genome–specific enrichment. The visualization reveals SGBs diversity in the functional potential for vitamin biosynthesis.

6. Conclusions and Future Perspectives

This study presents the most extensive metadata–curated catalogue of cheese microbiomes to date, integrating 1,593 shotgun metagenomic samples from international sources, including newly sequenced PDO and PGI cheeses. Through rigorous metadata harmonization and genome–resolved metagenomics, we reconstructed 12,579 MAGs, expanding current food microbiome repositories by around 60%. Our results revealed significant associations between cheese compartments and microbial α– and β–diversity metrics, highlighting distinct, compartment–specific microbiome signatures. Supervised machine learning models reliably classified cheese microbiomes based on metadata and identified potential microbial biomarkers. Critically, many of the reconstructed genomes represent previously undescribed microbial taxa, underscoring the hidden microbial diversity within cheese ecosystems. Functional profiling further underscored the value of genome–resolved metagenomics in identifying genes linked to flavor development, microbial safety, and health–related functions. These findings open the door to targeted applications in food biotechnology aiming to sustainable fermented foods optimization. Future work should aim to elucidate the mechanistic roles of microbial biomarkers identified here, particularly those involved in cheese ripening and bioactive compound production. Investigating the probiotic potential of cheese–associated microbes through functional assays and clinical validation could strengthen the link between fermented foods and health. Moreover, this resource lays the groundwork for advancing precision fermentation strategies. By using metagenome–informed design of minimal, functional microbial consortia, it may be possible to optimize cheese quality and sustainability, reducing reliance on resource–intensive production processes. This aligns with the growing demand for health–promoting, environmentally responsible foods and may help strengthen consumer trust in artisanal and industrial dairy products. In summary, this curated catalogue offers a robust foundation for microbiome–guided innovation in dairy science. It paves the way for future developments in sustainable fermentation, microbial authentication, and functional food development, with benefits extending across industry, consumers, and environmental sustainability.

7. Abbreviations

cFMD: curatedFoodMetagenomicData; CLR: Centered Log–Ratio; kSGB: Known Species–level Genome Bin; log1p: natural logarithmic scaling; MAGs: Metagenome–Assembled Genomes; PERMANOVA: Permutational Multivariate Analysis of Variance; PDO: Protected Designation of Origin; PGI: Protected Geographical Indication; SGB: Species–level Genome Bin; t–SNE: t–distributed Stochastic Neighbour Embedding; uSGB: Unknown Species–level Genome Bin.

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Closing the loop in agriculture: effects of a fermented tomato-based biostimulant on tomato growth and fruit quality

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Fertilizers from agri-food waste: a sustainable way to improve vegetative and productive performance of different crops

The aim of this PhD thesis was to explore sustainable strategies for enhancing crop performance through the use of fertilizers derived from agri-food waste. Specifically, the study investigated the application of biochar and various biostimulants produced via lactic acid fermentation of food residues, with the goal of improving the vegetative and productive traits of tomato and blueberry plants. This research aligns with the principles of circular economy, aiming to reduce waste, value by-products, and promote more sustainable agricultural practices.

Fertilizzanti da scarti agroalimentari: una via sostenibile per migliorare le performance vegetative e produttive di diverse specie di interesse agrario

L'obiettivo di questa tesi di dottorato è sviluppare strategie sostenibili per migliorare le performance colturali attraverso l'impiego di fertilizzanti derivati da scarti agroalimentari. In particolare, sono stati studiati l'utilizzo del biochar e di diversi biostimolanti ottenuti mediante fermentazione lattica di residui alimentari, con lo scopo di migliorare le caratteristiche vegetative e produttive del pomodoro e del mirtillo. Il lavoro si inserisce nell'ambito dell'economia circolare, mirando alla valorizzazione degli scarti, alla riduzione dell'impatto ambientale e alla promozione di pratiche agricole più sostenibili.

Key words: sustainability; circular economy; agri-food waste; biostimulants, crop performance.

1. Introduction

Approximately one-third of global food intended for human consumption is lost or wasted each year (Gustavson et al., 2011; Ishangulyyev et al., 2019), exacerbating the challenges posed by climate change and a growing global population (Yang et al., 2023). As agri-food systems face increasing demand for sustainable solutions, biostimulants have emerged as effective tools to improve plant performance while reducing environmental impact (Ertani et al., 2015; Szparaga et al., 2019; Shukla et al., 2022). Those derived from food waste are particularly promising, offering dual benefits of resource recovery and enhanced crop quality (Kumar et al., 2023; Dasgan et al., 2024). Fermentation technologies further support this approach by transforming waste into bioactive compounds with agronomic value (Ricci et al., 2019; Hadj Saadoun et al., 2021; Nazeer et al., 2024). This study evaluates the application of a fermented tomato-based biostimulant (FTB), produced from unsold market tomatoes, in a soilless tomato system in Emilia-Romagna, Italy. In addition to overall yield and quality effects, the study explores how FTB influences fruit quality gradients across plant trusses—an important factor in optimizing sustainability and reducing losses in tomato production (Bias et al., 2014; Ávila-Juárez et al., 2018; Yao et al., 2023).

2. Materials and Methods

2.1 Preparation of the experimental biostimulant

A 1:1 mixture of unsold green and red tomatoes from the 2023 harvest (Azienda Agricola Anzola) was homogenized and sterilized (121 °C, 20 min). Five lactic acid bacteria strains from the University of Parma Culture Collection, all isolated from food sources, were tested: *Weissella cibaria* 4253, *Lactiplantibacillus plantarum* 4188, *Pediococcus acidilactici* 3992, *Lacticaseibacillus zeae* 2240, and *Lacticaseibacillus rhamnosus* 1473. Strains were pre-cultured to 9 log CFU/mL and inoculated into the fermentation medium at 7 log CFU/mL. Fermentation conditions were optimized using an experimental design with one qualitative factor (microbial strain) and two quantitative factors (temperature 30–37 °C; time 24–48 h), with final microbial load (log CFU/mL) as the response variable. Twenty experimental conditions were tested in duplicate (40 trials total). Microbial counts were performed on MRS agar after incubation for 48 h. The best conditions were provided by *Weissella cibaria* 4253, whose performance was validated and used for scale-up, producing 7 kg of fermented tomato-based biostimulant (FTB).

2.2 Site description, plant material and experimental design

The greenhouse experiment was conducted from February to June 2024 (16 weeks) at Soc. Agricola Anzola (Boretto, Reggio Emilia, Italy), under controlled conditions. *Solarino* (Rijk Zwaan-RZ) tomato plants were grown from seeds sown in a local nursery in January 2024 and transplanted after 6 weeks into coconut fiber bags for soilless cultivation. A fermented tomato biostimulant (FTB) was applied weekly at three dilution levels (100, 200, 300 ml/L; 50 ml per plant) using a syringe. Treatments (FTB1, FTB2, FTB3) were arranged in a randomized block design with three replicates each (12 bags in total). Controls received the same amount of water.

2.3 Vegetative, Productive, and Quality Data Collection

Throughout the 16-week experimental period, tomato plants were monitored weekly for growth and yield. Plant height and number of leaves were recorded until week 12 (topping) and week 6 (defoliation), respectively, while stem diameter was measured biweekly. Fruits were harvested at full maturity (April–June), with data collected on fruit number, average weight, and total yield. Fruit size and quality were assessed by truss position (lower, medial, upper) during weeks 10, 13, and 16. For quality analyses, 50 fruits per treatment were sampled. Parameters included external color, texture (firmness), total soluble solids (TSS), titratable acidity (TA), electrical conductivity (EC), pH, total polyphenol content (TPC), antioxidant activity (DPPH assay), and lycopene content (LC). Color (CIE-Lab) was measured with a Minolta CM-36dG colorimeter, and firmness with a TA.XT2i Texture Analyzer. Juice was analyzed for TSS (°Brix), TA (as citric acid equivalents), EC (at 25°C), and pH. The TSS/TA ratio was used to evaluate sugar–acid balance. TPC was determined by the Folin–Ciocalteu method and expressed as mg GAE/kg FW, while antioxidant activity was assessed by the DPPH assay and expressed as mM TEAC; both analyses were performed on extracts obtained using methanol/acetone (70:30, v/v). Lycopene content was quantified by spectrophotometry after extraction with a hexane:acetone: ethanol mixture (2:1:1, v/v/v), using absorbance at 473 and 502 nm ($\varepsilon = 1280$).

2.4 Statistical analysis

Statistical analyses were performed using JASP (v.0.19.3). Data normality was assessed; parametric data were analyzed with Tukey's test ($p \leq 0.05$), while non-parametric data were evaluated using the Kruskal-Wallis test. For each harvest date, a one-way ANOVA was conducted considering "Treatment" as the main factor.

3. Results and discussion

The FTB influenced multiple vegetative, productive, and qualitative parameters of tomato plants. Notably, FTB1 treatment led to a 23% increase in total yield and an 8% increase in average fruit weight compared to the control (Table 1), confirming previous findings on plant-based biostimulants enhancing productivity (Distefano et al., 2022). Fruit size varied with truss position, with the largest fruits observed in the medial trusses of FTB1-treated plants (Table 2), in agreement with the enlargement effects of fulvic acid-based treatments reported by Suh et al. (2014). While no significant improvement in fruit skin redness (a* value) was recorded, FTB3 maintained more stable coloration across trusses (Table 3), a desirable trait for quality standardization (Rivelli et al., 2024). Fruit firmness was significantly enhanced by FTB, particularly in FTB1-treated plants, with increases of 29% (medial) and 16% (upper trusses) (Table 3), supporting previous findings on improved fruit texture due to biostimulants (El-Miniawy et al., 2013; Cardarelli et al., 2024). In terms of taste-related traits, Total Soluble Solids (TSS) increased progressively from medial to upper trusses, especially in FTB-treated plants, indicating enhanced sweetness and flavor; conversely, titratable acidity (TA) declined with maturity, more markedly in FTB3 fruits (Table 4). The resulting higher TSS/TA ratio—7% greater than control in FTB3 (Table 4)—indicates improved organoleptic quality (Distefano et al., 2022). Regarding functional compounds, both total polyphenol content (TPC) and lycopene levels were influenced by truss position and treatment (Table 4). While TPC typically declined over time, FTB1 and FTB2 mitigated this loss, maintaining higher values in later harvests. Lycopene content, which usually drops under high temperatures (Dumas et al., 2003), was preserved or even increased in FTB-treated plants, suggesting a protective effect of the biostimulants against heat-induced degradation. Consistent with previous studies (Rosales et al., 2011), this study found that fruits from the upper trusses showed the highest antioxidant activity (Table 4), likely due to increased temperatures during their development compared to fruits from the lower trusses. Temperature is known to influence antioxidant accumulation, with levels decreasing when it falls below 12°C or rises above 32°C (Dumas et al., 2003; Rosales et al., 2011).

Table 1: *Effect of Fermented Tomato Biostimulant (FTB) on number of fruits, Yield per plant, and individual fruit weight*.

Treatment	n° of fruits±SD	Yield/plant^{-1}±SD (g)	Fruit weight±SD (g)
FTB0	96.5±9.3^{a}	1170.3±121.3^{b}	12.41±0.44^{bc}
FTB1	118.3±9.0^{a}	1443.4±52.4^{a}	13.41±0.49^{a}
FTB2	108.8±14.3^{a}	1172.7±145.5^{b}	11.89±0.52^{c}
FTB3	93.7±2.2^{a}	1062.6±82.7^{b}	12.96±0.54^{b}

One-way ANOVA, Tukey's test, p≤0.05. Within each parameter, different letters indicate values statistically different.
FTB0: 0 ml/L of FTB, FTB1: 100 ml/L of FTB, FTB2: 200 ml/L of FTB, and FTB3: 300 ml/L of FTB.

Table 2: Effect of Fermented Tomato Biostimulant (FTB) on Fruit Morphological Parameters at different truss position.

Truss Position	Treatment	FL±SD (mm)	FW ±SD (mm)
Lower	FTB0	34.07±0.39^{ab}	24.74±0.43
	FTB1	35.21±0.39^{a}	25.33±0.31
	FTB2	33.81±0.35^{b}	25.46±0.19
	FTB3	34.00±0.23^{ab}	25.38±0.33
p-value		0.033	0.404
Medial	FTB0	37.04±0.45^{b}	26.00±0.40^{a}
	FTB1	39.33±0.71^{a}	26.17±0.43^{a}
	FTB2	38.08±0.57^{ab}	26.31±0.36^{a}
	FTB3	37.59±0.53^{ab}	24.23±0.36^{b}
p-value		0.049	0.002
Upper	FTB0	34.12±1.02	22.12±0.36
	FTB1	33.48±1.26	23.17±0.53
	FTB2	31.51±0.41	23.06±0.42
	FTB3	35.08±1.08	23.73±0.37
p-value		0.096	0.084

Statistical analysis was performed using one-way ANOVA for each truss position followed by Tukey's post-hoc test (p ≤ 0.05). Within each parameter, different letters indicate values statistically different. Results are expressed as mean ± standard deviation (SD). FTB0: 0 ml/L of FTB, FTB1: 100 ml/L of FTB, FTB2: 200 ml/L of FTB, and FTB3: 300 ml/L of FTB, FL: fruit length, FW: fruit width.

Table 3: *Influence of Fermented Tomato Biostimulant (FTB) on fruit color parameters and Texture in different truss position.*

Truss Position	Treatment	L*	a*	b*	a*/b*	Firmness (N)
Lower	FTB0	33.46±0.14	17.26±0.42^{a}	16.63±0.21	1.03±0.02^{a}	6.99±0.24^{a}
	FTB1	33.70±0.17	15.28±0.59^{b}	16.19±0.25	0.94±0.03^{ab}	6.55±0.23^{ab}
	FTB2	33.72±0.17	16.97±0.46^{ab}	16.93±0.24	1.00±0.02^{ab}	6.58±0.23^{ab}
	FTB3	33.66±0.11	15.43±0.52^{ab}	16.53±0.21	0.93±0.02^{b}	5.89±0.26^{b}
p-value		0.636	0.011	0.174	0.036	0.026

Medial	FTB0	33.86±0.16^{ab}	20.55±0.35^{a}	17.28±0.21^{a}	1.19±0.01^{a}	5.85±0.23^{b}
	FTB1	34.12±0.12^{a}	19.83±0.45^{a}	17.32±0.18^{a}	1.14±0.02^{ab}	7.57±0.32^{a}
	FTB2	34.25±0.14^{a}	20.14±0.36^{a}	17.78±0.22^{a}	1.13±0.01^{ab}	6.54±0.23^{ab}
	FTB3	33.42±0.13^{b}	17.88±0.39^{b}	16.22±0.23^{b}	1.10±0.02^{b}	6.91±0.29^{ab}
p-value		<0.001	<0.001	<0.001	0.028	0.002
Upper	FTB0	32.69±0.25^{b}	17.78±0.73	15.74±0.24	1.12±0.04	6.60±0.32^{ab}
	FTB1	32.53±0.21^{b}	17.2±0.71	15.97±0.2	1.07±0.04	7.66±0.25^{a}
	FTB2	32.96±0.20^{ab}	18.86±0.48	16.42±0.32	1.15±0.03	6.55±0.23^{b}
	FTB3	33.24±0.12^{a}	18.25±0.73	16.05±0.22	1.13±0.03	6.91±0.29^{ab}
p-value		0.025*	0.395*	0.307	0.645*	0.029

*Statistical analysis was performed using one-way ANOVA for each truss position followed by Tukey's post-hoc test ($p \leq 0.05$) for normally distributed data. For variables not meeting the normality assumption (indicated by *), the Kruskal–Wallis test was applied, followed by Dunn's post-hoc test. Within each parameter, different letters indicate values statistically different. Results are expressed as mean ± standard deviation (SD). FTB0= 0 mL/L, FTB1 = 100 mL/L, FTB2 = 200 mL/L, FTB3 = 300 mL/L of FTB.*

Table 4: *Influence of Fermented Tomato Biostimulant (FTB) on fruit quality parameters in different truss position*

Truss Position	Treatment	TSS±SD (°Brix)	TA±SD (% citric acid)	TSS/TA±SD	EC±SD (mS)	pH±SD	TPC±SD (mg GAE/Kg)	AO±SD (mM TEAC)	LC±SD mg/Kg
Lower	FTB0	8.23±0.08^{b}	0.83±0.002^{b}	9.88±0.15^{bc}	2.71±0.05	4.46±0.015^{a}	982.49±123.82^{a}	1.01±0.51	965.31±102.4^{a}
	FTB1	8.20±0.15^{b}	0.85±0.001^{a}	9.59±0.31^{c}	2.57±0.06	4.17±0.003^{c}	643.39±48.09^{c}	0.68±0.32	378.39±54.82^{c}
	FTB2	8.43±0.08^{ab}	0.79±0.015^{c}	10.57±0.03^{ab}	2.57±0.13	4.32±0.025^{b}	798.48±48.60^{bc}	0.59±0.27	507.98±118.81^{bc}
	FTB3	8.94±0.17^{a}	0.82±0.003^{b}	10.83±0.39^{a}	2.87±0.18	4.11±0.012^{c}	851.16±92.63^{ab}	0.83±0.27	752.55±190.06^{ab}
p-value		0.015	<0.001	0.001	0.329	<0.001	<0.001	0.397	<0.001
Medial	FTB0	8.60±0.10	0.72±0.022^{ab}	11.84±0.43^{ab}	2.63±0.13	4.19±0.005^{a}	734.91±50.13^{b}	1.03±0.06^{b}	651.15±205.71^{b}
	FTB1	8.40±0.11	0.74±0.015^{a}	11.30±0.25^{b}	2.81±0.02	4.13±0.005^{b}	952.14±39.49^{a}	1.21±0.08^{ab}	802.03±195.25^{ab}
	FTB2	8.44±0.13	0.76±0.006^{a}	11.06±0.22^{b}	2.67±0.07	4.15±0.005^{b}	650.26±38.45^{b}	1.11±0.20^{b}	666.89±62.10^{ab}
	FTB3	8.73±0.06	0.68±0.031^{b}	12.77±0.73^{a}	2.53±0.11	4.12±0.012^{b}	557.14±47.02^{c}	1.75±0.47^{a}	1015.48±182.17^{a}
p-value		0.178	<0.001	0.008	0.311	<0.001	<0.001	0.009	0.038
Upper	FTB0	8.97±0.03	0.68±0.007^{ab}	13.07±0.16	3.94±0.04	4.13±0.016^{b}	490.53±32.15	1.71±0.17^{ab}	725.76±158
	FTB1	9.64±0.36	0.65±0.014^{c}	14.7±1.25	3.98±0.01	4.27±0.017^{a}	467.76±74.34	1.17±0.12^{b}	882.44±65.84
	FTB2	9.64±0.43	0.69±0.01^{a}	13.94±1.00	3.96±0.03	4.34±0.037^{a}	651.78±178.73	1.35±0.47^{b}	873.08±119.9
	FTB3	10.03±0.52	0.66±0.006^{bc}	15.18±1.35	3.95±0.02	4.28±0.012^{a}	558.12±108.81	2.02±0.28^{a}	874.53±187.61
p-value		0.362*	0.007	0.075*	0.839*	<0.001	0.298*	0.007	0.366

*Statistical analysis was performed using one-way ANOVA for each truss position followed by Tukey's post-hoc test ($p \leq 0.05$) for normally distributed data. For variables not meeting the normality assumption (indicated by *), the Kruskal–Wallis test was applied, followed by Dunn's post-hoc test. Within each parameter, different letters indicate values statistically different. Results are expressed as mean ± standard deviation (SD). FTB0: 0 ml/L of FTB, FTB1: 100 ml/L of FTB, FTB2: 200 ml/L of FTB, and FTB3: 300 ml/L of FTB, TSS: Total Soluble Solids, TA: Titratable acidity, EC: Electrical Conductivity, TPC: Total Polyphenol Content, AO: Antioxidant Activity, LC: Lycopene Content.*

4. Conclusion

The application of FTB improved yield and fruit quality in tomato plants, particularly enhancing firmness, TSS, titratable acidity (TA), and bioactive compounds, especially in fruits from the upper trusses. It also helped counteract environmental stresses like heat-induced lycopene reduction. These results support using sustainable, food-waste-derived biostimulants to reduce waste, optimize resources, and produce value-added crops.

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Effect Of Biochar and Wood Distillate Application on Vegeto-Productive Performances of Strawberry Plants (cv. Charlotte), Under Controlled Conditions

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This PhD thesis aim is to highlight the effect of different bio-fertilizers like biochar and wood distillate on the growth and development, and yield and quality of the strawberry plant grown under controlled conditions.

Effetto dell'applicazione di biochar e distillato di legno sulle performance vegeto-produttive di piante di fragola (cv. Charlotte), in condizioni controllate

L'obiettivo di questa tesi di dottorato è quello di evidenziare l'effetto di diversi biofertilizzanti come il biochar e il distillato di legno sulla crescita e sullo sviluppo, sulla resa e sulla qualità della pianta di fragola coltivata in campo e in condizioni controllate.

Key words: Circular economy; *Fragaria x ananassa*; plant growth; sustainable agriculture; vegeto-productive performances.

1. Introduction

Strawberry (*Fragaria* x *ananassa*) is a globally important berry plant, the cultivation of which is increasing in the soilless system due to soil-related problems (Hernández-Martínez *et al.,* 2023; Woznicki *et al.,* 2024). Peat moss is considered as a primary substrate that is commonly used in the cultivation of plants. It also works as alternatives like biochar and wood distillate (Woznicki *et al.,* 2024, Bilderback *et al.,* 2005). Biochar is a carbon rich material derived from pyrolyzed biomass (Iacomino *et al.,* 2023). It improves the substrate properties, water retention and works as carbon sequestration (Oleszczuk *et al.,* 2012; Caldara *et al.,* 2024). Biochar plays an important role as a sustainable alternative to peat in the horticultural substrate (Dispenza *et al.,* 2016). It is studied that biochar when combined with the peatmoss has shown positive effects on strawberry growth, yield, and fruit quality (Blok *et al.,* 2019; Iacomino *et al,* 2023). Wood distillate is a byproduct that works as a biostimulants and contains bioactive compound like polyphenols and organic acid (Ofoe *et al.,* 2024). In strawberry and other crops, wood distillate application improves growth, yield and biochemical traits (Fedeli *et al.,* 2025; Mu *et al.,* 2006, Kårlund *et al.,* 2014; Travero and Mihara, 2016; Zulkarami *et al.,* 2011,).

In accordance with the Ph.D. thesis project, this report indicates the main results of one of the research lines pursued:

A1) impact of biochar on morpho-physiological, biochemical, yield and quality attributes of strawberry plants grown under soilless controlled conditions.

A2) impact of wood distillate on morpho-physiological, biochemical, yield and quality attributes of the strawberry plants grown under soilless controlled conditions.

2. Materials and Methods

2.1 Experimental Design

In this study, two experiments were carried out on strawberry plants (*Fragaria* x *ananassa*), cv. Charlotte, in the greenhouse, under soilless conditions. The first experiment was on the evaluation of the effect of biochar partially replacing the peat used as substrate for strawberry culture. The second experiment aimed at studying the effect of wood distillate, distributed as foliar spray and, at different concentrations, in the substrate. The different treatments tested per experiment is mentioned below in Table 1. Six plants per treatment were used in a completely randomized design. Plants were irrigated on a daily basis, while no synthetic fertilizers and pesticides were used.

2.2 Parameters Measured

Different morpho-physiological, biochemical and fruit quality attributes of both experiments were analysed. Data included plant height, leaf area, flavonoid content, chlorophyll content and SPAD. At harvest, the total number (n°) of harvesting fruit (yield) and fruit quality attributes lightness (L*), redness (a*), yellowness (b*), pH, total soluble sugar (TSS, brix°), titratable acidity (TA), the ratio between (TSS/TA) and root length, were recorded. Biochemical assays were assessed based on total phenolic content (TPC) and antioxidant (DPPH).

Plant height was measured by using the measuring tape from the base to the tip (Basak *et al.,* 2019). Leaf area was calculated by following the estimated leaf area from trifoliate leaf length and width (LA=ELA×0.74) (Abbott, 1968).
Physiological parameters including leaf flavonoid content (FLV in F660nm/F325nm), chlorophyll content (CCLa in T850nm/T720nm) and soil plant analysis (SPAD) were also recorded by using the Multiple Pigment Meter (MPM-100S, Bio Scientific Ltd, UK). Fruit quality attributes like (L*) lightness (dark to light), (a*) redness (green (-) to (+) red) and (b*) yellowness (blue (-) to (+) yellow) were analyzed by using CHROMA METER CR-400 (Konica Minolta, Tokyo, Japan), (Zuñiga *et al.,* 2020). During the biochemical analysis, the pH was measured by using a ph510 Meter, using a solution of 10 g juice/90 mL distilled water (El-Mogy *et al.,* 2019). TSS (°Brix) was analyzed with an optical portable refractometer (Hanna Instruments, Padova, Italy) in which 50µL of strawberry fruit juice was used (Pontesegger *et al.,* 2023). The TA was determined using the titration method described by Teka (2013). Acid ratio (TSS/TA) as described by Tigist *et al.* (2013). Ripened fruits harvested from all tested plants were used as a matrix for producing extracts analyzed for their Total Phenol Content (TPC) and antioxidant capacity (DPPH assay). The extracts were obtained from 1 g of fruit mixed with 20 mL of a methanol-water solution (70/30 ratio), mixed for 2 hours at room temperature; this procedure was partially modified starting from that described by Contessa *et al.* in 2013. The total phenol content (TPC) was evaluated by resorting to Folin–Ciocalteau's phenol reagent (Contessa *et al.,* 2013). The antioxidant capacity of fruit extracts was determined by applying the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays (Abram *et al.,* 2015).

2.3 Statistical Analysis

The experimental data collected was subjected to the analysis of one-way ANOVA using GLM (General Linear Model) procedures, with IBM SPSS Statistics 29.0.1.0 software, (SPSS Inc., Chicago, IL, USA). Mean separation comparisons have been performed resorting to Tukey's test ($p \leq 0.05$).

First Experiment (Biochar Treatments)

Treatment Code	Substrate Composition
C	Techno grow mix only (control)
2BC	Techno grow mix with 2% of biochar (w/w)
4BC	Techno grow mix with 4% of biochar (w/w)

Second Experiment (Wood Distillate Treatments)

Treatment Code	Foliar Spray	Substrate Composition
C	Tap water	Techno grow mix only (control)
SS	3mL/L	Tap water
2.5WD	3mL/L	2.5mL/L of wood distillate (v/v)
5WD	3mL/L	5.0mL/L of wood distillate (v/v)
10WD	3mL/L	10.0mL/L of wood distillate (v/v)

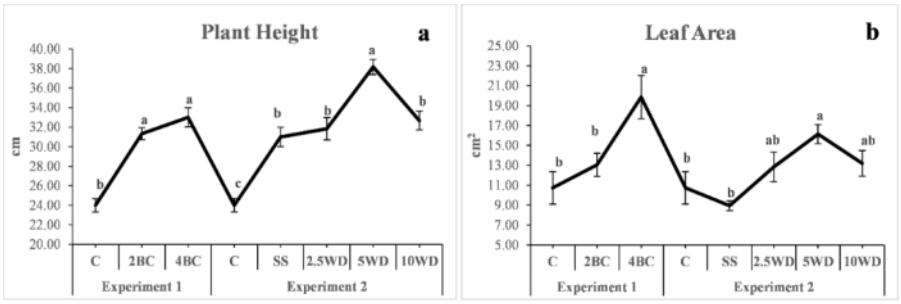
Table 1 *Treatments of both experiments*.

3. Result and Discussion

3.1 Influence of biochar and wood distillate on morpho-physiological attributes of strawberry plant.

In experiment 1, biochar treatments, regardless of concentrations, significantly increased plant height compared to the control (Fig.1a). Similarly, leaf area was highest under the highest biochar concentrations (Fig.1b). In experiment 2, wood distillate application at 5mL/L significantly enhanced plant height (Fig.1a).
Leaf area was also significantly higher with the 5mL/L wood distillate treatment (Fig. 1b).
Physiological parameters responded variably to the treatments. Experiment 1 showed significantly higher leaf flavonoid content (FLv) under both biochar concentrations compared to control.
Chlorophyll content (CCLa) and SPAD values were significantly increased only by the highest biochar concentrations (Table 2).
In experiment 2, FLv content was significantly highest under the 2.5mL/L wood distillate treatments. CCLa was significantly highest under 5ml/l treatments, while SPAD values were significantly elevated in both 5mL/L and foliar spray (SS) treatments compared to the control (Table 2).
The positive influence in the morpho-physiological parameters in the strawberry plants under the application of biochar and wood distillate suggested that it might be due to improvement in nutrient availability and particularly usage of optimal treatment concentration.

Figure 1 *Plant height (a) and leaf area (b) of the strawberry plants of both experiments represented.*



One-way ANOVA. Tukey's test, $p \leq 0.05$. Per each experiment, within each parameter different letters indicate statistically different values. C: control, 2BC: 2% of biochar, 4BC: 4%biochar, SS: foliar spray 3mL/L, 2.5WD: 2.5mL/L, 5WD: 5mL/L and 10WD: 10mL/L

Table 2 *Physiological attributes of the strawberry plants under both types of experiment.*

	FLv	CCLa	SPAD
Experiment 1			
C	0.27±0.02b	20.63±0.77b	37.87±0.52b
2BC	0.35±0.02a	20.12±0.81b	37.57±0.50b
4BC	0.36±0.01a	24.17±0.91a	39.84±0.48 a
Experiment 2			
C	0.27±0.02c	20.63±0.77b	37.87±0.52b
SS	0.37±0.02ab	25.02±0.94b	40.27±0.48 a
2.5WD	0.46±0.03a	24.11±0.89ab	39.84±0.45ab
5WD	0.45±0.02ab	25.96±0.71a	40.80±0.32 a
10WD	0.37±0.02b	24.11±1.41ab	39.60±0.72ab

One-way ANOVA. Tukey's test, $p \leq 0.05$. Per each experiment, within each parameter different letters indicate statistically different values. C: control, 2BC: 2% of biochar, 4BC: 4%biochar, SS: foliar spray 3mL/L, 2.5WD: 2.5mL/L, 5WD: 5mL/L and 10WD: 10mL/L. FLv: Flavonoid content (F660nm/F325nm), CCLa: Chlorophyll content (T850nm/T720nm) and SPAD index.

3.2 Influence of biochar and wood distillate on yield and fruit quality of strawberry plant.

Fruit yield (total number of fruit) was not significantly affected by biochar or wood distillate treatments in either experiment, despite numerical trends: the highest yield occurred with 4% biochar in experiment 1 and in the control for experiment 2 (Fig. 2).

The Fruit skin color attributes responded variably to the concentrations. In experiment 1, the control and 4% biochar showed significantly higher L* values than 2% biochar but did not differ from each other. In experiment 2, the control had significantly higher L* than the 2.5mL/L and 5mL/L wood distillate treatments.

a* in experiment 1, showed significantly higher values in control than 2% biochar, while control and 4% biochar did not differ. No significance in a* was observed among any wood distillate treatments in experiment 2.

b* in experiment 1, both the control and 4% biochar had significantly higher b* values than 2% biochar but were not significantly different from each other. In experiment 2, the control and 10mL/L treatments showed significantly higher b* than 2.5mL/L, with other treatments exhibiting intermediate values (Table 3).

Above mentioned results indicated that biochar and wood distillate had a limited impact on the reproductive stage of the strawberry. These results also suggested the varying results due to different concentrations levels on the vegetative stage.

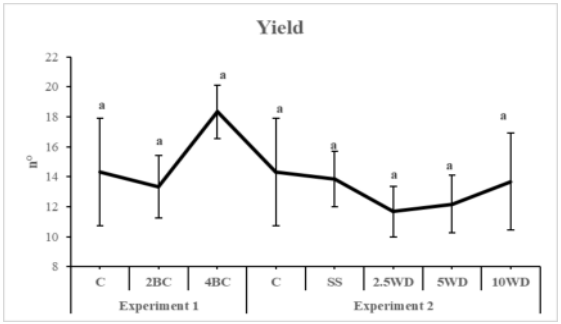


Figure 2 *Total number of harvested fruits of the strawberry plants of both experiments. Per each experiment, within each parameter different letters indicate statistically different values. ns: non statistically significant. C: control, 2BC: 2% of biochar, 4BC: 4%biochar, SS: foliar spray 3mL/L, 2.5WD: 2.5mL/L, 5WD: 5mL/L and 10WD: 10mL/L. One-way ANOVA. Tukey's test, $p \leq 0.05$*

Table 3. *Fruit quality attributes of the strawberry plant under both types of experiment*

	L*	a*	b*
Experiment 1			
C	39.00±0.33a	29.61±0.34a	28.83±0.44a
2BC	36.62±0.36b	28.16±0.45b	26.65±0.50b
4BC	38.36±0.47a	28.85±0.43ab	28.55±0.50a
Experiment 2			
C	39.00±0.33a	29.61±0.34a	28.83±0.44a
SS	37.66±0.54ab	27.91±0.55a	27.83±0.71ab
2.5WD	36.88±0.45b	28.26±0.45a	26.65±0.59b
5WD	37.11±0.42b	28.41±0.43a	28.01±0.55ab
10WD	38.39±0.46ab	28.34±0.47a	29.07±0.61a

One-way ANOVA. Tukey's test, $p \leq 0.05$. Per each experiment, within each parameter different letters indicate statistically different values. C: control, 2BC: 2% of biochar, 4BC: 4%biochar, SS: foliar spray 3mL/L, 2.5WD: 2.5mL/L, 5WD: 5mL/L and 10WD: 10mL/L. L*: lightness, a*: redness, b*: yellowness.

3.3 Influence of biochar and wood distillate biochemical analysis of strawberry plant.

In experiment 1, fruit from the 2% biochar treatment had a significantly higher pH compared to other treatments. In experiment 2, the 5mL/L wood distillate treatment resulted in a significantly higher pH, while 10mL/L showed a significantly lower pH (Table 4).

Titratable acidity (TA) was significantly increased by the highest concentrations: 4% biochar in experiment 1 and 10mL/L wood distillate in experiment 2 (Table 4).

Total soluble acid (TSS, Brix°) was significantly increased under 2% biochar in experiment 1. In experiment 2, the 2.5mL/L treatments submitted significantly higher TSS than other treatments, while the control showed the lowest TSS (Table 4).

The TSS/TA ratio was significantly higher in the control and 2% biochar treatments compared to 4% biochar in experiment 1, although the control and 2% biochar did not differ. In experiment 2, the 2.5mL/L treatments had a significantly higher ratio than 10mL/L (Table 4).

The total phenolic content and the antioxidant (DPPH), in both experiments did not show statistical significance (Table 4).

These results suggested that moderate biochar and wood distillate doses can improve the biochemical attributes by enhancing the nutrient availability and metabolic activity. These also could be due to improvement of sugar accumulation. Higher titratable acidity may result due to increased organic acid synthesis under mild stress. Lack of differences in the TPC and DPPH traits suggested that these treatments did not strongly trigger the secondary metabolic pathway.

Table 4 *Influence of biochar and wood distillate on biochemical attributes of the strawberries for of experiment.*

	pH	TA	TSS	TSS/TA	TPC	DPPH
Experiment 1						
C	3.62±0.00b	1.63±0.01c	5.77±0.03b	3.5±0.0a	1381.48±203.7a	6.55±1.27a
2BC	3.86±0.00a	2.02±0.01b	7.13±0.03a	3.5±0.0a	1752.77±67.6a	5.69±0.29a
4BC	3.56±0.01c	2.49±0.04a	5.07±0.03c	2.0±0.0b	1805.55±255.5a	6.99±0.06a
Experiment 2						
C	3.62±0.00d	1.63±0.01d	5.77±0.03c	3.5±0.0b	1381.48±203.7a	6.55±1.27a
SS	3.97±0.00b	1.91±0.08c	5.27±0.03d	2.8±0.1c	1533.33±159.2a	4.82±1.11a
2.5WD	3.92±0.00c	1.63±0.01d	7.83±0.03a	4.8±0.0a	1232.41±110.1a	5.45±0.66a
5WD	4.43±0.01a	2.15±0.07b	7.67±0.03b	3.6±0.1b	1159.26±133.3a	5.37±1.40a
10WD	3.56±0.01e	3.19±0.02a	7.63±0.03b	2.4±0.0d	2048.15±444.4a	5.50±0.25a

One-way ANOVA. Tukey's test, $p \leq 0.05$. Per each experiment, within each parameter different letters indicate statistically different values. C: Control, 2BC: 2%biochar, 4BC: 4% biochar, SS: wood distillate foliar spray; 2.5mL/L, 5mL/L and 10mL/L: wood distillate application on soil. TA: Titratable Acidity (%), TSS: Total Soluble Solids (°Brix), TSS/TA ratio between TSS TPC: Total Phenol Content (mg/ml), DPPH: Antioxidant Capacity (mg/ml).

4. Conclusion and Future Perspectives

Research highlights the positive impact of biochar (any concentration) and wood distillate (optimal at 5ml/l) on the morphological, physiological, and biochemical traits of the strawberry plants. Biochar, regardless of its concentration, significantly enhanced plant morphology and physiology, while the wood distillate showed a more promising effect at the 5mL/L concentrations. Although the yield was not improved, the fruit quality attributes were influenced by the specific treatments. Future research should explore the combined long-term effects of biochar and wood distillate across the different strawberry cultivars and the growth environments to optimize sustainable strawberry production strategies. Additionally, exploring different strawberry cultivars and various environmental conditions will be essential to develop optimized sustainable production strategies. By following these strategies, these can maximize both yield and fruit quality while supporting the plan health and soil fertility.

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Genomic and physiological insights into bacterial heavy metal resistance for environmental decontamination

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This PhD project investigates microbial resistance to heavy metals, with the aim of developing sustainable biotechnological strategies for the decontamination of industrial wastewater. Through a combination of physiological, biochemical, and genomic-transcriptomic approaches, the project characterizes key microbial mechanisms, such as biosorption, enzymatic reduction, and stress adaptation, in response to copper, chromium, and cadmium. Selected bacterial strains showed high metal-removal efficiency and plant growth-promoting abilities. These results support the design of eco-friendly bioremediation strategies.

Analisi genomiche e fisiologiche della resistenza batterica ai metalli pesanti finalizzate alla decontaminazione ambientale

Il presente progetto di dottorato si propone di studiare la resistenza microbica ai metalli pesanti, con l'obiettivo di sviluppare strategie biotecnologiche sostenibili per la decontaminazione delle acque reflue industriali. Attraverso un approccio integrato fisiologico, biochimico e genomico-trascrittomico, il progetto caratterizza i principali meccanismi microbici coinvolti, come la biosorbimento, la riduzione enzimatica e l'adattamento allo stress, in risposta a rame, cromo e cadmio. Alcuni ceppi batterici selezionati hanno mostrato un'elevata efficienza nella rimozione dei metalli e proprietà promotrici della crescita delle piante. Questi risultati supportano lo sviluppo di strategie di biorisanamento ecocompatibili.

Key words: Heavy metals; reduction; adsorption; transcriptomic analysis; bioremediation.

1. Introduction

This PhD project aims to deepen the understanding of heavy metals (HM) remediation by integrating microbial biosorption, enzymatic reduction, transcriptomic analysis, and plant-microbe interaction studies. This oral communication summarizes the key outcomes of the following three experimental activities:

A1) Evaluation of chromium reduction and transcriptomic responses in *Rhodococcus erythropolis* strain SC26
A2) Investigation of copper biosorption by *Serratia plymuthica* strain As3-5a(5) in both planktonic and biofilm systems, using real electroplating wastewater.
A3) Assessment of the role of plant growth-promoting bacteria (PGPB), particularly *Pseudomonas koreensis* strain 69RS, in alleviating cadmium toxicity in crops.

2. Heavy metals toxicity and environmental impact

Over the past few decades, various industrial activities, such as welding, dyes and pigments production, electroplating, leather tanning, agricultural, and wood preservation, have been identified as major contributors to HM contamination in water systems (Singh *et al.,* 2023). These pollutants, such as copper (Cu), chromium (Cr) and cadmium (Cd), significantly lower water quality and pose serious risks to both human health and ecosystems due to their high toxicity, persistence, and non-biodegradable nature (Nyika *et al.,* 2022). Their accumulation in crops not only compromises food safety but also undermines agricultural productivity and economic sustainability (Yaashikaa *et al.,* 2022). Addressing HM pollution has therefore become a pressing global challenge, requiring the development and implementation of sustainable and effective remediation strategies (Jain *et al.,* 2022).

3. Bacterial mechanisms for heavy metal decontamination

Bacteria counteract HM toxicity through several mechanisms such as biosorption, enzymatic reduction, active efflux and precipitation.

Extracellular polymeric substances (EPS), rich in functional groups like carboxyl, hydroxyl, and amino groups, play a crucial role in biosorption (Zadeh *et al.,* 2023). Composed primarily of proteins, polysaccharides, lipids, and nucleic acids, EPS forms a protective matrix around microbial cells that enhances resistance to environmental stressors and improves metal adsorption capacity (Liu *et al.,* 2024). EPS proteins, particularly those with carboxyl groups, exhibit high binding affinity for metal cations including Cu(II) (Liu *et al.,* 2024). Cr(VI), a highly toxic and mobile species, enters bacterial cells *via* sulphate transporters and is enzymatically reduced to Cr(III), a less toxic form through intra- or extracellular redox reactions (Ramli *et al.,* 2023). This reduction process induces

oxidative stress, which is mitigated by enzymes such as catalase, glutathione S-transferase (GST), and superoxide dismutase (SOD) (Thatoi *et al.,* 2014). Cadmium (Cd) is often accumulated in agricultural crops. Plant growth-promoting (PGP) bacteria can reduce Cd bioavailability and enhance stress tolerance by promoting immobilization, efflux, or conversion into insoluble forms (e.g., CdS, $CdCO_3$; Anand *et al.,* 2023). In crops like rice (*Oryza sativa*) and tomato (*Solanum lycopersicum*), Cd uptake occurs *via* transporters shared with essential nutrients like Zn(II), Fe(II), and Mn(II) (Peera Sheikh Kulsum *et al.,* 2023). These microbial mechanisms offer a sustainable and cost-effective alternative to conventional HM remediation approaches.

4. Experimental Procedure

In this PhD thesis, a five-stage experimental framework was developed to investigate bacterial interactions with HM and their applications in bioremediation. The study was structured as follows: i) characterization of *Rhodococcus erythropolis* strain SC26 to assess its ability to enzymatically reduce Cr(VI) to Cr(III); ii) transcriptomic and genomic analyses of *R. erythropolis* SC26 to identify redox pathways and metal resistance genes; iii) adsorption experiments using *Serratia plymuthica* strain As3-5a(5) in planktonic form to evaluate its removal efficiency toward Cu(II) ions; iv) biosorption tests on electroplating wastewater using both planktonic and biofilm-forming *S. plymuthica* cultures, to assess Cu(II) biosorption capacity; v) evaluation of PGP traits in *Pseudomonas koreensis* strain 69RS under Cd(II) stress, to determine its capacity to mitigate Cd(II) toxicity and its potential for assisted phytoremediation.

5. Materials and Methods

All experiments were carried out under laboratory-scale conditions to evaluate microbial responses to HM and their potential in bioremediation.
Cr(VI) reduction by *R. erythropolis* strain SC26 was assessed in Cr(VI) solutions (0.5–3 mM) or electroplating wastewater (2 mM). Metal concentration was determined using the diphenylcarbazide method (DPC; Dean and Beverly, 1958) and confirmed *via* inductively coupled plasma-mass spectrometry (ICP-MS). Growth kinetics were modelled using Monod and Haldane equations (Arutchelvan *et al.,* 2019). Enzymatic activity and localization was determined by using cell lysates and supernatants. Reactive oxygen species (ROS) production was analysed using the DCFH-DA assay (Firrincieli *et al.,* 2024; Min *et al.,* 2024). Transcriptomic profiling (RNA-Seq, DESeq2) was used to identify differentially expressed genes under Cr(VI) stress. Cu(II) biosorption was assessed using *S. plymuthica* As3-5a(5) in both planktonic and biofilm systems, using a synthetic $CuCl_2$ solution (3.14 mM) or electroplating wastewaters (0.5, 4.39, and 40.33 mM). EPS was extracted from cultures harvested at 24, 48, and 72 h using unwashed, water-washed, and buffer-washed cells (Zanetti *et al.,* 2022). Adsorbed Cu(II) was quantified spectrophotometrically (Pallenberg et al., 1995) and validated using ICP-MS. PGP and Cd resistance tests were conducted on *P. koreensis* 69RS. PGP traits included nitrogen fixation, phosphate solubilization, indole-3-acetic acid (IAA) production, siderophore release, EPS production (Melzi *et al*., 2024), and ACC deaminase activity (Dell'Amico *et al.,* 2008). Quantitative ACC deaminase assays were performed under Cd stress ($CdCl_2$). Osmotic stress tolerance was assessed using NaCl, PEG, and sodium arsenate (Sosa *et al.,* 2005). Germination assays under Cd exposure were performed on *Oryza sativa* and *Solanum lycopersicum* (Melzi *et al.,* 2024). *P. koreensis* 69RS Cd biosorption was investigated by ICP-MS.

6. Results and Discussion

6.1 Cr (VI) reduction by *Rhodococcus erythropolis* strain SC26 growing cells

Strain SC26 displayed a Cr(VI) MIC of 4 mM, consistent with previous studies (Banerjee *et al*., 2017). Growth modelling using Monod and Haldane equations identified an early inhibitory threshold at 0.38 mM and a growth suppression at 3.68 mM. In low-oxygen conditions (sealed vials), the strain achieved 98% reduction of 1 mM Cr(VI) within 169 hours. In flasks, 50% of the metal was reduced in 24 hours, reaching a maximum of 60% after 145 hours. These results highlight the strain's efficient Cr(VI) reduction, consistent with findings reported by Gautam *et al*. (2022).

6.2 Enzymatic activity

Cr(VI) exposure strongly induced chromate reductase activity, particularly in cell-free lysates, indicating an intracellular localization of the reductive pathway (Figure 2a). This contrasts with reports describing predominantly extracellular activity, suggesting an involvement of the extracellular chromate reductase or extracellular electron mediators (Chen *et al.,* 2018; Cao *et al.,* 2023).GST activity, associated with membrane fractions, peaked at 1 mM Cr(VI) but decreased at 2 mM, suggesting partial inhibition under higher stress (Figure 2b). ROS assays confirmed elevated oxidative stress in exposed cells, although continuous exposure appeared to induce enzymatic adaptation and reduced ROS levels.

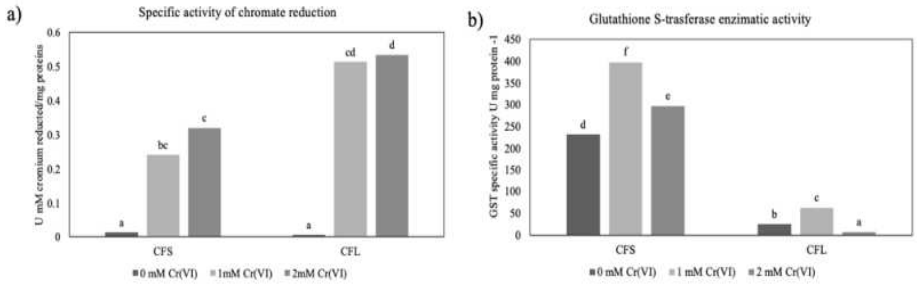


Figure 2 *Specific chromate reduction activity (a) and GST (b) activity of strain Rhodococcus erythropolis SC26 after 24h growth in the absence and presence of 1 and 2 mM of Cr(VI) determined in the cell free supernatant (CFS) and cell free lysate (CFL). (Tukey's test, $p \leq 0.05$).*

6.3 Whole genome-analysis and differential gene expression

Whole-genome sequencing revealed a genome of 6.9 Mbp, comprising one chromosome and four plasmids (two circular and two linear). Transcriptomic analysis revealed that 47% of genes were consistently upregulated under both Cr(VI) concentrations tested. These genes were mainly involved in cofactor biosynthesis, amino acid metabolism, and redox balance. As reported in other studies, classical chromate transporter or reductase genes were not detected, suggesting alternative mechanisms (Cao *et al.,* 2023). Two putative electron transfer pathways involved in Cr(VI) reduction were proposed: a faster NAD(P)H-driven route involving SDR oxidoreductases and quinone oxidoreductases; a secondary pathway with FAD-binding oxidoreductases transferring electrons *via* $FADH_2$ to nitroreductases.

6.4 Evaluation of *Serratia plymuthica* As3-5a(5) Cu(II) adsorption and EPS quantification

EPS composition influenced metal adsorption. Cells harvested at 72h and washed with buffer showed the highest Cu(II) removal efficiency (92%). Reduced polysaccharide content in EPS correlated with increased Cu(II) adsorption, highlighting the importance of tightly bound protein fractions. Spectroscopic data confirmed the preferential binding of Cu(II) to carboxyl groups in proteins. Supporting this hypothesis, Liu *et al*. (2024) reported that proteins exhibited significantly higher adsorption capacities for HM, such as Cu(II), Zn(II), and Cd(II), compared to polysaccharides. Interestingly, the adsorption process occurs within just 4 minutes of contact between the cells and the metal ions. Two-dimensional correlation spectroscopy further revealed that carboxyl groups in proteins preferentially and more rapidly bind Cu(II) ions than those in polysaccharides (Liu *et al.,* 2024). Flow cytometry confirmed a decrease in surface polysaccharides following buffer washing without affecting cell viability. SEM-EDS analysis validated Cu adsorption on the bacterial surface and revealed increased EPS production during late growth phases, which enhanced biofilm structure and metal interaction.

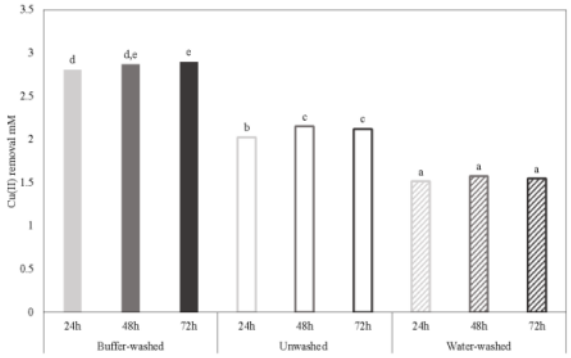


Figure 3 *Cu(II) adsorption measured at 24-, 48-, and 72-hours incubation following different cell treatments (buffer-washed, unwashed, and water-washed) (Tukey's test, $p \leq 0.05$).*

6.5 Biofilm formation and Cu(II) adsorption from real electroplating wastewaters

As3-5a(5) formed robust biofilms on sintered glass, which enabled up to 98% Cu(II) removal from synthetic solutions within 4 minutes. Mendrinou *et al.* (2021) demonstrated that bacterial biofilm of *Halomonas denitrificans*, formed on sintered glass, was able to completely remove a 1.5 mM Cu(II) solution. Sintered glass was used as solid support because its porous nature provided a large surface area, facilitating bacterial colonization (Jenjitwanich *et al.,* 2024). In real electroplating wastewaters (Cu: 0.5–40.3 mM), biofilm systems achieved higher Cu(II) removal than planktonic cultures, reaching up to 89% removal. Increasing planktonic biomass from 10^{10} to 10^{11} cells mL^{-1} enhanced removal from 10% to 97%, confirming the direct relationship between biomass and adsorption capacity.

6.6 PGP traits and cadmium tolerance of selected strains

Two strains, *P. koreensis* 69RS and *S. plymuthica* As3-5a(5), exhibited PGP functions and showed moderate to high cadmium (Cd) tolerance (up to 4 mM). Osmotic stress assays confirmed the resilience of *S. plymuthica* SC3-I2 to $NaAsO_2$, NaCl, and PEG 6000 treatments, identifying it as suitable candidate for drought- or salinity-stress relief. Testing the ability of PGP bacterial strains to tolerate osmotic stress generated by sodium arsenate is particularly interesting when considering their application in the bioremediation of rice aerobic fields, since Cd translocation to the grain is higher (Tripti *et al.,* 2018). Cd MIC assays performed in TMMG medium confirmed the highest tolerance in the *Serratia* strains, with MICs up to 1 mM. The results agree with MIC of these bacteria genera reported in literature (Diez-Marulanda *et al.*, 2023).

Quantitative ACCD assays showed significantly increased enzymatic activity under Cd stress in *P. koreensis* 69RS and *S. plymuthica* As3-5a(5), indicating a strain-specific response to metal-induced stress. Zainab *et al.* (2020) reported that the ACCD activity of two HM resistant bacterial strains increased under chromium stress.

6.7 Growth pouches assays under cadmium stress and Cd adsorption

Growth pouches assays demonstrated that *P. koreensis* 69RS and *S. plymuthica* As3-5a(5) significantly promoted root and shoot growth of *Oryza sativa* and *Solanum lycopersicum* under 0.1 mM and 0.5 mM Cd stress. These findings are consistent with previous studies reporting the ability of *Serratia* strains to enhance plant growth and mitigate Cd-induced stress. For instance, the application of *Serratia* to rice seedlings improved germination and early growth even in the presence of Cd (Kotoky *et al.,* 2019). Similarly, *P. koreensis* has been reported to promote maize growth under 0.5 mM Cd stress conditions (Vincze *et al*., 2024).

Cd adsorption assays showed that *P. koreensis* 69RS removed 80% of Cd(II) within 4 minutes, suggesting a rapid surface adsorption. This aligns with previous reports of efficient Cd detoxification *via* ACCD–positive strains that alleviate plant toxicity (Dell'Amico *et al.,* 2008).

7. Conclusions and Future Perspectives

This study highlights the effectiveness of microbial strategies such as biosorption, enzymatic reduction, and PGP mechanisms as promising approaches for the remediation of HM including copper, chromium, and cadmium. *S. plymuthica* As3-5a(5) exhibited high Cu(II) adsorption capacity in both planktonic and biofilm systems, with adsorption efficiency influenced by the composition of EPS. Biofilm formation on sintered glass carriers significantly enhanced Cu(II) removal from electroplating wastewater, indicating its potential for scalable biofilter applications. *R. erythropolis* SC26 demonstrated Cr(VI) reduction capacity, supported by transcriptomic evidence showing upregulation of oxidoreductase genes and stress response pathways, along with enzymatic data confirming intracellular Cr(VI) reduction activity. *Pseudomonas koreensis* 69RS and *S. plymuthica* As3-5a(5) exhibited strong PGP traits, Cd(II) tolerance, and modulation of ACCD activity in response to metal exposure, promoting germination under Cd stress.

Overall, these findings support the use of microbial resources as sustainable and adaptable tools for environmental cleanup, with potential applications ranging from industrial wastewater treatment to the restoration of polluted agroecosystems. Future research should focus on validating these results under field conditions, and integrate microbial biotechnologies into scalable, low-impact treatment systems aligned with circular economy principles. Studies should evaluate microbial persistence, colonization dynamics in the rhizosphere, and interactions with native microbiota. Additionally, the implementation of biofilm-based bioreactors in industrial wastewater treatment represents a promising technological advance. Finally, genome editing may help produce optimized microbial strains for targeted metal remediation and plant support, enhancing future bioremediation strategies.

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Microbiome Mapping in Meat Food Chain from Farm to Fork

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Meat spoilage is majorly driven by microbial activity. Therefore, this longitudinal study investigates the structural and functional profiles of the microbiome in beef processing chains from farm-to-fork. We collected 712 samples per two seasons, from four beef processing chains, covering 4 animals per facility, contact and non-contact surfaces, in different locations (farm, slaughter hall, chiller hall, boning hall, retail packing hall, carcasses, processing final meat product, and matured meat (continuous vs retail stage maturation). Leveraging shotgun metagenomics, de novo assembly (MEGAHIT), and PhageBox2, we identified virulent phage contigs and matched them against Kraken2-derived host abundances. Mixed-effects modelling revealed both suppressive and, surprisingly, augmentative phage-host associations. Our results highlight key spoilage and pathogenic taxa whose abundances are driven down by phages as well as those enriched in phage-rich niches shedding new light on phage-mediated ecological shifts during meat processing environments and meat products. These insights pave the way for harnessing native phages as targeted biocontrol tools to extend shelf life and bolster food safety.

Mappatura del microbioma nella catena alimentare della carne, dal campo alla tavola

Il deterioramento della carne è principalmente causato dall'attività microbica. Pertanto, questo studio longitudinale indaga i profili strutturali e funzionali del microbioma nelle catene di lavorazione della carne bovina dalla fattoria alla tavola. Abbiamo raccolto 712 campioni per due stagioni, da quattro catene di lavorazione della carne bovina, coprendo 4 animali per struttura, superfici di contatto e senza contatto, in luoghi diversi (azienda agricola, sala di macellazione, sala di refrigerazione, sala di disassamento, sala di confezionamento al dettaglio, carcasse, lavorazione del prodotto finale a base di carne e carne frollata (maturazione continua rispetto alla fase di vendita al dettaglio)). Sfruttando la metà genomica shotgun, l'assemblaggio de novo (MEGAHIT) e PhageBox2, abbiamo identificato contigui di fagi virulenti e li abbiamo confrontati con le abbondanze di ospiti derivate da Kraken2. La modellazione a effetti misti ha rivelato associazioni fago-ospite sia soppressive che, sorprendentemente, aumentative. I nostri risultati evidenziano taxa chiave di deterioramento e patogeni le cui abbondanze sono ridotte dai fagi così come quelli arricchiti in nicchie ricche di fagi, gettando nuova luce sui cambiamenti ecologici mediati dai fagi durante gli ambienti di lavorazione della carne e i prodotti a base di carne. Queste intuizioni aprono la strada allo sfruttamento dei fagi nativi come strumenti di bio-controllo mirati per prolungare la durata di conservazione e rafforzare la sicurezza alimentare.

Key words: Meat spoilage, microbial dynamics, antimicrobial resistance genes, bacteriophages, mobile genetic elements

1. Introduction

The Ph.D. thesis project aims to evaluate and compare the taxonomic and functional potential of the microbiome in four distinct beef processing chains. In accordance with the previously described PhD thesis project, the main activities during this project were:

(A1) Comprehensive sampling from the four beef processing chains. DNA extraction from surface swabs and meat samples. Samples preparation for shotgun metagenomic sequencing.
(A2) Raw sequencing filtering, metagenomic assembly, and metagenomic-assembled genomes development.
(A3) Taxonomic and functional profiling of the assembled contigs for spoilage related genes, antimicrobial resistance genes, transposons, plasmids, integrons and bacteriophages.
(A4) Statistical analysis and data visualization.

2. Materials and Methods

In October 2023, and March 2024, four beef processing facilities in different Irish cities were visited, and 712 samples were collected. These included swabs from carcasses, various meat contact and non-contact surfaces, post-cleaning and during production phases, and operator's hands. Samples were stored at −80 °C and then shipped to Italy in the subsequent months. They were pre-processed with PBS buffer, followed by Stomacher for microbial cells detachment. DNA extraction was performed using the using the Molysis Complete5 kit. DNA was quantified with the Qubit HS Assay (Thermo Fisher Scientific, US). Metagenomic libraries were prepared (Illumina, US) and sequenced on an Illumina NovaSeq platform leading to 2 × 150 bp, paired end reads. Bioinformatic analysis involved filtering the host reads using BMTagger tool, and quality-checking and raw-reads filtering, were performed using Prinseq-lite (Schmieder and Edwards, 2011). Taxonomic profiles were obtained using Kraken2.

While functionality, especially for antimicrobial resistance genes, biocides resistance genes, mobile genetic elements and spoilage related genes were generated using Card, Bacmet, Mobile Genetic Elements Finder, and a custom database of spoilage related genes respectively (Blanco-Míguez et al. 2023). The phage contigs were obtained using the Phabox2 tool. Data visualization and statistical analysis were performed in R environment. Kruskal-Wallis and Wilcoxon rank sum tests were used to assess significant differences. The 'vegan' package was used to compute alpha diversity indices and bray-curtis distance dissimilarity were calculated using the vegdist function of the vegan package. While 'ggplot2′ package was used for visualizations (Sequino et al. 2024).

3. Results and Discussion

3.1 Determination of the Microbial Diversity:

Primarily, the taxonomic resolution using alpha diversity indices (Shannon, Simpson, and Richness) revealed significant differences in microbiome diversity (Shannon and Simpson) among the different major locations across the two seasons. However, this significance was lost among the continuous and retail-stage mature meat. The diversity among the mature meat products was found maximum in the initial stages of maturation but then this significance was also lost among the major locations was also lost (Fig. 1). Similarly, the beta-diversity among the major locations across the two seasons was found significantly different. Most of the ellipses were found overlapping among the major locations. However, the summer samples were not very distant as compared to the winter samples (Fig. 2). The taxonomic composition among the major locations across the two seasons was significantly different. The top 20 species were mostly abundant in summer samples, while its abundance in winter samples in most locations were absent. Similarly, the top 20 species abundances were quite similar in both seasons in all major locations. However, there were some taxa differences among both the continuous and retail-stage meat samples (Fig. 3). These results reveal the significant impact of seasonal temperatures, major location-based temperature change along the processing stages, and the different maturation protocols on the bacterial taxonomic composition and abundance.

3.2 Analysis of the Bacteriophages in the entire Processing Chains:

The phabox2 identified phage related contigs in all contigs generated by megahit tool. Then we used several mixed effects models taking major locations, seasons, microbial load, bacterial hosts, and facilities as fixed and random effects in all these models. A total of 12 different models were employed and evaluated based on AIC and BIC scores and selected the best fit model among them. The phage impact on microbial host abundance was found different for different hosts and in different locations. For some bacterial hosts it showed suppressive and for some it showed augmentative role, but these associations were entirely location dependent. For example, for some bacterial hosts in a specific location it showed suppressive role while for the same bacterial host it showed augmentative role in another location (Fig. 4). These results reveal that phages take-on active killing action in some locations to show its suppressive role, while the augmentative role may be due to the high number of bacterial hosts in some locations and phage infection dynamics in the entire processing chain.

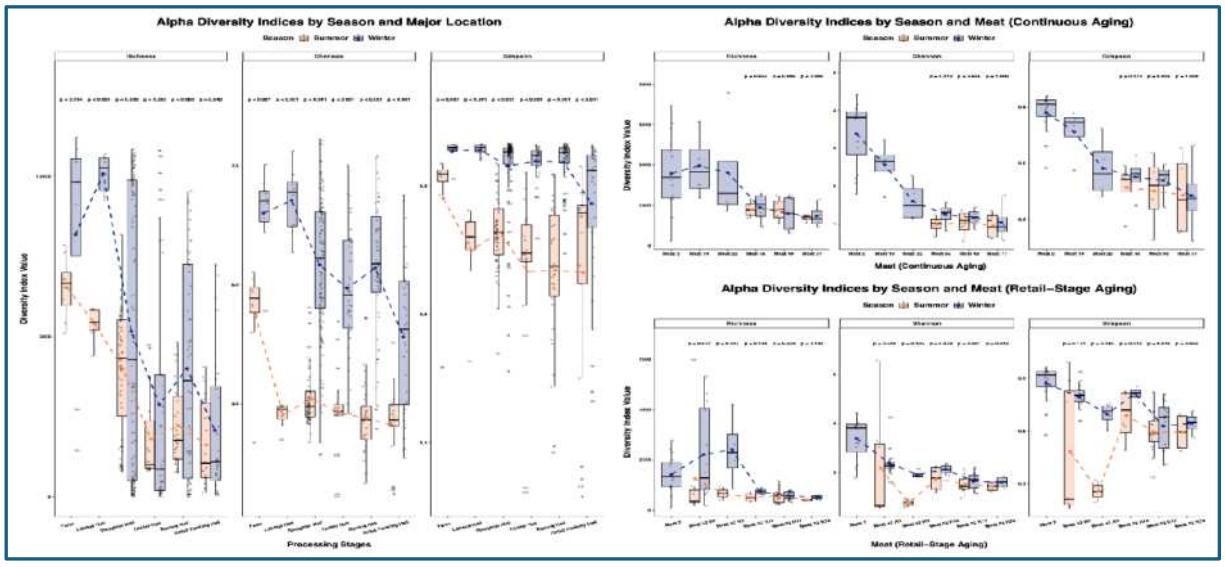


Figure. 1: This figure demonstrates the alpha-diversity indices (Shannon, Simpson, and Richness) among the major locations across the two seasons in the processing stages in the processing stages and then the two vertical plots show the alpha-diversity indices among the continuous stored meat (without interruption) and retail-stage matured meat.

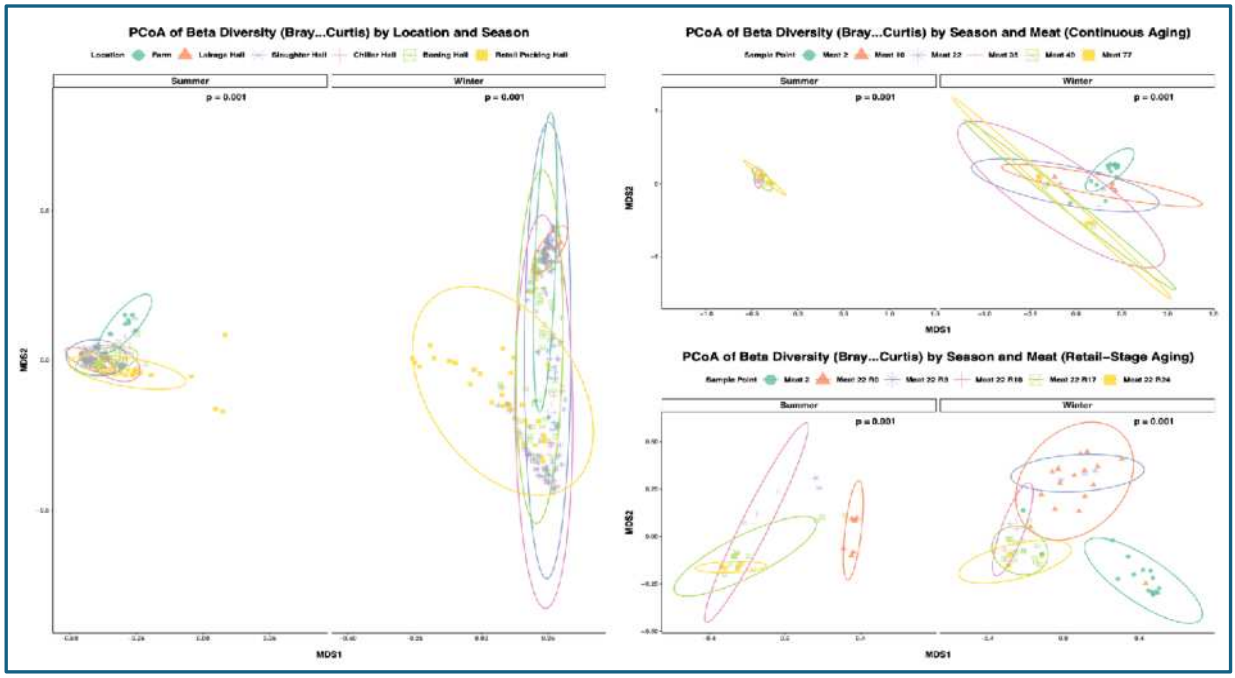


Figure. 2: This figure shows the beta-diversity index (using Bray-Curtis dissimilarity method) among the major locations across the two seasons in the processing stages in the processing stages and then the two vertical plots show the beta-diversity indices among the continuous stored meat (without interruption) and retail-stage matured meat.

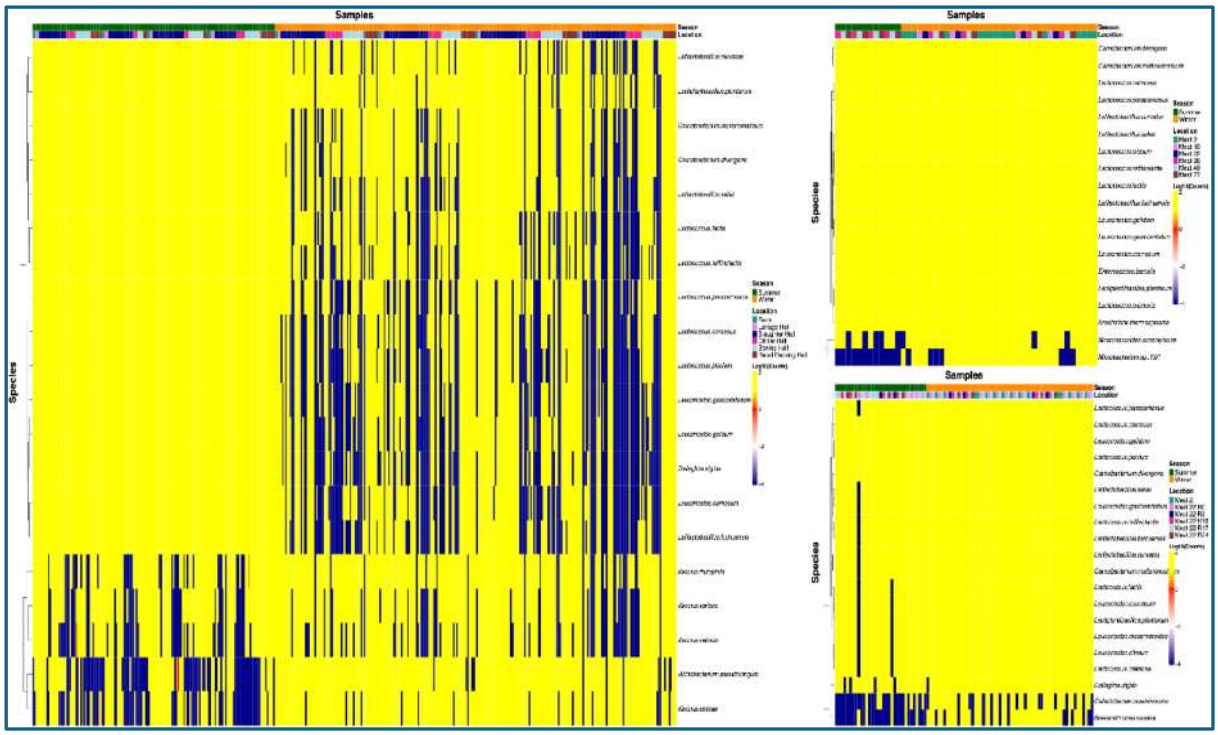


Figure. 3: This figure shows the taxonomic composition and abundance of top 20 bacterial taxa among the major locations across the two seasons in the processing stages and then the two vertical plots show this difference among the continuous stored meat (without interruption) and retail-stage matured meat.

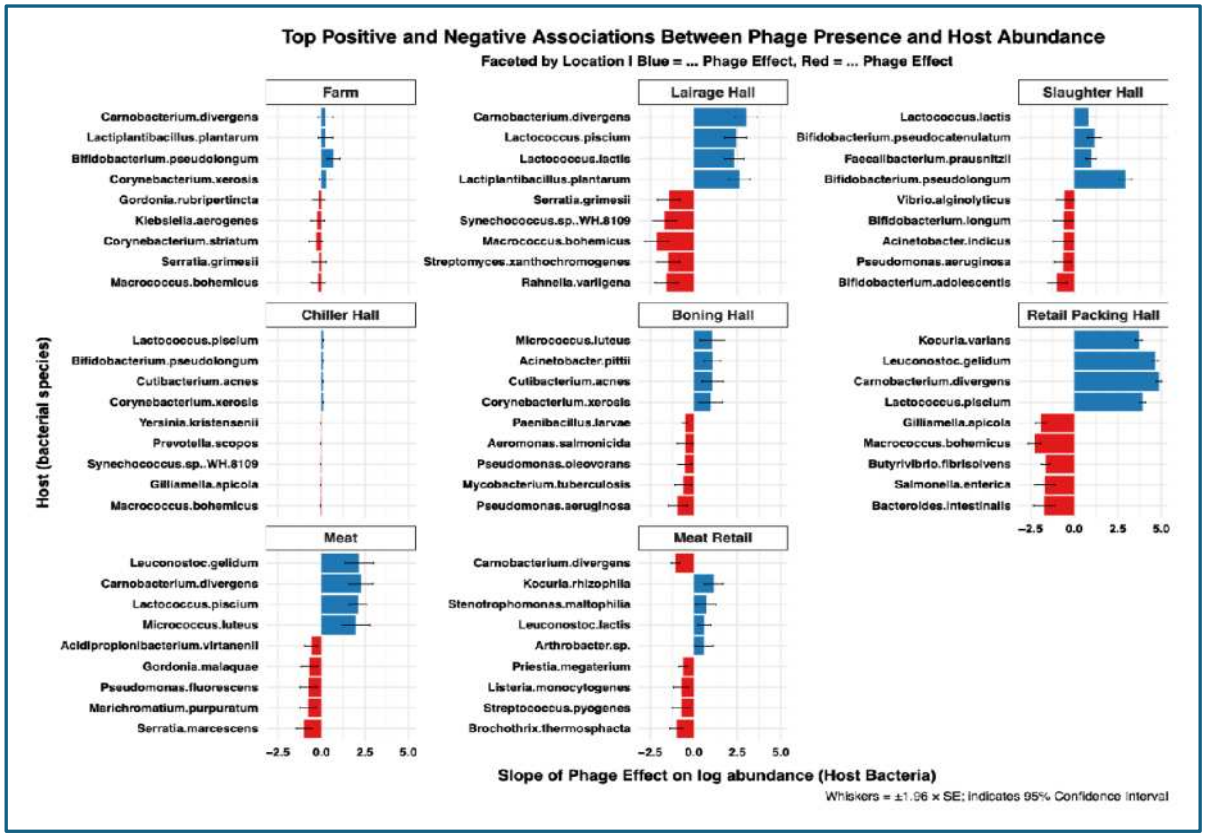


Figure. 4: This figure shows the impact of bacteriophages on top 5 suppressed (red color) bacterial hosts and 4 augmented (blue color) across the major locations. The whiskers show the 95% confidence intervals of each bacterial-host and phage association.

4. Conclusions and Future Perspectives

This work demonstrates that naturally occurring, virulent bacteriophages are not mere bystanders but active architects of the microbial community structure throughout the beef processing and wet meat aging. By mapping phage-host interaction slopes at each processing stage, we reveal a dual role for phage's: they can both suppress spoilage organisms and, in select contexts, indirectly promote certain bacteria, likely through competition or infection dynamics. This level of longitudinal resolution is unprecedented in meat microbiome research. Practically, these findings unlock the potential to deploy tailored phage consortia at critical control points, transforming phages from passive metagenomic signals into precision biocontrol agents that enhance product quality, safety, and consistency across the farm-to-fork continuum.

5. References

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Oral presentation sessions
PhD students – 3rd year
Abstracts

PARALLEL SESSION 12
Food Microbiology and Biotechnology (2)

Innovative bioprocesses and extraction techniques of high value molecules and products from agricultural biomasses

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This PhD project, which belonged to the framework of the Agritech project, aimed to recover value-added compounds from agricultural biomasses by applying an integrated approach between bioprocesses, like fermentation and mild extraction techniques. To reach this goal, after a preliminary proximate composition analysis of wastes, lactic acid fermentation was applied to produce antimicrobials, aroma compounds and biofertilizers and mild extraction techniques were used to recover proteins and fermentable sugars. The potential implementation of sequential processes to achieve a cascade biorefinery approach was also explored.

Sviluppo di bioprocessi e tecniche di estrazione di molecole e prodotti ad alto valore da biomasse agricole residuali

L'obiettivo di questo progetto di tesi di dottorato, inserita all'interno del progetto Agritech, è stato il recupero e la valorizzazione di composti con un valore aggiunto da sottoprodotti della produzione agricola mediante l'applicazione di un approccio integrato tra diversi bioprocessi, come la fermentazione e processi di estrazione in fase acquosa o con l'ausilio di enzimi. A questo fine, la fermentazione è stata applicata per produrre composti con attività antimicrobica, aromi e biofertilizzanti, mentre processi di estrazione acquosa sono starti usati per recuperare proteine e zuccheri fermentescibili. È stata inoltre esplorata la potenziale implementazione di processi sequenziali per realizzare un approccio di bioraffineria a cascata.

Key words: agricultural biomasses, value-added compounds, lactic acid fermentation, enzymatic-assisted extraction

1. Introduction

In accordance with the PhD thesis project previously described (Fontechiari, 2023), this oral communication reports the main results of the following four activities directed to:

A1) Determine the effects of pre-treatments on biomasses. Different pre-treatments (milling, homogenization, ultrasounds and acid) were applied to a collection of agricultural biomasses (e.g., kiwifruit, Brassicaceae leaves, tomato). These techniques prepared the matrices for the next steps.
A2) Assess the chemical composition of the biomasses: a chemical composition analysis was performed on the different agricultural biomasses. This step was mandatory for deciding the next ones.
A3) Design of fermentation processes. Lactic acid fermentation was applied to recover antimicrobials, aroma compounds and biofertilizers from agrifood biomasses. Experimental designs (DoE) were applied to study the process parameters, optimize the fermentation protocol and build a predictive model.
A4) Evaluate the effect of mild extractions. Different enzyme reactions were tested to promote the extraction of valuable compounds from the selected residues. Reaction was first optimized at a laboratory scale and then scaled up.

2. Bioprocesses for the upcycling of agricultural biomasses

Agricultural waste is a globally recognized concern that impacts the economy, society, and the environment. The current management strategies are not considered sustainable or present environmental implications, such as greenhouse gas emissions and landfill accumulation. According to FAO (Food and Agriculture Organization of the United Nations) 1.3 billion tons of food are lost or wasted per year, with fruit and vegetables representing the largest food loss contributor. Fruit and vegetable wastes are generated all over the supply chain, from harvesting to sales, and it is estimated that a total of 30% of their production is wasted worldwide (Liu et al., 2023). Agricultural biomasses (e.g.: husk, seeds, stems, roots, pulp, bagasse, and peels) are still rich in value-added molecules, which can be upcycled into high-value products (Gómez-García et al., 2021). Following these directions and given their abundance, low cost, and limited competition with food resources, agricultural wastes are a valuable feedstock for the production of high-added-value compounds through green biotechnological processes. Fermentation and enzymatic-assisted extraction (EAE) have gained increasing attention in recent years as viable and environmentally friendly approaches to upcycle agricultural wastes and their potential was highlighted on various matrices. However, many factors limit their application and many gaps are still present, promoting additional research (Vilas-Franquesa et al., 2024).
Fermentation is an environmentally friendly process used to synthesize value-added compounds, using low energy and water and generating minimal waste. The upcycling of food waste through fermentation is based on the

metabolic ability of microorganisms to convert the organic constituents of the substrates into primary and secondary metabolites. These metabolites can enhance the bioactive content of the biomass or can be extracted as value-added compounds. Following this direction, the PhD project focused on the exploitation of strains belonging to the University of Parma Culture Collection (UPCCO), which comprises Lactic Acid Bacteria (LAB) isolated from food and food related environments over the past 25 years. LAB are generally recognized as safe (GRAS) microorganisms characterized by robustness against several stresses, such as low pH and osmotic stress, simple metabolisms and high nutrient uptake. For this reason, they are considered attractive cell factories for industry (Sauer et al., 2017) and can be used to upcycle agricultural wastes. In particular, this thesis aimed at the obtainment of antimicrobials, aroma compounds and plant biostimulants from the fermentation of agricultural biomasses thanks to LAB metabolism. Many LABs can produce metabolites with antimicrobial activity against some of the most common foodborne pathogens during the fermentation of fruit and vegetable by-products (Ricci et al., 2021). Moreover, LAB possesses metabolic pathways that lead to certain aromatic compounds and aroma precursors like aldehydes, esters, and metabolites derived from phenolic acids, lipids, peptides and proteins (Hadj Saadoun et al., 2021). Bacterial species belonging to the genera of *Pseudomonas, Bacillus, Bacteriodes* have been used in the production of biostimulants from food by-products (Areeshi, 2022) and in recent years lactic acid fermentation processes have been explored to convert agrifood wastes into plant biostimulants. Generally speaking, the production of valuable compounds is strictly related to strains and species employed in the fermentation process and to the characteristics of the fermented agricultural wastes.

To provide a broader overview of strategies for recovering high value-added compounds from agricultural biomass, the PhD project also focused on the exploration and optimization of mild extraction techniques. Enzyme-assisted extraction (EAE) is a specific and efficient method that works under mild conditions and has a low environmental impact. This technique takes advantage of the ability of enzymes to hydrolyze cell wall components, reducing the requirement of chemical solvents and high temperatures, promoting the extraction of the desired biomolecules and preserving their structure and functions. Different enzymes used in enzyme-assisted extraction enable the selective recovery of a wide range of vlue-added compounds which depend on the enzyme's specificity and on the composition of the substrate. EAE, when combined into a biorefinery framework, has been successfully used in the sequential recovery of proteins, oils, sugars, and fibers from a variety of fruit and vegetable by-products, including seeds, kernels, peels, leaves, pomace, and other agro-industrial residues (Fuso et al., 2022). The nature and quality of the products obtained through enzyme extractions are strictly related to the proximate composition and physicochemical properties of the biomass used. When proteases are used, the protein fraction of agricultural wastes can be recovered in the form of peptides and free amino acids, leading to the production of enzymatic protein hydrolysates with significant technological and functional qualities. These hydrolysates found different applications in various industries like food, animal feed, and nutraceuticals. Of note is the application of protein hydrolysates originating from plant waste as biostimulants, a practice that has demonstrated positive impacts on crop performance, resilience to stress, antioxidant capacity, and the compositional quality of cultivated plants (Puglia et al., 2021). Another application of EAE takes advantage of the ability of enzymes to break down the polysaccharides contained in lignocellulosic biomass, enabling the production of sugars, which can be successively fermented by target microorganisms to produce molecules of industrial interest. Given the recalcitrant structure of agricultural biomasses and in order to enhance enzymatic hydrolysis efficiency and improve its digestibility, a range of pretreatments can be applied. A wide range of microorganisms can be employed to ferment the obtained sugars, leading to the production of valuable products, including ethanol and polyhydroxyalkanoates (PHAs). The selection of an appropriate microorganism is crucial to achieving the desired product (Diaz et al., 2018).

3. Experimental Procedure

In this PhD thesis a cascade process was set up and applied to different agricultural side-streams received by producers located in the Emilia Romagna region of Italy. The following steps were followed to exploit the various agricultural biomasses: i) pre-treatments of the matrices (milling, homogenization, ultra-sound, acid treatment); ii) proximate composition (official AOAC methods) and chemical analysis; iii) design of fermentation processes using DoE; iv) design of mild extraction process ; v) evaluation of the results and scale-up of the best experimental conditions. The first two steps were fundamental to understanding how to better value the agricultural side-streams, and based on the results, iv) or v) was chosen. In alignment with circular economy principles, this PhD project focused on exploring the potential implementation of sequential processes to reach a cascade biorefinery approach for the valorization of agricultural biomass.

4. Materials and Methods

All samples employed during this PhD project (kiwifruit discarded for their size, broccoli leaves, unsold green and red tomatoes) were provided in the framework of the Agritech project (funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16/12/2021 of Italian Ministry of University and Research funded by the European Union – NextGeneration EU). The proximate composition of the residues was determined using the official AOAC methods, and GC-MS was used to assess their chemical composition. These results were evaluated to decide the best valorization route for the matrices.

Laboratory scale fermentation was performed using strains belonging to the University of Parma Culture Collection (UPCCO). Plate count was used to monitor the processes and DoE was employed as a tool for better understanding them and evaluating the interactions between the various factors involved. All DoE were created with MODDE® Pro v. 13.0.0. Software (MKS Umetrics, Umeå, Sweden). Enzymatic-assisted extractions protocols were tested on a laboratory scale, and their products were analysed to determine their effects (yields, products,..). After data analysis, the best conditions found for the bioprocesses were scaled up to a medium laboratory scale, in order to produce bigger quantities of the products.

5. Results and Discussion

The case study of kiwifruit waste valorization is reported as an example of the application of the previously described workflow. In particular, the focus of these works was the valorization of kiwifruit discarded on the field due to their non-compliant size for sale. First, kiwifruit was separated into pulp, peels and seeds, and then the proximate composition of the different parts was studied to establish the best way to valorize them.

5.1 Kiwifruit pulp fermentation to produce volatile compounds

Kiwi pulp was found to be primarily composed of water (85.7 ± 0.1 %) and digestible carbohydrates (81.6 % dw), especially sugars. The main monosaccharides found with GC-MS were glucose (3,1 g/100g fw), fructose (3,5 g/100g fw), and galactose (1,5 g/100g fw). Following these directions, fermentation was selected to exploit kiwifruit pulp and experiments were designed following a mathematical model using MODDE Pro v13.00 software. A linear model was developed based on three quantitative factors (fermentation time, temperature, and pH of the kiwifruit) and one qualitative factor, which was the microbial strains used for fermentation. The volatile profile of both fermented and unfermented kiwifruit by-products was characterized by various compounds belonging to different chemical classes, including aldehydes, alcohols, and esters. These compounds may be present in the raw matrix or generated during the fermentation process, as previously reported for other fruits (Ricci et al., 2019).

Interestingly, a higher growth rate of microbial strains does not always correlate with increased production of aromatic compounds. Hierarchical clustering analysis revealed that samples grouped in four clusters (Fig. 1), with 2 of them which exhibited higher levels of volatile organic compounds (VOCs) but were not associated with positive microbial growth. The opposite correlation was found for the two remaining clusters. Similar findings have been reported by other authors (Van Mastrigt et al., 2018), suggesting that the VOC synthesis is not strictly related to significant microbial proliferation. In fact, certain aromatic compounds can be produced even under near-zero growth conditions.

That study identified the key parameters that significantly impact both microbial growth and the production of specific compounds or compound classes. Successively, the model was applied to identify the best growth condition, which was tested as a biostimulant in the field.

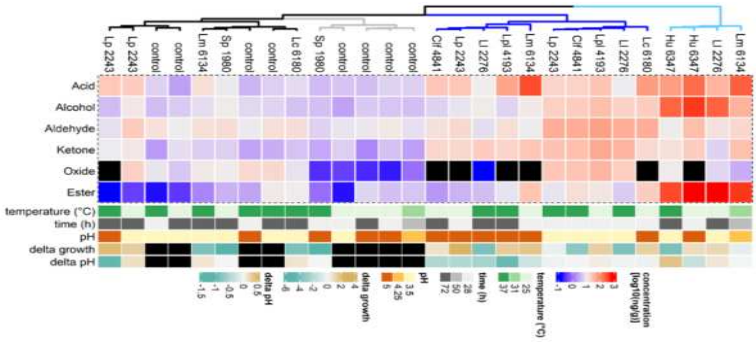


Fig. 1: Heatmap displaying the clustering of experimental conditions based on differences in concentration of volatile compounds (Hadj Saadoun et al., 2024)

5.2 Antimicrobial activity assessment of extract obtained from fermented kiwifruit pulp

To further valorize kiwifruit pulp, all the fermented samples obtained in the previously described DoE were subjected to a water/ethanol extraction performed with an Accelerated Solvent Extractor. The extracts were dried and resuspended into Tryptic Soy Broth TSB (VWR, Milan, Italy) to reach a final concentration of 10 mg/mL, and their antimicrobial activity against *Listeria monocytogenes* LGM 13305 and *Escherichia coli* ATCC 25922 was assessed using a microplate reader. Data resulting from the microplate reader were fitted to Baranyi's DMFit (USDA ComBase) to obtain numerical values of the Lag phase and the Maximum growth rate, which were integrated as responses in the previous experimental design. MODDE Pro v13.00 software (MKS Umetrics, Umea, Sweden) was used to analyze the data and check which of the factors was significant in the model. The software analysis highlighted that fermentation conditions (pH, temperature and time) affected the Lag phase and the maximum growth rate of both *L. monocytogenes* and *E. coli*. The two parameters were also significatively influenced by some of the microbial strains employed in fermentation. To confirm this observation Minimum Bactericidal Concentration (MBC) of fermented and unfermented extract was assessed, this time increasing the concentration to a final value of 100 mg/mL. The results were 50 mg/mL for *L. monocytogenes* and 100 mg/mL for *E. coli*. These data highlighted that the first test showed a positive effect of the kiwifruit pulp extracts even if

tests were conducted at subinhibitory antimicrobial concentrations. According to literature, lactic acid fermentation of fruit and vegetable by-products can produce metabolites with antimicrobial activity (Ricci et al., 2021) and various antimicrobials have been reported to inhibit the rate of bacterial growth at subinhibitory concentrations (Zhanel et al., 1992). Not only was the fermentation of kiwifruit pulp found to be capable of producing aromatic compounds, but extracts obtained from this fermented matrix also demonstrated antimicrobial activity, suggesting new potential applications, which should be further explored through future research.

5.3 Protein hydrolysate production from kiwifruit seeds

Based on the protein content found in kiwi seeds (9.36 ± 2.00% dw), enzymatic hydrolysis of kiwifruit seeds with Alcalase® (1% w/w), a protease derived from *Bacillus licheniformis,* was carried out and optimized to produce a protein hydrolysate. The resulting seed protein hydrolysate was then characterized in terms of protein yields, degree of hydrolysis (DH%) and protein profile including peptide and total amino acid composition, with a focus on potential application as plant biostimulant. Two reaction times (2 and 4 hours) were tested to determine the effect of time on extraction and hydrolysate characteristics. The results indicated that the protease significantly enhances protein extraction, particularly within the first 2 hours. In particular, the highest protein extraction yield (65 ± 10%) was achieved after 2 hours of enzymatic treatment, increasing the yield of the 2-hour control sample without protease (7.1 ± 0.9%). Alcalase-assisted extractions from other plant matrices have reported yields of up to 80% (Ahmad Nadzri et al., 2021).

SDS-PAGE qualitatively confirmed these results and showed a significant reduction in protein bands with molecular weights above 10 kDa, indicating the effectiveness of the EAE. Peptidomic analysis confirmed the effective breakdown of kiwifruit proteins by Alcalase, primarily yielding di- or tripeptides and free amino acids. Peptides derived from Actinidin, Actinidain, and Kiwillin were identified in similar proportions in all samples, with a single Thaumatin-like protein peptide detected only in the enzyme-treated hydrolysates. That revealed no substantial differences between the 2-hour and 4-hour hydrolysis times in terms of peptide profile and only a limited number of identified peptides were found: 9 and 12 in the enzyme-treated samples, compared to 12 and 4 in the controls. Regarding peptide size, the identified oligopeptides in both the 2-hour and 4-hour hydrolysates had an average length of 9 amino acids, while controls averaged 7. This difference suggests that Alcalase effectively hydrolyzed the kiwifruit proteins. To explore potential applications, the total amino acid profile of the 2-hour extract was characterized. The analysis revealed that the most abundant amino acids (g/100 g extract) in the kiwifruit seed extract were glutamic acid (0.91±0.04 g/100g extract) and cysteine (0.87±0.09 g/100g extract), followed by aspartic acid (0.52±0.03 g/100g extract), leucine (0.42±0.004 g/100g extract), and methionine (0.39±0.02 g/100g extract). A promising application for the extract is its use as a plant biostimulant. Protein hydrolysates, rich in amino acids and peptides, have demonstrated biostimulant activity by enhancing seedling growth and germination, improving plant and fruit growth and quality, and increasing stress tolerance (Rouphael and Colla, 2020). In the framework of the Agritech project, this hydrolysate was tested as biostimulant on tomato plants (*Solanum lycopersicum)* on the field, along with two fermented conditions found in the previously described DoE.

5.4 Fermentable sugars production from kiwifruit peels

Kiwi peels are lignocellulosic residues and their main component was found to be dietary fibers, with both soluble and insoluble fibers averaging above 30% dm. Due to this high polysaccharide content, the peels were subjected to enzymatic hydrolysis using cellulase to break down complex carbohydrates and produce hydrolysates rich in reducing sugars, particularly glucose (Van Dyk and Pletschke, 2012). A significative increase of 10 g/L in reducing sugars was reached within the first hours of hydrolysis. Acid and ultrasound pretreatments were also tested to evaluate if that can cause physical and chemical changes in lignocellulose biomass, increasing its solubility and the sugars production. However, no significant changes were detected in reducing sugars production, suggesting that no pretreatment was the best condition, also considering its lower costs and environmental impact. The resulting hydrolysates can be fermented using selected microorganisms to obtain high-added-value products with a wide range of applications. Ethanol production is a promising one, and *Saccharomyces cerevisiae* has been highly used for ethanol fermentation due to its high yields and general robustness to environmental stresses (Diaz et al., 2018). The last research of this project would be focused on this route, since preliminary results were encouraging.

5.5 Valorization of other biomasses

The previously described workflow was applied to other agricultural biomasses processed during the project.

Broccoli leaves were valorized through enzymatic-assisted extraction by applying the same protocol developed for kiwifruit seeds. After two hours of enzymatic treatment with Alcalase, a protein extraction yield of 45.2 ± 1.5% was achieved, and the main amino acids found were glutamine, alanine, and proline. Protein hydrolysates rich in peptides and amino acids showed positive effects on plants when employed as plant biostimulants (Rouphael and Colla, 2020). For that reason, broccoli leaves hydrolysate was tested as plant biostimulant on the field during a collaboration in the framework of the Agritech project. To achieve a cascade biorefinery approach for the valorization of this agricultural biomass, the hydrolysis residue was recovered, dried, and subsequently implemented in a sequential enzymatic-assisted hydrolysis process. This time, cellulase was used to break down

the polysaccharides and produce fermentable sugars, along with acid and ultrasound pretreatment. Without pretreatment, reducing sugars increased by 10 g/L during 72 hours of hydrolysis, while the same increase was achieved in only 2 hours of hydrolysis when acid-pretreated was applied. This data follows the literature, which reports that diluted acid pretreatments led to an increase in enzymatic hydrolysis yields (Zheng et al., 2013). On the other hand, ultrasound pretreatment showed no significant improvements in reducing sugar production. The two most promising conditions were selected and employed as a substrate for ethanol fermentation using *Saccharomyces cerevisiae,* together with kiwifruit peels hydrolysate, during the last stage of this PhD project.
Unsold green and red tomatoes were other agricultural biomasses used with the aim to produce biostimulant for soilless greenhouse tomatoes were mixed and homogenized, and lactic acid fermentation was selected to valorize this mixture. The experiments were conducted following a Design of Experiment. According to the model prediction, the most suitable strain and the optimal fermentation conditions were validated (*Weissella cibaria* 4253, 24 hours, 30 °C) and scaled to a medium laboratory scale to produce a fermented tomato-based biostimulant. According to Di Cagno et al. (2009), *Weissella* strains were previously isolated from tomatoes and have already demonstrated the ability to ferment and positively influence these matrices. In collaboration with the plant production unit of the University of Parma, the final product was applied as a plant biostimulant in a soilless tomato cultivation system. Fermentation was employed as a strategy to reduce tomato waste and generate a value-added product capable of enhancing crop performance, as well as improving fruit growth and quality

6. Conclusions and Future Perspectives

Fermentation and enzymatic-assisted extraction (EAE) have gained increasing attention in recent years as viable and environmentally friendly approaches to upcycle agricultural wastes and their potential was applied on various matrices.
First, this project demonstrated a viable pathway for completely valorizing undersized kiwifruit waste, discarded during a post-harvest selection, by extracting valuable compounds from its by-products (i.e., pulp, seeds and peel). Fermentation of the kiwifruit pulp not only resulted in the production of aromatic compounds but also showed a correlation with antimicrobial activity, suggesting new potential applications for biomass upcycling. EAE, using different enzymes, effectively valorized both kiwifruit seeds and peels. In particular, an effective breakdown of kiwifruit seeds proteins was reached by Alcalase, generating short peptides and free amino acids and suggesting a potential application of the kiwifruit seed hydrolysate as a plant biostimulant. These findings highlight the potential of underutilised agricultural residues for value-added bioconversion processes which should be further studied through future research in order to explore their industrial scalability and practical implementation.

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Effect of GABA production on biomass yield and stress robustness of *Levilactobacillus brevis*

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The PhD thesis project was focused on the production of γ-aminobutyric acid (GABA) by *Levilactobacillus brevis* LB12. The factors affecting GABA production in LB12 (*i.e.* pH, glutamate concentration, anaerobic vs aerobic conditions, metabolic shift) were evaluated in synthetic medium by using controlled cultivation in bioreactors. The effect of glutamate to GABA conversion on the biomass production and stress robustness of LB12 was also investigated. The molecular mechanisms that activate and regulate the expression of *gad* operon genes and, therefore, the synthesis of GABA, were also evaluated. Finally, an *in-silico* analysis on the *gad* operon of lactic acid bacteria was addressed to understand the evolution of GABA system in *Lvb. brevis* (the only LAB species with the complete GABA genetic equipment). The activities of PhD thesis were aimed at optimizing the GABA production by *Lvb. brevis* LB12, in order to use it as supplement in fermented foods (*in-situ* production) and improve nutritional and functionality of products.

Effetto della produzione di GABA sulla resa di biomassa e sulla resistenza allo stress di *Levilactobacillus brevis*

Il progetto di tesi di dottorato è stato concentrato sulla produzione di acido γ-amminobutirrico (GABA) da parte di *Levilactobacillus brevis* LB12 I fattori che influenzano la produzione di GABA in LB12 (ovvero pH, concentrazione di glutammato, condizioni anaerobiche rispetto a aerobiche, shift metabolico) sono stati valutati in mezzo sintetico utilizzando una coltivazione controllata in bioreattore. È stato inoltre studiato l'effetto della conversione da glutammato in GABA sulla produzione di biomassa e sulla tolleranza cellulare di LB12 nei confronti dello stress. I meccanismi molecolari che attivano e regolano l'espressione dei geni dell'operone *gad* e, di conseguenza, la sintesi di GABA, sono stati anch'essi oggetto di indagine. Infine, è stata condotta un'analisi in silico sull'operone *gad* dei batteri lattici per comprendere l'evoluzione del sistema GABA in *L. brevis* (l'unica specie di LAB dotata dell'intero corredo genetico per la produzione di GABA). Le attività della tesi di dottorato sono state finalizzate all'ottimizzazione della produzione di GABA da parte di *L. brevis* LB12, al fine di impiegarlo come supplemento negli alimenti fermentati (produzione *in situ*) e migliorare il valore nutrizionale e funzionale dei prodotti.

Key words: *Levilactobacillus brevis,* γ-aminobutyric acid (GABA), glutamate decarboxylase (GAD), controlled cultivation.

1. Introduction

In accordance with the PhD thesis project, the results of the following activities are reported below:
Optimisation of GABA production by *Lvb. brevis* LB12. Controlled batch cultivations were performed to investigate the effect of pH and glutamate supplementation on the GABA synthesis, biomass yield and stress response (acid, osmotic, freeze-drying conditions) in *Lvb. brevis* LB12. Cultivation shift, based on sublethal acid adaptation, was also performed to evaluate the effect on stress robustness.

2. State of Art

Lvb. brevis is a heterofermentive and ubiquitous lactic acid bacterium (LAB), used as starter and/or adjunct culture for the production of several fermented foods (mainly vegetable and cereal-based products; Zheng et al. 2020; Feyereisen et al. 2019).
Most of *Lvb. brevis* strains are able to synthetize the ɣ-aminobutyric acid (GABA), a non-proteinogenic amino acid recognised as the key inhibitory neurotransmitter in mammalian nervous system. GABA acts in several physiological functions, including promotion of brain development, regulation of neurological disorders (Dhakal et al. 2012; Xu et al. 2017). Because of this GABA is exploited as bioactive compound in food, pharmaceutical and cosmetic industries and the attention on its health-promoting potential is continuously increasing.
GABA can be obtained via chemical synthesis or through microbial metabolism. In several microorganisms, glutamate decarboxylase (GAD) and glutamate/GABA antiporter (GadC) systems are responsible, respectively, for the irreversible decarboxylation of glutamate to GABA (in presence of PLP, with consumption of a proton H^+

and production of CO_2) and for the external secretion of GABA. GAD and Glu/GABA antiporter are encoded by *gad* operon. Among LAB, the species *Lvb. brevis* has been recognised as GABA-producer and most of strains harbour the complete *gad* operon (including the transcriptional regulator *gadR*; Liu et al. 2024).
Besides genetic equipment, other factors may affect the functionality of GABA production system. Among them, pH values, temperatures, time of fermentation, composition of growth substrate and cell density are certainly the most important. The biosynthesis of GABA is mainly regulated by pH since the catalytic activity of bacterial GAD is favored at pH ranging from 3.8-5.2: Moreover, an efficient conversion of glutamate to GABA requires high cell density and, therefore, the optimal growth conditions of GABA-producers should be satisfied.
Based on these considerations, the aim of PhD thesis was the optimization of GABA production, based on growth modelling (fermentation processes, by testing and selecting appropriate conditions in bioreactor cultivation) and genomic analysis (*in-silico* gene analysis, genome sequencing), in order to identify the best condition for the synthesis of this bioactive compound by *Levilactobacillus brevis* LB12.
In this report, the trials and results related to effect of pH and glutamate supplementation on biomass yield, GABA production and stress responses are illustrated.

3. Materials and Methods

3.1 Bacterial Strain and Culture Conditions

Levilactobacillus brevis LB12 (isolated from sourdough) was used in this study. The strain was maintained as freeze-dried stocks (11% w/v skim milk with 0.1% w/v ascorbic acid) in the Culture Collection of Industrial Microbiology Laboratory, Università degli Studi della Basilicata (Potenza, Italy), and routinely propagated in Weissella Medium Broth pH 6.8 (WMB; Zotta et al., 2012) before each assay.
The strain was used to study the effects of glutamate and acid adaptation on GABA production, biomass yield and tolerance to acid, oxidative stress and freeze-drying.

3.2 Batch cultivation in bioreactor

WMB-preculture (24 h, 30°C) of *Lvb. brevis* LB12 were standardized (final absorbance at 650 nm, A650, of 1.0; SmartSpec™Plus Spectrophotometer, Bio-Rad Laboratories Inc., Milan, Italy) and successively cultivated in 1-L bioreactor (2% v/v inoculum; (Applikon, Schiedam, The Netherlands) as described in **Table 1**.
Cultivations were carried out for 46 h, at 30°C, under both controlled (pH 6.5) and uncontrolled pH, with or without monosodium glutamate (10 g/L) supplementation.
For all conditions (**Table 1**), after 40 h of cultivations (stationary phase), 5 g/L of MSG and 5 g/L of glucose were added to support further cell growth and GABA production. Moreover, in pH-controlled cultivations (F3, F4), after the stationary phase (40 h), a pH-lowering profile (from 6.5 to 4.5; hold to 4.5 for 4 h) was applied to promote adaptation to acid stress and GABA production. pH values were automatically adjusted using sterile 4 N NaOH and 2 N HCl solutions. Temperature was maintained at 30°C using a probe connected to an ezControl Controller (Applikon, Schiedam, Netherlands), and agitation was set at 150 rpm using a magnetic stirrer.
Process parameters (temperature, pH, base/acid addition volume, DOD) were recorded with Lucullus® PIMS software (Securecell AG, Zurich, Switzerland). Two independent biological replicates were performed for each condition.

Table 1 *Batch cultivation conditions used to evaluate the effect of glutamate and acid adaptation on GABA production and stress tolerance of Lvb. brevis LB12*

Cultivation	pH [b]	MSG induction [a]	pH profile [c]
F1	uncontrolled	none	-
F2	uncontrolled	10 g/L	-
F3	6.5	none	pH from 6.0 to 4.5
F4	6.5	10 g/L	pH from 6.0 to 4.5

[a] WMB with and without MSG supplementation
[b] Control of the pH value 6.5 during fermentation (up to stationary phase, 40 h of cultivation)
[c] pH profile from 6.5 to 4.5 was gradually decreased (-0.5 point/30 min) and pH 4.5 was kept for 4 h.

3.3. Biomass yield and GABA production

Biomass yield: Samples were aseptically withdrawn at 2 h interval up to 16 h (exponential phase; E) and then at 40 h (stationary phase; S) and 46 h (end of cultivation/adaptation phase; A) for the measurement of A_{650}. At E, S and A phases, standard curves were created to correlate A_{650} values to cell dry weight (CDW; washed biomass was dried at 105 °C for 24 h, g/L). The kinetics parameters of growth curves were estimated with the dynamic model of Baranyi and Roberts (1994) using DMFit v 3.5 for Excel.

GABA production: in S and A phases, supernatants were collected (8000 rpm, 5 min, 4°C) and used to verify the production of GABA with Thin Layer Chromatography (TLC; Zotta et al., 2022). Harvested cells were washed twice (8000 rpm, 15 min, 4°C) with 20 mM potassium phosphate buffer pH 7 (PB7), standardized to a final biomass of 1 g/L and resuspended in 50 mM sodium acetate buffer, supplemented with 0.1 mM pyridoxal-5′-phosphate (PLP) and 10 g/L of MSG (reaction buffer), adjusted to pH values 4.5. Reaction mixtures were incubated at 37°C for 4 h (150 rpm, orbital shaker, Solaris 4000 R Large Incubated and Refrigerated Benchtop Orbital Shaker, Thermo Scientific™, Germany) as reported in Zotta et al. (2022). At the end of incubation, the pH values, cell density (A_{650}), and GABA accumulation (TLC) were measured. TLC images were analyzed using NIS-Element software to calculate the spot integrated density. Two technical replicates were carried-out for each condition.

3.4 Stress Tolerance Assays

Cell biomass, collected in S and A phases, was harvested by centrifugation (12,000 × g, 5 min, 4°C), washed twice with PB7 buffer, standardized to A_{650} = 1.0, and used to assess the following stresses:

***Acid stress*:** incubation in 20 mM potassium phosphate buffer (PB) at pH 2.5, for 30 min and 1 h, at 30°C (as described by Lyu et al., 2018).

***Oxidative stress*:** incubation in 50 mM hydrogen peroxide (H_2O_2), for 30 min and 1 h, at 30°C (as described by Ianniello et al., 2016).

For both acid and oxidative stresses, after each incubation time, aliquots were serially diluted in PB7 (10-fold dilution) and plated on Weissella Medium Agar (WMA) and incubated anaerobically at 30°C for 48 hours. Unstressed cells (incubation in PB7) were used as control.

***Freeze-drying stress*:** standardized cells were resuspended in 300 µL of 11% skim milk and 0.1% ascorbic acid. Samples were frozen overnight at -80°C and then freeze-dried at -50°C and 0.1 hPa for 24 h using a Heto Drywinner 3 bench-top freeze dryier (Ianniello et al., 2016). The number of viable cells was estimated after 120 days of storage at -20°C by plate counting on WMA, as described for acid and oxidative stresses.

3.5 Genome sequencing LB12

Total DNA of *Lvb. brevis* LB12 was extracted with the Wizard® HMW DNA Extraction Kit and was sent to an external service for the Whole Genome Sequencing (WGS) with Oxford Nanopore Technology. WGS of LB12 will allow to identify the sequences of genes belonging to *gad* operon (*gadB*/*gadA*; glutamate decarboxylase, *gadC*, glutamate:gamma-aminobutyrate antiporter; *gadR*, transcriptional regulator, *gltX*, glutaminyl-tRNA synthetase or glutamate-tRNA ligase) useful for gene expression analysis (section 3.6).

3.6 Gene Expression Analysis

Total RNA was extracted from cells collected after the stationary phase (40 h) and after the adaptation phase (46 h) by using ZR Fungal/Bacterial RNA Kit (Zymo Research, Irvine, CA, US); an on-column DNase digestion (DNase I RNase-free; Invitrogen™, Burlington, Ontario, Canada) was applied. RNA was quantified using a NanoDrop spectrophotometer (Thermo ScientificTM) and used to synthesize cDNA with Maxima H Minus Master Mix (Thermo ScientificTM). Specific primer for gad operon genes was designed. qRT-PCR was performed using a StepOnePlus system (Applied Biosystems, Foster City, California, USA) and SYBR Green Master Mix (Applied Biosystems, Vilnius, Lithuania), following a defined running protocol. Negative controls without cDNA were included. For each condition XX replicates were carried-out. Gene expression was analyzed using the ΔΔCt method, with *gapdh* (glyceraldehyde-3-phosphate dehydrogenase) as a control reference gene.

4. Results and Discussion

4.1 Biomass yield and GABA production

Results demonstrated that after 40 h of cultivation (stationary phase), biomass yield was significantly higher in cultivation carried-out under pH-controlled conditions. Specifically, biomass concentrations of 1.36 ± 0.01 g/L and 1.44 ± 0.06 g/L were, respectively, measured in pH-controlled fermentations with and without MSG (F3 and F4), while lower values were observed in uncontrolled-pH cultivation (0.93 ± 0.02 g/L in F2 and 0.90 ± 0.02 g/L in F1). pH values within the optimal ranges (6.5), in fact, promoted growth also in the late stages of fermentation, reducing metabolic stress. The supplementation of glutamate slightly improved biomass production, as already reported by Zotta et al. (2022). During the adaptation phase, the further increase of biomass yield was probably due to interaction of glucose and glutamate supplementation.

As expected, GABA production was evident in cultivation with glutamate supplementation; moreover, the densiometric analysis of TLC spot confirmed the inductive effect of glutamate of GABA synthesis in both pH-controlled and uncontrolled cultivation (the results were supported also by expression of *gad* gene).

The addition of glutamate is essential for activating the GABA system in lactic acid bacteria (LAB), as it provides the substrate required by the GAD enzyme. However, it is critical to optimize glutamate concentration to maximize GABA yield without negatively impacting cell growth (Icer et al., 2024).
pH plays a crucial role in GABA production by modulating the activity of glutamate decarboxylase (GAD) during growth. The results showed that GABA accumulation was higher in uncontrolled cultivations, because of the lower pH values during the growth. Acidification profile applied in pH-controlled cultivations promoted GABA production, confirming the GAD activation under acid conditions.

4.2 Stress tolerance

The resistance of LB12 to acid, oxidative, and freeze-drying stress was evaluated at the end of the stationary (40 h) and adaptation (46 h) phases.
Surprisingly, glutamate supplementation reduced acid stress resistance (4.92 ± 0.50 log cycles, F3) in pH-controlled cultivation after 30 minutes of incubation; on the contrary, the reduction in survival in pH-uncontrolled growth was lower (1.14 log cycles, F1). This behavior was also confirmed during longer acid stress exposure (60 min).
After the adaptation phase (46 h), glutamate supplementation and low pH decreased survival under acid stress after 30 minutes (2.74 ± 0.10, F3 and -2.03 ± 0.42, F4 log cycles). Even after 60 minutes of incubation, the absence of glutamate supplementation and uncontrolled pH conditions demonstrated the best resistance to acid stress.
The effect of pH was variable, and adaptation to acidic conditions provided better tolerance to acid stress. Although our results contrast with other data in the literature, they need further investigation.
Also, resistance to oxidative stress depends on glutamate supplementation and growth phase. In the stationary phase (40 h), after 30 minutes, glutamate supplementation increased oxidative stress (0.37 ± 0.08 log cycles, F4) in pH-controlled cultivation but increased survival in non-glutamate supplementation at pH-uncontrolled conditions (1.39 ± 0.25 log cycles, F1). Long stress exposure (60 min) reduced the number of survivors with glutamate supplementation and non-controlled pH cultivation, but survival increased with glutamate supplementation in pH-controlled cultivation (F3).
At the end of the adaptation phase (46 h), after 30 minutes, glutamate supplementation reduced oxidative stress in pH-controlled cultivation (5.63 ± 0.39 log cycles, F3); on the contrary, no glutamate supplementation and pH-uncontrolled conditions showed better oxidative stress resistance (F4). After 60 minutes of incubation, survival was reduced with glutamate supplementation and pH-uncontrolled conditions (3.28 ± 0.30 log cycles, F2). Glutamate supplementation reduced oxidative stress (-2.27 ± 0.19 log cycles, F3) in pH-controlled cultivation.
The assessment of survival rates right after freeze-drying showed only a slight decrease in cell viability across all fermentation conditions. At the end of the stationary phase (40 h), glutamate supplementation promoted survival after the freeze-drying process and mid-term preservation in pH-uncontrolled cultivation (F2). At the end of the adaptation phase (46 h), the highest survival was observed in cultivation with uncontrolled pH and without glutamate supplementation.

4.3 Gene Expression Analysis

The RT-PCR data revealed that the GAD operon genes (*gadB*, *gadC*, *gadR*) showed different levels of expression across the 4 cultivation conditions. Glutamate supplementation led to the overexpression of gadB genes, while the regulator *gadR* was mainly affected by pH values, according to the previous data on other *Lvb. brevis* strains (Lyu et al., 2018; Gong et al., 2019). The relative expression of gadC (Glu/GABA antiporter), on the contrary, was variable and dependent by interaction glutamate supplementation/pH values.

5. Conclusions and Future Perspectives

The results demonstrated that pH control during cultivation significantly affects biomass yield, GABA production and cell response to environmental stresses by modulating the activation of defense mechanisms. Cultivation under pH-controlled conditions showed greater resistance to oxidative stress and freeze-drying, suggesting an improved redox balance and greater physiological stability of the cells.
Glutamate supplementation promoted GABA production but did not always result in improved acid stress resistance (as indicated also by gene expression analysis), and this aspect needs further investigation.
Adaptation to low pH increased robustness to acid stress.
This study demonstrated that strategies based on metabolic shifts could be useful to improve GABA production and robustness in *Lvb. brevis* LB12.

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Valorisation of Alternative Protein Sources by Tailored Biotechnological Processes and Non-Thermal Technologies to Obtain New Ingredients to Be Used in the Formulation of Innovative Foods

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This PhD thesis deals with the valorisation of alternative protein sources, such as legumes, through the development of tailored biotechnological processes using selected microbial strains, to enhance the chemical-physical, functional, sensorial and nutritional properties of the matrix. The thesis further explores the use of the resulting value-added ingredients in the formulation of innovative, sustainable and gut health-promoting plant-based food products.

Valorizzazione di nuove fonti proteiche, attraverso processi biotecnologici su misura e tecnologie non termiche, per l'ottenimento di ingredienti da utilizzare nella formulazione di alimenti innovativi

Questa tesi di dottorato ha riguardato la valorizzazione di fonti proteiche alternative, come i legumi, attraverso lo sviluppo di processi biotecnologici su misura utilizzando ceppi microbici selezionati, con l'obiettivo di migliorare le caratteristiche chimico-fisiche, funzionali, sensoriali e nutrizionali della matrice. Inoltre, la tesi esplora l'utilizzo degli ingredienti a valore aggiunto così ottenuti nella formulazione di prodotti alimentari a base vegetale innovativi, sostenibili e che promuovono il benessere intestinale.

Key words: Alternative protein sources, biotechnological processes, value-added ingredients, innovative foods.

1. Introduction

In accordance with the PhD thesis project previously described (Amadei, 2024), this oral communication reports the main results of the following four activities directed to:

A1) identify and characterize the main alternative protein sources as well as a range of safe, food-relevant microorganisms;
A2) determine the optimal growth conditions for the selected strains within the target matrix, and evaluate their capacity for producing innovative ingredients;
A3) assess the nutritional, functional, organoleptic, and safety properties of the obtained ingredients;
A4) develop an innovative food product incorporating the selected ingredient, characterize the formulation for its organoleptic properties and the final product for its functionality.

2. Legumes as Alternative Protein Sources

Global demand for sustainable and functional protein sources is rising, driven by population growth and increasing economic prosperity. Therefore, both research and industry are intensifying their efforts to identify viable alternatives to animal-based proteins. Legumes, including beans, peas, lentils, lupins, and chickpeas, are recognised as valuable plant-based protein sources, with protein contents ranging from 17% to 46% depending on the species and cultivar (Arbab Sakandar *et al.*, 2023). In addition, legumes are rich in bioactive compounds such as polyphenols and dietary fiber, which are associated with a lower risk of metabolic and cardiovascular diseases. Among legumes, chickpea (*Cicer arietinum* L.) is the second most cultivated one and consumed worldwide. Chickpea flour is notable for its balanced macronutrient profile, consisting of about 17-21% proteins, 60% carbohydrates, 5-7% lipids, and essential micronutrients. The main protein fractions include globulins (53-60% of the total protein content), glutenins (19-25%), albumins (8-12%), and prolamins (3-7%). Chickpea flour is also rich in essential amino acids, such as lysine, but it is deficient in sulfur-containing amino acids such as methionine and cysteine. The functional nutrients of chickpea flour, such as peptides, fiber, and micronutrients show health benefits for consumers, for example antioxidants, antidiabetic, antihypertensive, anticancer, and anti-inflammatory activity. Its nutritional potential is limited by the presence of antinutritional compounds, such as phytic acid, raffinose-family oligosaccharides and enzyme inhibitors, which can impair the digestibility of proteins, as well as aldehydes, which also contribute to undesirable sensory properties (Begum *et al.*, 2023). To overcome these aspects, modern processing techniques such as fermentation, germination, and extrusion have been developed to reduce the undesired factors, thereby enhancing protein bioavailability and decreasing gastrointestinal side effects.

Recent studies demonstrated that chickpea flour has good technological properties and can be incorporated into various food products such as baked goods, pasta, dairy alternatives, and snacks. Its application significantly increases protein content, dietary fiber, and polyunsaturated fatty acids, while maintaining or improving sensory acceptability (Patil *et al.*, 2024).

3. Biotechnological Processes

Biotechnological approaches using selected safe microorganisms (bacteria and yeasts) are increasingly applied to food systems to enhance their organoleptic, nutritional, technological, and functional attributes (Sáez *et al.*, 2022). Fermentation processes can extend shelf life, increase protein bioavailability, enrich the profile of aroma compounds, and confer health-promoting benefits, while simultaneously reducing the presence of antinutritional factors (Abbaspour, 2024). For these reasons, nowadays this approach is widely investigated in plant-based food. Several studies have demonstrated that fermentation improves the technological properties, protein content, and sensory characteristics of legume flour (Garrido-Galand *et al.*, 2021). In particular, selected strains of lactic acid bacteria (e.g. *Lactobacillus* spp.) and yeasts (e.g. *Saccharomyces cereviasiae* and non-conventional yeasts) can increase the total phenolic content and antioxidant activity of the matrix, reduce levels of antinutritional compounds, and contribute to the development of desirable aroma profiles in chickpea flour (Chiacchio *et al.*, 2025; Pable *et al.*, 2014). Moreover, fermentation enhances the quality and functionality of chickpea proteins by hydrolysing them into smaller polypeptides, free amino acids, and bioactive peptides, thereby improving their essential amino acid composition and overall nutritional value. Fermented chickpea flour also exhibits better technological properties compared to untreated flour (Sáez *et al.*, 2022). These functional and structural modifications are influenced by various factors, including the genus, species, and strain of the microorganisms applied; their specific metabolic activities; the synergistic effects arising from co-inoculation; the nutrient composition of the substrate; and the conditions of the fermentation process (Garrido-Galand *et al.*, 2021). Additionally, the application of fermented legume flours, in particular fermented chickpea flour, into food formulations has been shown to impart multiple benefits to the final products. These include enhanced antioxidant activity, improved flavour profile, higher technological performance in breadmaking, extended shelf life in pasta (De Pasquale *et al.*, 2021; Schettino *et al.*, 2020), and, in general, an improvement of nutritional quality, such as reduced phytic acid content, along with an enhancement of colour and aroma characteristics compared to formulations containing non-fermented flour (E. Gadallah and Aljebreen, 2023).

4. Experimental Procedure

In this PhD thesis an experimental procedure was set up by performing several trials that can be divided into four main phases: 1) identification of the most promising legume matrix based on its nutritional potential, accessibility and suitability for microbial fermentation. Chickpea flour was selected as the target substrate due to its high protein content, favourable amino acid profile, and increasing relevance in plant-based food applications. At the same time, safe and food-grade bacteria and yeast strains were screened and selected based on their proteolytic and lipolytic activity. The ability of these selected strains to grow on chickpea flour was evaluated through growth kinetics studies; 2) biotechnological processes were designed and optimized for the fermentation of chickpea flour using the previously selected strains. Fermentation parameters, including duration, temperature, and initial microbial inoculum load, were selected to produce a value-added ingredient; 3) fermented chickpea flour ingredients were characterized for the proximate chemical composition, microbial safety, nutritional, functional and organoleptic properties; 4) the fermented chickpea-based ingredients were incorporated into an innovative food formulation (fortified pasta), and the resulting prototypes were evaluated for their nutritional, technological and sensorial properties.

5. Materials and Methods

Chickpea flour was hydrated with mineral water (1:2 w/w) and inoculated with selected yeasts, namely *Yarrowia lipolytica* (Y3) and *Debaryomyces hansenii* (Y15A), either as a single strain or in co-culture with LAB strains, namely *Lactiplantibacillus plantarum* (LP23), *Lacticaseibacillus paracasei* (L), and *Lactobacillus sakei* (M12A). The initial concentration was about 5 and 6 log CFU/g for yeasts and LAB, respectively. The incubation was carried out for 48 h in sterile flasks at 30 °C. A control sample was obtained without any added inoculum and analysed immediately after the formulation. During the incubation period, microbiological analysis and pH measurement were performed. At the end of fermentation, after freeze-drying, the ingredients were evaluated for their peptide content (OPA assay) and amino acid profile (RP-HPLC), sugars concentration (K-RAFGL 08/23, Megazyme), damaged (AACC 76-31.01 method), digestible and resistant starch (K-DSTRS, Megazyme). Moreover, they were evaluated for their antioxidant activity (DPPH and ABTS assays), total phenolic content (Folin-Ciocalteu method), volatile molecules profile (SPME/GC-MS) and sensorial acceptability (internal panel test). The presence/absence of *Escherichia coli* O157:H7 and STEC was performed on the samples at the end of fermentation according to SureTect Escherichia coli O157:H7 and STEC Screening PCR Assay. Based on the

results, two fermented flours (LP23 and LP23+Y15A), along with their respective formulations (pasta fortified with 30% of the new ingredient), were subjected to standardized gastrointestinal digestion using the INFOGEST protocol. Subsequently, they were evaluated through a 24-hour in vitro gut fermentation model, consisting of a batch culture system with controlled pH, to assess changes in gut microbiota composition and alterations in short-chain fatty acid and amino acid profiles (UHPLC-MS/MS), which may have potential beneficial effects on human microbiota. The significant differences between the means of all samples were evaluated using one-way ANOVA, apart from short-chain fatty acid analysis where two-way ANOVA was applied, followed by Tukey HSD Post-Hoc Test at $p < 0.05$. The volatile molecule profiles were used for the principal component analysis (PCA). Statistical analyses were performed with Statistica software (v. 8.0; StatSoft, Tulsa, OK, USA).

5. Results and Discussion

5.1 Microbiological characterization and pH

Chickpea flour represented a proper substrate for microbial growth, indeed, all the selected strains, either in single or in co-culture, were able to grow at the selected conditions (Fig. 1). Since this matrix can harbor spoilage bacteria, *Enterobacteriaceae* count was determined and at the end of fermentation period the lowest values were found in samples inoculated with LAB strains. This is due to the significant decrease of pH (from about 6.5 to 4), that is expected since LAB produces organic acids, while in samples inoculated only with yeast strains, the pH decreased slowly because they typically convert sugars into alcohols and carbon dioxide or perform aerobic metabolism (Maicas, 2023).

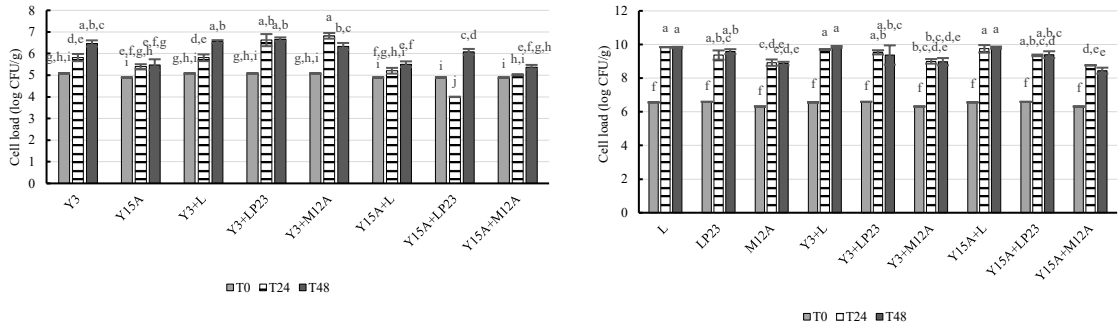


Figure 1 *Cell loads (log CFU/g) of yeast (**a**) and bacteria strains (**b**) in chickpea flour immediately after inoculation (T0) and after 24 (T24) and 48 hours (T48) of incubation at 30 °C. The results are the meaning of three independent replicates (n=3) ± standard deviation. Different letters indicate significant differences (p<0.05).*

5.2 Chemical-physical composition

At the end of incubation period, a significant increase in peptide content was observed in some fermented samples when compared to the control: it highlights the ability of the selected microbial strains to hydrolyse proteins, thereby potentially enhancing the digestibility, bioavailability, and bioactivity of the resulting ingredients. After the incubation period, a significant increase in the content of some essential amino acids, including leucine, isoleucine, valine, lysine, phenylalanine, and methionine were noticed. Moreover, fermentation resulted in elevated levels of citrulline, and γ-aminobutyric acid (GABA), which are recognized for their benefits on health (Table 1).

Table 1 *Amino acid content (mg AA/100 g) of chickpea flour not-inoculated (CONTROL) and inoculated with single and in co-culture microbial strains at the end of the incubation period. The results are the meaning of three independent replicates (n=3) ± standard deviation. Different letters indicate significant differences (p<0.05).*

	CONTROL	Y3	Y15A	L	LP23	M12A	Y3+L	Y3+LP23	Y3+M12A	Y15A+L	Y15A+LP23	Y15A+M12A
Asp	0.4 ± 0.0 ^{i}	2.2 ± 0.2 ^{g,h}	8.0 ± 0.2 ^{a}	3.7 ± 0.2 ^{d,e}	3.9 ± 0.2 ^{d}	6.5 ± 0.2 ^{b}	3.2 ± 0.1 ^{e,f}	2.1 ± 0.1 ^{h}	4.9 ± 0.2 ^{c}	3.4 ± 0.1 ^{d,e}	2.7 ± 0.1 ^{fg}	6.9 ± 0.3 ^{b}
Thr	0.9 ± 0.0 ^{h}	4.4 ± 0.1 ^{b}	7.6 ± 0.1 ^{a}	3.2 ± 0.1 ^{e}	3.0 ± 0.0 ^{e,d}	1.7 ± 0.0 ^{f}	2.8 ± 0.0 ^{d}	2.2 ± 0.0 ^{e}	1.2 ± 0.0 ^{g}	2.4 ± 0.1 ^{e}	1.9 ± 0.1 ^{f}	1.4 ± 0.1 ^{g}
Ser	1.0 ± 0.0 ^{e}	1.3 ± 0.1 ^{b}	3.2 ± 0.0 ^{a}	1.3 ± 0.1 ^{b}	0.3 ± 0.1 ^{f}	0.8 ± 0.0 ^{d}	1.2 ± 0.0 ^{b}	0.4 ± 0.0 ^{f}	0.6 ± 0.0 ^{e}	0.8 ± 0.0 ^{d}	0.3 ± 0.1 ^{f}	0.7 ± 0.0 ^{d,e}
Asn	2.2 ± 0.1 ^{h}	2.3 ± 0.0 ^{g,h}	5.9 ± 0.1 ^{a}	3.8 ± 0.1 ^{d}	4.9 ± 0.1 ^{b}	2.6 ± 0.1 ^{fg}	3.1 ± 0.2 ^{e}	4.4 ± 0.2 ^{c}	1.6 ± 0.1 ^{i}	2.8 ± 0.1 ^{e,f}	4.4 ± 0.1 ^{c}	2.4 ± 0.1 ^{g,h}
Glu	10.0 ± 0.4 ^{b}	1.4 ± 0.1 ^{f}	3.9 ± 0.1 ^{d}	2.4 ± 0.2 ^{e}	10.4 ± 0.5 ^{b}	2.4 ± 0.2 ^{e}	2.4 ± 0.1 ^{e}	4.1 ± 0.2 ^{d}	3.9 ± 0.2 ^{d}	5.6 ± 0.2 ^{c}	12.5 ± 0.7 ^{a}	5.4 ± 0.4 ^{c}
Gln	0.0 ± 0.0 ^{e}	0.0 ± 0.0 ^{e}	0.0 ± 0.0 ^{e}	0.0 ± 0.0 ^{e}	1.5 ± 0.1 ^{a}	0.4 ± 0.1 ^{d}	0.3 ± 0.0 ^{d}	0.7 ± 0.0 ^{c}	0.7 ± 0.0 ^{c}	0.0 ± 0.0 ^{e}	0.0 ± 0.0 ^{e}	0.9 ± 0.1 ^{b}
Gly	1.5 ± 0.1 ^{h}	4.4 ± 0.2 ^{b}	5.8 ± 0.1 ^{a}	3.7 ± 0.2 ^{c,d}	3.1 ± 0.1 ^{e,f}	4.6 ± 0.2 ^{b}	2.7 ± 0.1 ^{e,fg}	2.4 ± 0.1 ^{g}	3.2 ± 0.2 ^{d,e}	3.0 ± 0.1 ^{e,f}	2.6 ± 0.2 ^{fg}	4.2 ± 0.3 ^{b,c}
Ala	0.3 ± 0.0 ^{h}	8.9 ± 0.3 ^{a}	7.1 ± 0.1 ^{b}	2.9 ± 0.1 ^{e}	1.3 ± 0.0 ^{g}	5.1 ± 0.2 ^{c}	2.6 ± 0.1 ^{e}	2.0 ± 0.1 ^{f}	3.6 ± 0.2 ^{d}	2.6 ± 0.1 ^{e}	1.4 ± 0.1 ^{g}	3.9 ± 0.3 ^{d}
Cit	0.1 ± 0.1 ^{i}	6.1 ± 0.2 ^{a}	4.3 ± 0.2 ^{d,e,f}	5.1 ± 0.3 ^{b}	4.4 ± 0.3 ^{c,d,e}	4.1 ± 0.2 ^{e,f}	3.7 ± 0.1 ^{f}	2.1 ± 0.1 ^{g}	4.9 ± 0.3 ^{b,c,d}	5.0 ± 0.2 ^{b,c}	1.0 ± 0.1 ^{h}	3.9 ± 0.3 ^{e,f}
Val	0.6 ± 0.1 ^{h}	5.1 ± 0.3 ^{b}	7.6 ± 0.1 ^{a}	2.4 ± 0.1 ^{e}	2.3 ± 0.1 ^{e,f}	4.9 ± 0.1 ^{b}	1.9 ± 0.0 ^{fg}	1.7 ± 0.1 ^{g}	3.1 ± 0.1 ^{b,c,d}	1.6 ± 0.1 ^{b,c}	1.6 ± 0.1 ^{h}	4.3 ± 0.3 ^{e,f}
Met	0.4 ± 0.0 ^{g}	2.5 ± 0.1 ^{c}	3.4 ± 0.1 ^{a}	1.7 ± 0.1 ^{e}	1.7 ± 0.1 ^{e}	3.0 ± 0.2 ^{b}	1.3 ± 0.0 ^{f}	1.3 ± 0.0 ^{f}	2.1 ± 0.1 ^{d}	1.2 ± 0.1 ^{f}	1.3 ± 0.1 ^{f}	2.9 ± 0.3 ^{b}
Ile	0.6 ± 0.0 ^{f}	3.9 ± 0.2 ^{b}	5.6 ± 0.2 ^{a}	1.6 ± 0.2 ^{e}	2.1 ± 0.2 ^{d}	4.3 ± 0.2 ^{b}	1.7 ± 0.1 ^{d,e}	1.5 ± 0.1 ^{e}	2.9 ± 0.1 ^{c}	1.3 ± 0.1 ^{e}	1.5 ± 0.1 ^{e}	4.0 ± 0.3 ^{b}
Leu	1.3 ± 0.0 ^{h}	10.5 ± 0.4 ^{c}	13.9 ± 0.4 ^{a}	7.1 ± 0.5 ^{d,e}	8.1 ± 0.4 ^{d}	12.2 ± 0.1 ^{b}	5.3 ± 0.4 ^{fg}	6.4 ± 0.3 ^{e,f}	8.2 ± 0.2 ^{d}	4.7 ± 0.2 ^{g}	7.0 ± 0.4 ^{d,e}	11.7 ± 0.9 ^{b,c}
Tyr	0.8 ± 0.1 ^{e}	0.0 ± 0.0 ^{d}	0.0 ± 0.0 ^{d}	0.0 ± 0.0 ^{d}	0.6 ± 0.0 ^{c}	0.0 ± 0.0 ^{d}	0.0 ± 0.0 ^{d}	0.6 ± 0.0 ^{c}	0.7 ± 0.1 ^{c}	0.0 ± 0.0 ^{d}	1.3 ± 0.1 ^{b}	1.6 ± 0.2 ^{a}
Phe	1.0 ± 0.1 ^{f}	7.0 ± 0.3 ^{b}	7.8 ± 0.2 ^{a,b}	4.2 ± 0.2 ^{d,e}	4.4 ± 0.2 ^{d}	7.7 ± 0.4 ^{a,b}	3.4 ± 0.2 ^{e}	4.1 ± 0.2 ^{d,e}	5.7 ± 0.2 ^{c}	3.4 ± 0.2 ^{e}	4.4 ± 0.2 ^{d}	8.0 ± 0.7 ^{a}
Gaba	4.3 ± 0.3 ^{f}	29.1 ± 1.5 ^{a}	29.4 ± 1.4 ^{a}	22.5 ± 1.5 ^{b}	13.7 ± 0.8 ^{d,e}	26.5 ± 1.4 ^{a}	17.5 ± 1.0 ^{c}	16.9 ± 1.0 ^{c,d}	18.6 ± 0.8 ^{c}	18.7 ± 0.8 ^{c}	12.2 ± 0.7 ^{e}	23.0 ± 1.7 ^{b}
Orn	0.2 ± 0.0 ^{g}	6.0 ± 0.2 ^{d}	20.6 ± 0.6 ^{a}	17.9 ± 0.9 ^{b}	9.6 ± 0.4 ^{e}	21.2 ± 0.9 ^{a}	16.4 ± 1.0 ^{b}	3.2 ± 1.0 ^{e,f}	11.0 ± 0.4 ^{c}	6.8 ± 0.3 ^{d}	1.6 ± 0.1 ^{fg}	3.8 ± 0.3 ^{e}
Lys	2.3 ± 0.2 ^{f}	2.6 ± 0.1 ^{f}	5.9 ± 0.2 ^{a}	0.3 ± 0.0 ^{h}	5.1 ± 0.2 ^{b}	3.3 ± 0.2 ^{d,e}	1.7 ± 0.0 ^{g}	1.8 ± 0.0 ^{g}	3.7 ± 0.2 ^{c,d}	0.6 ± 0.0 ^{h}	3.9 ± 0.2 ^{c}	3.1 ± 0.3 ^{e}
His	1.1 ± 0.0 ^{e}	0.4 ± 0.0 ^{f}	2.4 ± 0.1 ^{b}	1.9 ± 0.0 ^{c}	2.0 ± 0.1 ^{c}	1.1 ± 0.0 ^{e}	1.0 ± 0.0 ^{e}	1.6 ± 0.0 ^{d}	2.4 ± 0.1 ^{b}	2.4 ± 0.1 ^{b}	1.9 ± 0.1 ^{c}	2.7 ± 0.2 ^{a}
Arg	35.3 ± 1.4 ^{a}	0.4 ± 0.1 ^{f}	0.5 ± 0.1 ^{f}	2.5 ± 0.0 ^{f}	20.6 ± 1.0 ^{c}	7.7 ± 0.5 ^{e}	0.7 ± 0.0 ^{f}	21.7 ± 0.0 ^{c}	9.4 ± 0.5 ^{e}	16.1 ± 0.7 ^{d}	36.3 ± 1.9 ^{a}	30.0 ± 2.6 ^{b}
Pro	2.8 ± 0.1 ^{e}	3.0 ± 0.1 ^{e}	8.0 ± 0.2 ^{a}	5.6 ± 0.4 ^{b}	5.5 ± 0.1 ^{b}	5.7 ± 0.1 ^{b}	4.4 ± 0.3 ^{c,d}	4.3 ± 0.3 ^{d}	4.3 ± 0.1 ^{d}	5.3 ± 0.7 ^{b,c}	4.4 ± 0.3 ^{c,d}	6.2 ± 0.4 ^{b}
Total	67.1 ± 2.8 ^{g}	101.5 ± 3.9 ^{c,d}	150.9 ± 4.1 ^{a}	93.8 ± 4.9 ^{c,d,e}	108.5 ± 4.4 ^{c}	125.8 ± 5.0 ^{b}	77.3 ± 3.6 ^{fg}	85.5 ± 3.7 ^{e,f}	96.7 ± 4.1 ^{c,d,e}	87.7 ± 4.1 ^{d,e,f}	104.2 ± 6.0 ^{c}	131.0 ± 10.0 ^{b}

Regarding the carbohydrate fraction, a slight reduction in damaged starch content was observed in the fermented samples compared to the control. The starch fraction predominantly present was the slow-digesting type, which could positively modulate the postprandial glycaemic response. Moreover, the biotechnological process led to a significant reduction in the levels of glucose, sucrose, and, especially, raffinose family oligosaccharides, which are generally undesirable due to their association with bloating and flatulence during digestion. From an initial content of about 0.93 g/100 g, the most significant decrease of these sugars was observed in the sample inoculated with *Y. lipolytica* Y3 and *L. paracasei* L reaching a value of about 0.07 g/100 g.

5.3 Functional properties

From a functional perspective, antioxidant activity was evaluated using both the ABTS and DPPH assays. The results revealed an increase in antioxidant activity in all the inoculated samples compared to the control. From the DPPH results, the highest antioxidant activity was observed in the sample inoculated with *D. hansenii* Y15A and *L. paracasei* L (Fig. 2). These results are consistent and follow the same trend as those observed for total phenolic content, as determined by the Folin-Ciocalteu assay.

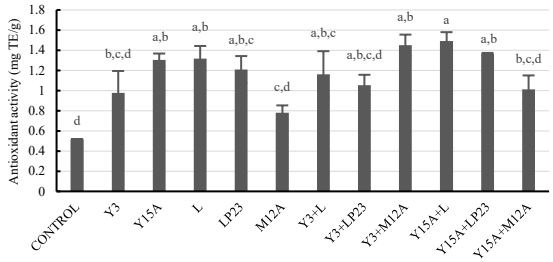


Figure 2 *Radical scavenging activity (mg TE/g) of chickpea flour not-inoculated (CONTROL) and inoculated with single and in co-culture microbial strains at the end of the incubation period. The results are the mean of three independent replicates (n=3) ± standard deviation. Different letters indicate significant differences (p<0.05).*

5.4 Organoleptic properties

The volatile molecules profile was modified differently depending on the microbial strains used (Fig. 3). Compared to the control, a decrease in the abundance of aldehydes and alcohols, along with an increase in acids, ketones, and esters, was observed in all the inoculated samples. Specifically, the concentration of hexanal, an aldehyde associated with beany and fatty aroma, and of 1-hexanol, an alcohol contributing to the characteristic leguminous note, were reduced. On the other hand, fermentation increased the levels of esters, including ethyl acetate (which imparts a fruity aroma), acids, that are primary metabolites of lactic acid bacteria and provide a milky aroma, and ketones, such as acetoin, which imparts buttery notes.

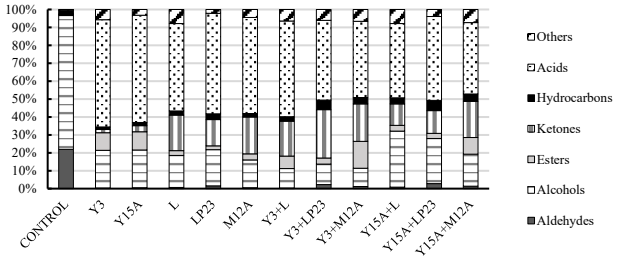


Figure 3 *Volatile molecule profiles (expressed as relative abundance, %) of chickpea flour not-inoculated (CONTROL) and inoculated with single and in co-culture microbial strains at the end of the incubation period.*

The combination of various microbial strains has led to the formation of highly varied and complex aromatic profiles, which in some cases resembled dairy products and in others, bakery products. Overall, the biotechnological process resulted in a significant improvement in the aromatic profile compared to the raw chickpea flour, as confirmed by the internal panel test.

5.5 Impact of the Fermented Ingredient and the respective formulations in In Vitro Colon Fermentations

Based on the results obtained from chemical-physical, functional, and organoleptic characterization but also on safety parameters, in particular the absence of *Escherichia coli* STEC, two fermented products were selected: a) chickpea flour fermented with *L. plantarum* LP23 and b) chickpea flour fermented with the mix *L. plantarum* LP23 and *D. hansenii* Y15A. A non-fermented control sample was used as a reference. Fresh pasta was formulated with 30% of these selected ingredients. The dough was homogenized, rolled and cut into strips (tagliatelle). The microbial flora present in the dough was determined through microbiological sampling. From a technological perspective, differences were observed between the samples: in particular, the colour of the pasta dough made with the fermented ingredients was lighter and less yellow than the one made with non-fermented chickpea flour. Moreover, the pH value of the new formulations presented a lower pH (average 4.1) than the control made with non-fermented ingredients (6.3). While the water activity value did not vary significantly (around 0.98), different water absorption capacities during the mixing of the ingredients were observed (data not shown). Pasta was then cooked in salted water, and the volatile compound profile was analysed both in raw and cooked pasta. Both the fermented ingredients and the new formulations underwent to INFOGEST digestion and then evaluated through a 24 h *in vitro* gut model. The preliminary results of the tested ingredients showed an increase in acetate concentration over time, particularly in the sample inoculated with the ingredient fermented with LP23+Y15A

(Fig. 4). This suggests enhanced microbial metabolic activity, which may contribute to the reinforcement of intestinal barrier integrity. On the other hand, the increase in butyrate and propionate concentrations during fermentation was not significant compared to the control. A slight increase in branched-chain fatty acids was observed, particularly in the sample inoculated with the ingredient fermented by LP23+Y15A. This increase can be attributed to the lower availability of simple sugars in the fermented ingredients and the higher availability of peptides and amino acids in this sample, resulting from the prior fermentation.

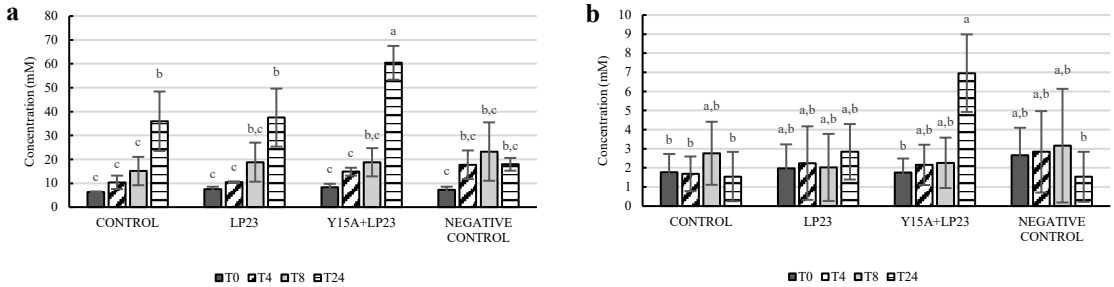


Figure 4 *Concentration (mM) of acetate (**a**) and branched fatty acids (**b**) in the selected digested ingredients and in a negative control during the in vitro gut fermentation (T0, T4, T8, T24). The results are the meaning of four independent replicates (n=4) ± standard deviation. Different letters indicate significant differences (p<0.05).*

6. Conclusions and Future Perspectives

This study demonstrated the potential of tailored biotechnological processes using selected microbial strains to enhance the nutritional, functional, and sensorial properties of chickpea flour, that is considered an interesting alternative protein source. Fermentation significantly changed its protein and amino acids profile, improved antioxidant activity, and volatile compound composition; moreover, its feasibility in pasta formulations was confirmed. Changes in gut microbiota composition after the in vitro fermentation of the ingredients and the formulations are still under analysis. Future research should focus on scaling up fermentation processes, validating *in vivo* health effects, exploring the use of other legume matrices and other technologies, such as non-thermal treatments, to optimize functional ingredients development for sustainable and health-promoting food systems.

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Optimization of Fermentation Parameters for Biomass Production of *Streptococcus thermophilus* BT01 with Low or High Urease Activity

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This PhD funded by Beingpharma Srl and the Italian Ministry of Research under the DM 352, aimed to optimize biomass production parameters to modulate the metabolic activities of *Streptococcus thermophilus* that are directly or indirectly related to probiotic traits. Once the biomass production parameters were optimized, a pilot-scale industrial production was carried out to provide material for intervention studies on specific subject groups. These studies aimed to evaluate host-cell physiological interactions using a double-blind, crossover design. In particular, the research focused on modulating the urease activity of *S. thermophilus* to develop a novel precision functional probiotic product intended to alleviate symptoms of Irritable Bowel Syndrome and improve patients' health and well-being.

Ottimizzazione delle condizioni di fermentazione per la produzione di biomasse del probiotico *Streptococcus thermophilus* BT01 con bassa o alta attività ureasica

Questo dottorato finanziato da Beingpharma Srl e dal Ministero Italiano della Ricerca sotto il DM 352 ha avuto come obbiettivo l'ottimizzazione di parametri di produzione della biomassa per la modulazione delle attività metaboliche di *Streptococcus thermophilus*, direttamente o indirettamente correlate alle caratteristiche probiotiche. Una volta ottimizzati i parametri di produzione della biomassa, è stato prodotto un lotto pilota di produzione industriale, disponibile per studi di intervento su categorie definite di soggetti, al fine di valutare le interazioni fisiologiche ospite-cellula utilizzando un approccio crossover in doppio cieco. In particolare, lo studio si è concentrato sulla modulazione dell'attività dell'ureasi di *S. thermophilus* per lo sviluppo di un nuovo prodotto probiotico di precisione, progettato per ridurre i sintomi della sindrome dell'intestino irritabile e migliorare lo stato di salute e il benessere dei pazienti.

Key words: *Streptococcus thermophilus*; enzymatic activities; modulation; IBS, precision probiotics

1. Introduction

Streptococcus thermophilus is widely used in the dairy industry for the manufacturing of fermented milk and cheeses and several probiotic formulations. *S. thermophilus* evolved from closely phylogenetically related pathogenic streptococci through loss-of-function events counterbalanced by the acquisition of relevant traits, such as lactose and urea utilization for the adaptation to the milk environment which represents a unicum among lactic acid bacteria (Bolotin et al., 2004; Hols et al., 2005). The role of urease activity in *S. thermophilus* in modulating environmental pH and its involvement in cell physiology and energetic metabolism have already been investigated (Mora et al, 2005; Arioli et al, 2010; Arioli et al., 2022). Urease, through the hydrolysis of urea in ammonia and CO_2, has a pivotal role in the physiology of *S. thermophilus*. This activity not only enhances *S. thermophilus* fitness and catabolic efficiency, but can benefit neighbouring bacteria, boosting fermentation kinetics (Arioli et al., 2017). Irritable Bowel Syndrome (IBS) is a multifactorial disorder characterized by alterations in gut motility, visceral hypersensitivity, and gut symptoms. A significant proportion of IBS patients show dysbiosis in mucosal and fecal microbiome compared with healthy controls (Singh & Lembo, 2021). Particularly, IBS is associated with increased levels of potentially harmful bacteria, which are linked to higher nitrogen consumption and high urease activity levels, which lead to increased competitiveness during infection stage (Ni et al., 2017; Xu et al., 2020). Several interventions targeting the gut microbiome have opened the potential for new IBS treatments. Previous studies (Bazzocchi et al., 2002; Brigidi et al., 2001; Guandalini et al., 2010) observed that the administration of VSL#3 (Yovis, Sigma-Tau, Pomezia, Italy) a pharmaceutical-grade probiotic containing *S. thermophilus* BT01 comprising 2.0×10^{11} CFU/g lyophilized bacterial cells, led to increased fecal β-galactosidase activity, decreased urease activity and a reconstituted gut microbiota, showing significant clinical improvements in subjects with IBS. More recently, a randomized controlled cross-overstudy revealed the ability of *S. thermophilus* BT01 to modulate urease activity in healthy subjects' fecal samples depending on the biomass production process. Based on these observations, this study was devoted to the optimization of the biomass production process to develop two probiotic formulations of *S. thermophilus* BT01 characterized by a high and a low level of urease activity to be tested on subjects suffering of IBS.

2. Materials and Methods

2.1. Modulation of *S. thermophilus* Urease activity through culturing parameters control

S. thermophilus BT01 biomass was produced in 1L bench-scale bioreactors at controlled pH (pH 6.0, pH 6.5 and pH 6.8). Fermentation pH was controlled using two different buffering solutions (A or B). Growth kinetic parameters have been measured by flow cytometry using a live and dead double staining according to the ISO19344:2015. Metabolites (lactose, galactose, glucose, and L-lactic acid) have been quantified enzymatically (Arioli et al., 2022). Urease activity was quantified spectrophotometrically by phenol-red assay according to Martinović et al. (2023). Transcriptional analysis of *ureE*, *ureC*, *LacZ*, and *Ldh* genes was carried out by RT-qPCR (Arioli et al., 2010). The optimized production parameters suitable to obtain a lyophilized biomass with high or low urease activity were transferred to CSL S.r.l. (Zelo Buon Persico, Lodi, Italy) for the production of capsules (size 0) containing 50 billion of CFU/cps.

2.2. Statistical analysis

Statistical analyses were performed using GraphPad Prism 5 software, version 8.4.3. All data were checked for normality and homoscedasticity, and then parametric or non-parametric statistics were applied. The significance of differences was assessed by Student's t-test, ANOVA, Wilcoxon test. Differences of $p < 0.05$ were considered significant.

3. Results and Discussion

3.1 Culturing conditions can modulate *S. thermophilus* BT01 urease activity

Fermentation pH significantly impacted *S. thermophilus* BT01 viability, metabolic activity, and urease expression. Culture grown at pH 6.0 showed higher viable cell density compared to pH 6.5 and 6.8, while no significant variation in generation time was recorded across the three pH conditions tested (Tab. 1). Moreover, biomass obtained at pH 6.5 and 6.8 with buffer B showed a decreasing of cell viability (>20%) after 5h (data not shown).

Table 1: *Live and dead cell quantification of* S. thermophilus *BT01 cultured at different pH values*

Values are presented as mean ± SD. Superscript different letters indicate statistically significant pairwise differences ($p < 0.05$, unpaired t-test). pH comparisons were conducted within buffer A, while comparison between buffers was limited to the pH 6.0 conditions.

Buffer	pH	Live cells	Dead cells	AFU%	Generation time
		Log_{10}(FU/mL)	Log_{10}(FU/mL)	AFU%/TFU	min
A	6.0	9.60 ± 0.01^{a}	7.68 ± 0.24^{a}	98.80 ± 0.37^{a}	21.93 ± 1.86^{a}
	6.5	9.54 ± 0.03^{b}	7.88 ± 0.20^{a}	97.88 ± 0.42^{b}	22.78 ± 2.21^{a}
	6.8	9.25 ± 0.05^{c}	8.07 ± 0.11^{b}	93.78 ± 1.08^{c}	24.37 ± 2.60^{a}
B	6.0	9.71 ± 0.06^{d}	8.40 ± 0.07^{c}	95.27 ± 0.16^{d}	23.18 ± 2.30^{a}

Although growth kinetic parameters ($p = 0.637$), lactose consumption ($p = 0.284$) and lactic acid production ($p = 0.148$) were not significantly different between pH 6.0 and 6.5, urease activity was significantly higher in cells cultured at pH 6.0 compared to those measured in cells cultured at pH 6.5 ($p < 0.001$) and 6.8 ($p < 0.001$) (Fig. 1).

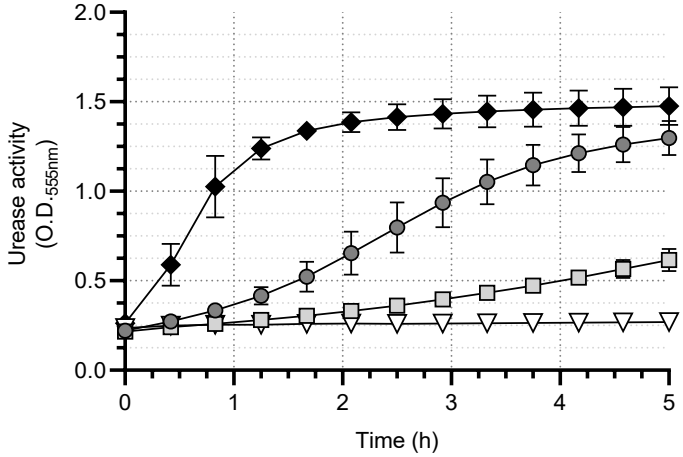
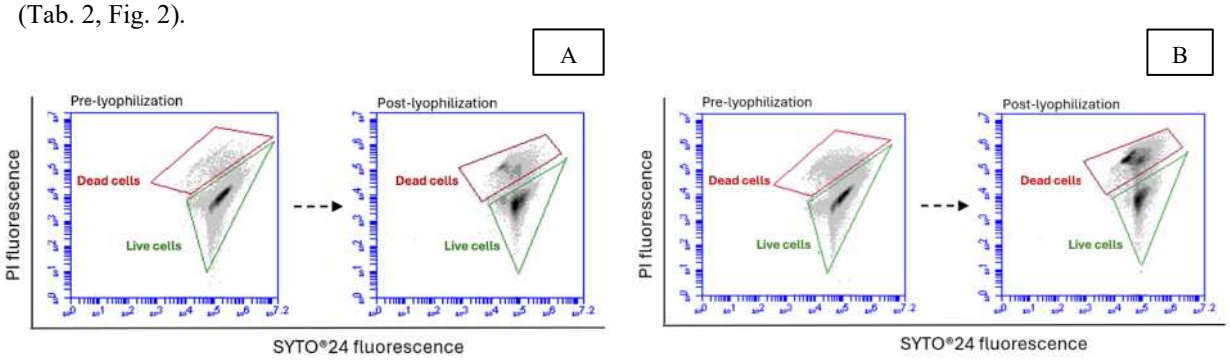


Figure 1: *Kinetic of urease activity of* S. thermophilus *BT01 cells grown at controlled pH 6.0 (circles), pH 6.5 (squares) and pH 6.8 (triangles) using buffer A, and at pH 6.0 (rhombuses) using buffer B*

Following lyophilization, cell viability strongly decreased in cells cultured at pH 6.5 and 6.8 compared to pH 6.0 (Tab. 2, Fig. 2).



Replacing buffer A with buffer B for pH control at 6.0 led to 65% increase of urease activity (Fig. 1).
The increase in urease activity depending on the buffer composition was supported by data generated by gene expression analysis carried out on cell biomass collected during the exponential phase during the growth at pH 6.0 controlled using buffer A or buffer B.

Table 2*:* S. thermophilus *BT01 live cells on total cells percentage before and after freeze-drying process*

Buffer	**pH**	**Before lyophilization**	**After lyophilization**
		AFU%/TFU	AFU%/TFU
A	6.0	98.80 ± 0.37[a]	79.26 ± 4.25[a]
	6.5	97.88 ± 0.42[b]	43.10 ± 0.56[b]
	6.8	93.78 ± 1.08[c]	30.82 ± 3.55[c]
B	6.0	95.27 ± 0.16[d]	79.26 ± 3.83[a]

Values are presented as mean ± SD. Superscript different letters indicate statistically significant pairwise differences ($p < 0.05$, unpaired t-test). pH comparisons were conducted within buffer A, while comparison between buffers was limited to the pH 6.0 conditions.

In specific, a significant higher expression level of the urease accessory protein encoding genes, *ureE*, was measured in cells cultured at pH 6 buffered using the solution B. Conversely *lacZ* gene expression was markedly higher in cells cultured at pH 6 using solution A (Fig. 3). Gene expressions were normalized using as reference the transcription of *rpoB* and *polC* genes.

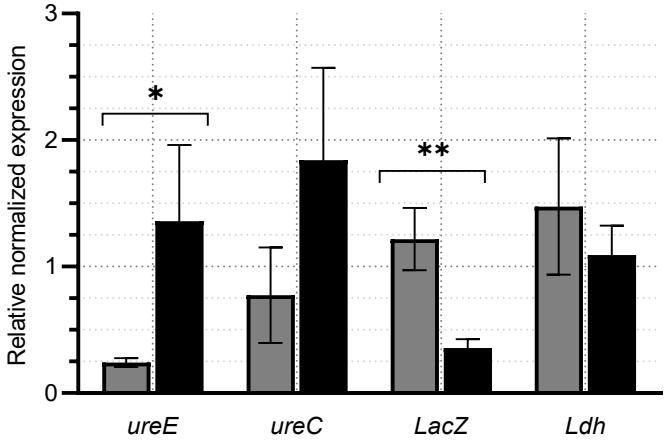


Figure 3: *Expression of* ureE, ureC, lacZ *and* Ldh *genes measured in biomasses cultured at pH 6.0 with buffer A (grey bars) or buffer B (black bars). Gene expressions were normalized using as reference the transcription of* rpoB *and* polC*. Data are expressed as* $\Delta\Delta C_T \pm SD$.

The lyophilization process determined a reduction of *S. thermophilus* BT01 cell viability, and consequently a reduction of urease activity (Fig. 4).

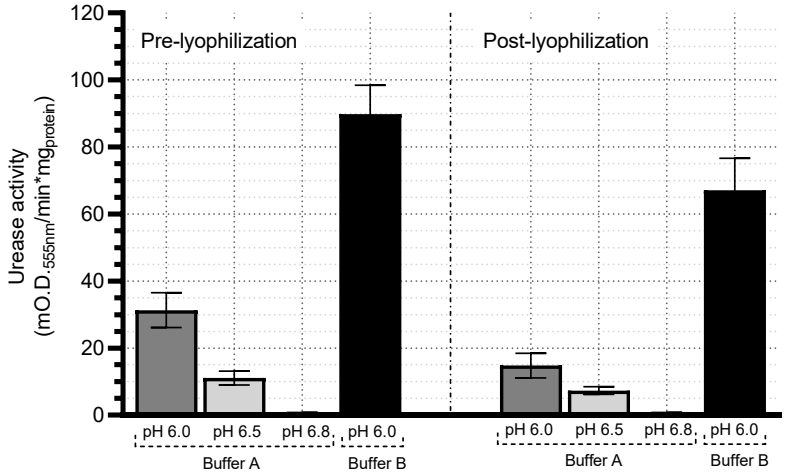


Figure 4*: Freeze-drying impact on* S. thermophilus *BT01 urease activity, expressed as* $mOD_{555nm}/min*mg_{protein}$.

Industrial production of *S. thermophilus* BT01 by CSL Srl, based on the transfer of the optimized fermentation parameters, led to the production of batch n. H059 and H060 (expiration date 06/2027) packaged in capsule size 0 containing 50 billion CFU/cps. The two batches were subjected to live and dead cell quantification and urease activity quantification. The results obtained (Tab. 3) showed that for batches H059 and H060 live cells were respectively 11.79 ± 0.01 and 11.88 ± 0.01 log_{10} (FU/g), whereas urease activity was respectively 102.34 ± 3.82 and 59.86 ± 5.03 $mOD_{555nm}/(min*mg_{protein})$.

Table 3*: Live and dead cell quantification and urease activity quantification of* S. thermophilus *BT01 batches n H059 and H060.*

Sample batch ID	Live cell Log_{10} (FU/g)	Dead cells Log_{10} (FU/g)	AFU% AFU%/TFU	Urease MaxV $mOD_{555nm}/min*mg_{protein}$
H059	11.79 ± 0.01^{a}	10.96 ± 0.03^{a}	87.30 ± 0.53^{a}	102.34 ± 3.82^{a}
H060	11.88 ± 0.01^{b}	11.42 ± 0.02^{b}	74.30 ± 0.49^{b}	59.86 ± 5.03^{b}

Values are presented as mean ± SD. Superscript different letters indicate statistically significant pairwise differences ($p < 0.05$, unpaired t-test).

The data obtained from industrial batches H059 and H060 suggested that the production process optimized at lab scale was successfully transferred at industrial scale, even if it was expected a lower value of urease activity for the batch H060. The two batches of *S. thermophilus* BT01 are now ready to be used in a double-blind controlled crossover study on IBS patients.

4. Conclusions and Future Perspectives

This study provides valuable insights into the influence of culture parameters on the biomass production of *S. thermophilus* in an industrial context, particularly with regard to the modulation of urease activity. The results highlight how understanding the physiology and genetics of a probiotic can be a key factor in producing biomass with a specific enzymatic activity, which must be modulated to assess its effects on host physiology. In particular, the partial consistency of results between industrial and laboratory-scale production underscores the need to further optimize the technology transfer process.

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PhD Student Dissertation projects
1st year

Food Contact Compatibility and Shelf-life Effects of New Packaging Materials (P-I-1)

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The PhD thesis project adopts a systemic perspective on food packaging by developing new biobased packaging solutions from agri-food residues to improve the sustainability of food-contact materials, without compromising the shelf life of food products. The project incorporates the principles of the circular economy and aligns with EU goals on waste reduction, innovation, and sustainability.

Idoneità all'uso alimentare ed effetti sulla shelf-life di nuovi materiali di confezionamento

Il progetto di tesi di dottorato adotta una visione sistemica del packaging alimentare prevedendo lo sviluppo di nuove soluzioni di materiali di confezionamento biobased a partire da residui agro-alimentari, per migliorare la sostenibilità dei materiali a contatto con gli alimenti senza compromettere la shelf-life dei prodotti alimentari. Il progetto integra i principi dell'economia circolare e si allinea agli obiettivi dell'UE sulla riduzione dei rifiuti, innovazione e sostenibilità.

1.Introduction

In the current context of climate change, resource scarcity, and excessive waste generation, the food industry is at the center of an essential transformation. One of the most dynamic areas within this transition is food packaging, where active packaging is positioning itself as a key innovation, not only for its protective functions but also for its potential to enhance environmental sustainability. Active packaging represents an innovative strategy and one of the most advanced frontiers in food preservation. Unlike conventional packaging, which acts as a passive barrier against moisture, oxygen, or microbial contamination, active packaging is engineered to release or absorb specific functional compounds. Its dual purpose is to prevent spoilage while enhancing the sensory and microbiological quality of food products during storage. Antimicrobial agents used in active packaging are often of natural origin, such as nisin, lysozyme, or essential oil extracts, or may be incorporated as metallic nanoparticles. These substances are not simply added into the packaging system but are embedded within the material matrix or in polymer systems that allow their controlled release. The release mechanism may occur through simple diffusion or in response to environmental conditions, ensuring that the antimicrobial agent is deployed only when needed. In addition to antimicrobials, antioxidants constitute another key class of substances incorporated into active packaging. These compounds prevent lipid oxidation, which leads to rancidity, color loss, and nutrient degradation, especially in products with high fat or vegetable oil content. Substances such as ascorbic acid (vitamin C), tocopherol (vitamin E), and plant-derived flavonoids are commonly integrated into packaging systems for this purpose. Compared to the direct addition of preservatives into food, the active release approach has demonstrated superior efficacy. It allows for targeted application, minimizes waste, reduces direct food contact, and enhances the sustainability of the preservation process (Realini and Marcos, 2014).

Active packaging can be developed through the direct incorporation of bioactive compounds in the material before the extrusion, during which the typical high melting temperatures can lead to activity loss, or through the application of an active coating. The latter can be applied either to the inner surface of the packaging or, in some cases, directly onto the food product. These coatings are typically made from biodegradable natural polymers such as chitosan, alginate, or carrageenan and can be enriched with antimicrobial, antioxidant, or gas-absorbing agents (Khaledian and Basiri, 2022).

Among emerging biodegradable polymers, polybutylene adipate terephthalate (PBAT) is an aliphatic-aromatic copolyester of fossil origin that is fully biodegradable and compostable. It is synthesized through a polycondensation reaction of butanediol (BDO), adipic acid (AA), and terephthalic acid (PTA). Its monomers can also be produced from renewable sources, further enhancing its environmental sustainability profile. Thanks to its mechanical properties PBAT is suitable for plastic film applications, such as food packaging films. Due to its complete biodegradability, it is possible to use it in blends with other biopolymers, incorporating natural or waste-derived fillers. This not only improves mechanical and barrier properties but also helps to reduce the overall material costs. According to FAO estimates, more than one-third of food produced globally is wasted each year, with substantial implications for natural resource use and greenhouse gas emissions (Nile et al., 2020). Active packaging addresses this issue on two fronts: by utilizing agro-industrial residues in packaging material production and by extending product shelf-life through physical-chemical barriers that slow deterioration. This dual action enhances both the economic efficiency of food systems and their overall environmental sustainability (Russel et al., 2014).

Another important aspect of this research involves the use of a high-performance reactor, a device designed to operate under elevated temperatures and pressures, to support complex reactions such as autohydrolysis or organosolv treatments and facilitate the extraction of bioactive compounds and functionalize the agri-food residues for a better incorporation as fillers into polymers (Cipriani et al., 2024). The primary objective of its use is twofold: to sustainably extract active compounds from lignocellulosic residues, and to valorize the remaining fibrous biomass as a natural filler in biodegradable polymer composites. The reactor enables precise control of temperature, pressure, and solid-to-liquid crucial parameters for optimizing both extraction efficiency and the structural quality of recovered fibers. This integrated approach supports a circular bio-economy, combining green extraction processes with the creation of eco-friendly, high-performance materials (Abolore, Jaiswal & Jaiswal, 2024).

2. PhD Thesis Objectives

Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1. **Optimization of reactor parameters** aims to calibrate the operating conditions of the high-pressure reactor to achieve maximum efficiency in both fractionation processes (autohydrolysis followed by organosolv) and extraction processes for the recovery of bioactive compounds (A1.1) and filler material. The dual purpose is to obtain structural biopolymers such as cellulose and lignin (A1.2) with high purity or a solid residue to be used as a natural filler in biobased materials, as well as functional extracts with antioxidant or antimicrobial activity, for applications in active packaging.

A2. **Coating development** is aimed at the development of coating solutions tailored to the selected substrate (conventional or biobased plastic or paper), using the previously obtained active compounds.

A3. **Characterization of the new materials**: all the developed materials and coating solutions will be characterized for food contact compatibility according to current European legislation (A3.1), antimicrobial and/or antioxidant activity (A3.2); additional safety aspects required for food contact authorization (A3.3).

A4. **Upscaling and sustainability evaluation** selected solutions from previous activities will be analyzed to evaluate feasibility for an upscaled production (A4.1) and sustainability aspects based on an LCA approach (A4.2).

A5. **Writing and Editing of the PhD thesis**, scientific papers, and oral and/or poster communications.

Table 1 *Gantt diagram for this PhD thesis project.*

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1	***Optimization of Reactor Parameters***																								
	1. Optimization of process parameters for bioactive compouds																								
	2. Process optimization for natural fillers																								
A2	***Coating Development***																								
A3	***Characterization of the New Materials***																								
	1. Food contact compatibility																								
	2. Evaluation of packaging activity (e.g., antimicrobial and antioxidant effects)																								
	3. Additional safety aspects																								
A4	***Upscaling and Sustainability Evaluation***																								
	1. Evaluation for an upscaled production																								
	2. Environmental sustainability (LCA)																								
A5	***Writing and Editing of the PhD Thesis***																								

3. Selected References

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Quality evolution during storage of Spices and Herbs: understanding organoleptic and chemical (VOCs and main bioactive compounds) decay during Primary and Secondary Shelf Life (P-I-2)

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The primary aim of this PhD project, co-financed by the company Italpepe2 s.r.l. (RM), is to evaluate the quality profile in terms of organoleptic and chemical profiles of some spices and herbs, selected from the company lines, focusing on the study of their shelf life. The obtained quality profile will be used to select quality indicators in order to evaluate their evolution during their shelf life.
The approach of this project is to integrate different types of analysis, including chemical and sensory, to achieve multidisciplinary research with the aim of understanding the connections between chemical and sensory characteristics.

"Evoluzione della qualità durante la conservazione di spezie ed erbe: comprensione del decadimento organolettico e chimico (VOC e principali composti bioattivi) durante la Shelf Life Primaria e Secondaria"

L'obiettivo primario di questo progetto di dottorato, cofinanziato dall'azienda Italpepe2 s.r.l. (RM), è valutare il profilo qualitativo, in termini sia organolettici che chimici, di alcune spezie ed erbe aromatiche selezionate dalle linee aziendali, concentrandosi sullo studio della loro shelf life. Il profilo qualitativo ottenuto sarà utilizzato per selezionare indicatori di qualità, al fine di valutarne l'evoluzione durante la loro shelf life.
L'approccio di questo progetto è quello di integrare diversi tipi di analisi, comprese quelle chimiche e sensoriali, per realizzare una ricerca multidisciplinare con l'obiettivo di comprendere le connessioni tra caratteristiche chimiche e sensoriali.

1. State of the art

Spices and herbs (SH) have been part of the cuisine and traditional medicine of many people since ancient times [1]; these are easily distinguished by the parts of the plant used for production. Precisely, spices are defined as the dried part of aromatic plants (seeds, roots, barks, flowers, fruits, rhizomes) with exception for the leaves that represent herbs [1]. The importance over time of SH is due to their secondary metabolism that confers a range of beneficial properties thanks to their biocomponents, which are not only important for human health but also play an important role in the food technologies contributing both to technological and organoleptically characteristics [1]. Given the importance of SH and their economic value, their quality must be properly preserved from harvest to sale, considering that different processing technologies, storage conditions as well as formulations (whole, ground, powdered) affect degradation phenomena.

In this context, it is necessary to understand the changes that occur in the product during its primary (PSL) and secondary shelf life (SSL), by studying the evolution of specially chosen quality indicators for each raw material under analysis, indispensable to define the acceptability limit.

SL is defined as primary (PSL) when talking about a finite length of time after production and packaging during which the food product retains a required level of quality under well-defined storage conditions; the secondary SL is instead defined as the period after opening of the packaging during which a food retains its characteristics [2]. It is therefore important, for each product, to define a quality level, discriminating against the ones that are still acceptable for consumption from those no longer acceptable (acceptability limit) [2].
However, there are few articles in the literature that study the evolution of SH quality during their SL [3], [4], [5]. Given the high concentration of bioactive compound of SH, it is therefore necessary to understand what happens during the storage of these products so that we can improve the overall quality of SH that reaches the consumer's table and the food industry. According to Italpepe2's needs, the research focuses on:

Piper nigrum L. in its main variants black pepper (BP), white pepper (WP) and green pepper (GP), which differ among them as a function of ripeness and production technology [6].

Origanum spp. L. (with exception of *O. majorana* L.)
Hot red pepper (HRP) obtained from various species belonging to the genus *Capsicum*, valued for its distinctive pungency, aroma, color, etc.

2. PhD Thesis Objectives and Milestones

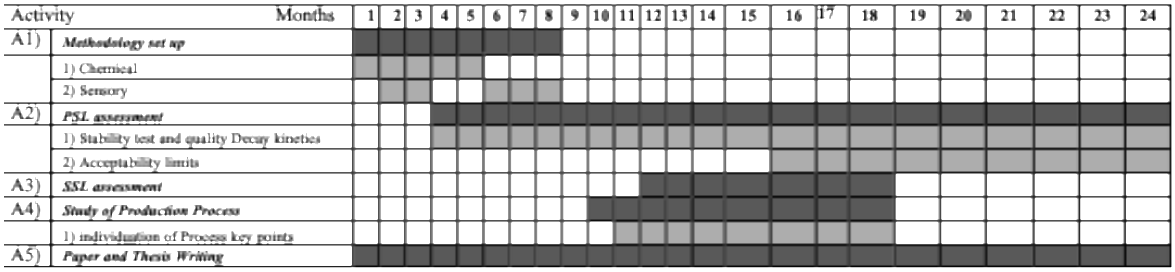
In line with the overall objective outlined above, Table 1 provides an overview of the materials and methods used in this PhD thesis project, while Table 2 outlines the breakdown of activities as scheduled in the Gantt chart:

	Methods	Objective	Instrument
Chemical methods	DPPH, FRAP, ABTS	Antioxidant Activity	UV-VIS
	Folin-Ciocalteau, $AlCl_3$	Bioactive compounds	UV-VIS
	Ethanol + UAE extraction	Piperine, capsaicinoids	UV-VIS /HPLC
	Liquid/Solid Extraction + UAE	Pigments (Chlorophylls, Carotenoids)	UV-VIS /HPLC
	Headspace solid-phase microextraction	Volatile Organic Compound (VOC) Analysis	HS-SPME/GC-EIMS
Sensorial	Descriptive	Olfactory, visive, taste	Panel test

Table 1 Main methodologies utilized for the different analysis.

A1) Methodology set up: to develop chemical and sensory methods suitable for the case study
A2) Primary Shelf-life assessment: To calculate the decay kinetics of the quality indices found through **PSL** trials
A3) Secondary Shelf-life assessment: To calculate the decay kinetics of the quality indices through **SSL** trials
A4) Study of the production process: to study the production process in order to identify the process key points to achieve a quality product
A5) Paper and Thesis writing.

Table 2 Gantt diagram for this PhD thesis project.



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Development of novel photodynamic materials to reduce microbial contamination in foods (P-I-3)

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This PhD project aims to develop advanced photodynamic materials tailored for food applications, utilizing a green approach in which the polymer and photosensitizer are derived from processing waste and biomass. These materials will provide a safer, more efficient means of reducing microbial contamination while preserving the quality and integrity of food products.

Sviluppo di nuovi materiali fotodinamici per ridurre la contaminazione microbica negli alimenti

Questo progetto di dottorato mira a sviluppare materiali fotodinamici avanzati per le applicazioni alimentari, utilizzando un approccio *green* in cui i polimeri e i fotosensibilizzatori verranno recuperati da scarti di lavorazione e biomasse. Questi materiali potranno fornire un mezzo sicuro ed efficiente per ridurre la contaminazione microbica, preservando la qualità e l'integrità dei prodotti alimentari.

1. State-of-the-Art

Microbial contamination poses a critical threat across the entire food chain, from farm to fork. Ensuring food safety is, therefore, an urgent global challenge. Traditionally, food preservation relies on chemical and thermal-based technologies to reduce microbial contamination. While effective, the use of chemicals can have adverse effects on human health and the sensory properties of food and can also lead to serious bacterial resistance. On the other hand, excessive heat treatment can cause undesirable nutritional, sensory, and functional changes in the treated foods. In response to these limitations, Photodynamic Inactivation (PDI) has emerged as a groundbreaking alternative for combating microbial contamination in food systems (do Prado-Silva et al., 2020; Penha et al., 2017). This innovative technique utilizes light to activate a photosensitizer (PS), which, in turn, generates reactive oxygen species (ROS) that effectively destroy microorganisms. Despite its potential, the direct application of photosensitizers in food has proven problematic, as it can negatively impact on the food's chemical, physical, and sensory properties. In fact, depending on the irradiation conditions applied and the exogenous PS used, it is possible to witness temperature increases in the product or its discoloration.
An innovative and yet underexplored approach in the food context is the immobilization of PS within a polymeric support that ensures direct contact between the matrix to be treated and the site of ROS generation, with no leaking of the PS. This approach currently has only medical and environmental applications (Byrne et al., 2018; Singh et al., 2024), while there is no literature regarding its exploitation in the food context. To design a photoactive material, a supporting polymer and a PS are required. In a green approach, both components can be obtained from processing waste and biomass. For example, PET-mimicking polymers can be made from lignin, which is extremely abundant in waste from the paper and food industries, as well as from plant biomass. On the other hand, molecules that can act as PS are widely present in both plant-derived raw materials (such as pigments and polyphenols) and microbial biomasses. Therefore, the development of materials starting from these sources can simultaneously provide tools for protecting the hygienic and health safety of foods and for generating products with high added value from processing waste. In light of these considerations, this PhD project aims to develop novel photodynamic materials for use in the food context to reduce microbial contamination.

2. PhD Thesis Objectives and Milestones

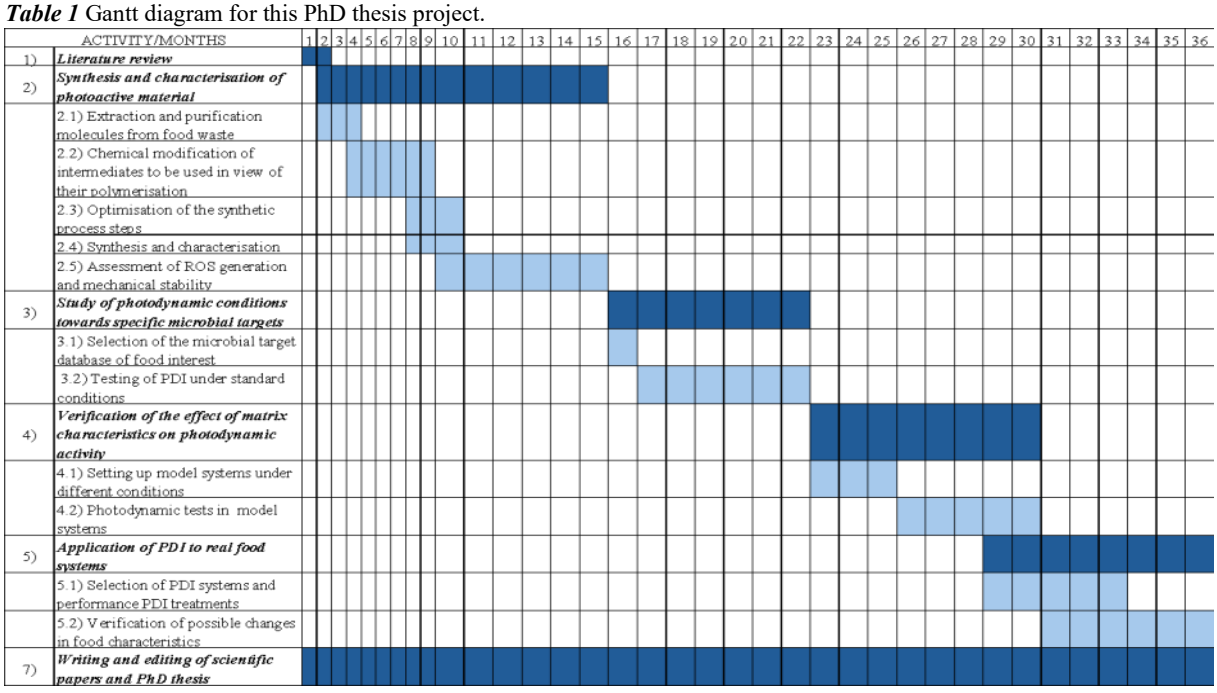
Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

1) **Literature review**: To identify the possible starting materials for the synthesis of the photoactive materials and the possible sources from which to obtain them. Additionally, information on extractive and synthetic processes employing a green chemistry approach will be collected.
2) **Synthesis and characterisation of novel materials**
   Steps of the research:
   2.1 Extraction and purification of molecules of interest from food waste, and evaluation of yields
   2.2 Chemical modification of intermediates to be used in view of their successive polymerisation

2.3 Optimisation of the synthetic process steps
2.4 Synthesis, chemical-physical and morphological characterisation of materials
2.5 Assessment of ROS generation and mechanical stability

3) **Assessment of PDI activity on specific microbial targets of food interest**: This phase will assess the photodynamic effect of the new materials against pathogenic and food-spoilage microorganisms. Steps of the research:
   3.1 Selection of microbial targets
   3.2 PDI test under standard conditions (physiological buffer, irradiation conditions, treatment time)
4) **Estimation of the matrix effect on PDI activity**: Considering the potential interference of food matrices with photodynamic activity, the new materials will be tested on model systems simulating real conditions at this stage. Steps of the research:
   4.1 Setting up model systems under different conditions of temperature, pH, carbohydrate/protein/fat concentration, salt concentration, viscosity, transparency, and aeration
   4.2 Photodynamic tests in model systems
5) **Application of PDI to real food systems**: Real foods will be selected to conduct PDI tests both in liquid and packaged foods to confirm the potential of the new materials and identify any potential negative effects on the foods. Steps of the research:
   5.1 Selection of real fluid and gaseous food systems
   5.2 PDI treatments of real food systems
   5.3 Assessment of possible physico-chemical and sensory changes in food
6) **Writing and editing** of scientific papers, PhD thesis and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.



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Cheese Food Safety: biocontrol systems through bacteriophages and active compounds (P-I-4)

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Food safety is essential for public health and depends on all stages of production, processing, storage, and consumption. Appropriate procedures and packaging systems are required to preserve physicochemical and microbiological properties and prolong shelf-life. Traditional fresh cheeses, although appreciated, are highly perishable due to elevated moisture and rich nutrients that support growth of spoilage and pathogenic microorganisms. Vacuum packaging, commonly used, fails to maintain quality or guarantee safety. This proposal aims to design and evaluate an innovative antimicrobial packaging system for fresh cheese stored under different refrigeration conditions, improving safety, extending shelf-life, and enhancing market competitiveness.

Sicurezza alimentare del formaggio: sistemi di biocontrollo attraverso batteriofagi e composti attivi
La sicurezza alimentare è essenziale per la salute pubblica e dipende da tutte le fasi della produzione, della lavorazione, dello stoccaggio e del consumo. Sono necessarie procedure e sistemi di confezionamento adeguati per preservare le proprietà fisico-chimiche e microbiologiche e prolungare la durata di conservazione. I formaggi freschi tradizionali, sebbene apprezzati, sono altamente deperibili a causa dell'elevata umidità e dei ricchi nutrienti che favoriscono la crescita di microrganismi patogeni e responsabili del deterioramento. Il confezionamento sottovuoto, comunemente utilizzato, non riesce a mantenere la qualità né a garantire la sicurezza. Questa proposta mira a progettare e valutare un innovativo sistema di confezionamento antimicrobico per formaggi freschi conservati in diverse condizioni di refrigerazione, migliorando la sicurezza, prolungando la durata di conservazione e aumentando la competitività sul mercato.

1. State-of-the-Art

Among Sicilian fresh cheeses, Canestrato Siciliano is a traditional cooked, hard cheese classified as fresh, semi-mature or mature. It is made exclusively from whole raw cow's milk and sometimes with small additions of sheep's milk (Ministerial Decree 16 February 2018). It is a distinctive representation of Sicily's regional cheese-making traditions, and it is consolidated as one of the island's most prized traditional cheeses (Carrà et al., 2013). However, in spite of the renowned quality of fresh cheeses (Todaro et al., 2014), the microbiological risk and limited shelf-life, with the current vacuum packaging system, reduce their marketing on a large scale. In this context, the focus on this product brings numerous benefits, first and foremost strengthening the trust relationship between consumers and producers. In particular, extending the shelf-life of fresh cheeses, in general, represents the most important challenge facing the production sector. Indeed, the high moisture content and nutritional profile of fresh cheeses provide ideal conditions for the growth of bacteria, spoilers and even pathogens, limiting their preservation even under refrigerated conditions. Given that food safety and awareness of the importance of the diet-health pair play a crucial role in consumer attitudes great value is placed on the choice of ingredients, production process and packaging. The development of micro-organisms in food depends on many factors, intrinsic and extrinsic, and can occur at any point in the production chain. After milking, milk, while presenting an endogenous microbiota, can support the growth of coliforms, yeasts, heterofermenting lactic acid bacteria responsible for eyeing and pathogens, such as Listeria monocytogenes (Claeys et al, 2013) and psychrotrophic microorganisms, such as Bacillus cereus and Pseudomonas aeruginosa (Sorrentino et al., 2012), which can produce large quantities of hydrolytic enzymes. Proteases, phospholipases and lipases can remain active even after bacterial cell death. The growth of sporigenic bacteria poses a threat to consumer health due to the production of toxins, and the heat resistance of spores. In their latest report on zoonoses (2023), the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) highlight that L. monocytogenes infections, characterised by encephalitis and sepsis, with a mortality rate of 20-30% are still among the most commonly reported in developed countries (Hilliard et al., 2018). The ability to grow in food is related to the species' ability to grow at refrigeration temperatures, resistance to acidic and osmotic conditions and, last but not least, the ability to form biofilms in production facilities and food. B. cereus is a ubiquitous bacterium that is pathogenic to humans and has been particularly focused on due to the high thermoresistance of its spores. P. aeruginosa is an opportunistic, antibiotic-resistant pathogen capable of forming biofilms. It is generally observed that the systems traditionally applied to eliminate pathogens affect the quality of the finished product with an impact on its sensory profile, human health and the environment, influencing consumer choices, especially, for dairy products. Great interest is being paid to innovative systems involving the addition of natural substances (bioactive molecules, post-biotics and bacteriophages) that can improve the microbiological profile without compromising quality. Among natural molecules, particular attention is paid to post-biotics and bacteriophages. Several phage preparations have already been approved by FDA for use in food products to control L. monocytogenes, Escherichia coli and Salmonella

spp., opening up prospects for the study and characterisation of further phage cocktails (Li et al., 2024). Probiotics have been extensively studied as functional ingredients with many food applications, mainly in dairy products and recent scientific evidence shows that the addition of probiotics in the form of dead cells and/or their components has a significant impact not only on consumer health but also on inhibiting the development of microorganisms in food. The use of postbiotics therefore appears very promising as it overcomes the problem of strain viability during the food production and storage process and, recently, attention has been paid to reduce the risk of transferring antibiotic resistance to humans. Some postbiotic preparations, obtained from probiotic lactic acid bacteria (LAB), have been used to improve the technological properties and extend the shelf-life of foods as an alternative to the application of live bacteria in antimicrobial active packaging systems (Moradi et al., 2020).

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 2:

A1) Evaluation of commercially available antimicrobial compounds as a function of the technological production process of Canestrato Siciliano Fresco. In vitro evaluation of efficacy against targets to determine the correct dose.

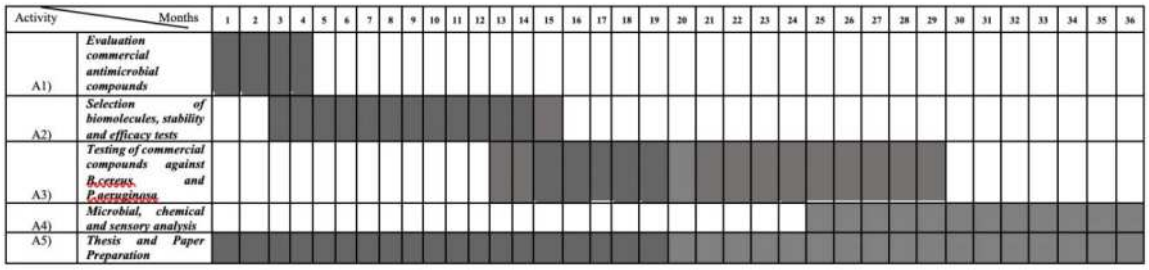
A2) Setting up ways of use. Selection of post-biotics and bacteriophages, stability and efficacy tests: 2.1 Experimental production of contaminated and control cheeses and subsequent treatment with the addition of post-biotics and bacteriophages. 2.2 Monitoring the effect against target bacteria and selection of the best bacteriophage concentration, LAB or bioactives molecules to be added. 2.3 Chemical analyses of cheese (moisture content, fat, protein and pH); SMart Nose; GC-MS. 2.4 PCR of the tested target bacteria.

A3) Testing of commercial (non-food grade) lytically acting bacteriophages against B. cereus and P. aeruginosa for efficacy in food: 3.1 Selection of B. cereus and P. aeruginosa strains for subsequent inoculation into cheese; 3.2 Selection of bacteriophages and post-biotics against B. cereus and P. aeruginosa based on the technological production process. In vitro evaluations of physico-chemical parameters to determine the dose to be used. 3.3 Testing the activity of bacteriophages and post-biotics in the food matrix. 3.4 Setting up a suitable system for using bacteriophages and postbiotics in cheese. 3.5 Production of experimental and traditional cheeses and evaluation of shelf-life.

A4) Microbiological, chemical and sensory monitoring of cheeses obtained in the previous phase: 4.1 Microbiological analyses of cheeses for target monitoring, concentration of bacteriophages, LAB. 4.2 Chemical analyses of cheeses, determination of moisture, fat, protein and pH content, fatty acid analysis; SMart Nose; GC-MS. 4.3 PCR of the targets considered

A5) Writing and Editing of the PhD thesis, scientific papers and oral and/or poster communications.

Table 2 Gantt diagram for this PhD thesis project.



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Technological Strategies for Developing Sustainable and Functional Plant-based Ingredients and their Applications in Innovative Food Products (P-I-5)

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This PhD research project focuses on the techno-functional, nutritional, and sensory valorization of dry-fractionated ingredients obtained from pulses and/or cereals by means of sustainable physical, technological, and biotechnological processes. The aim is to improve the techno-functional, nutritional and sensory properties of protein and starch ingredients obtained with dry fractionation. To reach this goal, enzymatic hydrolysis, ultrasound, and fermentation will be explored as suitable strategies that will be used for the development of innovative, sustainable formulations and food products aligned with current market and environmental demands.

Sviluppi tecnologici per la produzione sostenibile di ingredienti funzionali plant-based per l'applicazione in alimenti ad alto grado di innovazione

Questo progetto di ricerca di dottorato si focalizza sulla valorizzazione tecno-funzionale, nutrizionale e sensoriale di ingredienti ottenuti da frazionamento a secco di legumi e/o cereali, mediante l'uso di trattamenti fisici, tecnologici e biotecnologici sostenibili. L'obiettivo è migliorare le caratteristiche tecno-funzionali, nutrizionali e sensoriali delle frazioni proteiche e amidacee ottenute mediante frazionamento a secco. Per raggiungere questo obiettivo, processi di idrolisi enzimatica, ultrasuoni e fermentazione saranno esplorati come strategie per lo sviluppo di formulazioni e prodotti alimentari innovativi e sostenibili, in linea con le attuali esigenze di mercato e di sostenibilità ambientale.

1. State-of-the-Art

Global agrifood systems, particularly the meat-industry, severely contribute to greenhouse gas emissions, and have high environmental impact (FAO, 2024). Therefore, strategies to mitigate this phenomenon have been proposed, among which the dietary shift toward more plant-based diets. This includes the adoption of plant-based proteins in substitutions of traditional animal proteins. In this context, dry fractionation has gained attention as a sustainable method to obtain plant-based proteins and starches. Compared to the traditional wet extraction methods, dry fractionation is less energy- and water- demanding, and it helps to preserve protein native structure (Pulivarthi et al, 2023). However, dry-fractionated ingredients, particularly the proteins, often exhibit low water absorption capacity, and particularly undesirable sensory traits, which limit their application in plant-based product development (Gasparre et al, 2025). Similarly, the remaining starch-rich fraction remains underutilized, despite being the main output of dry fractionation. Recent literature has explored enzymatic hydrolysis, ultrasound treatments, and fermentation as tools to improve protein characteristics and overcome such limitations (Périé et al, 2025). Specifically, enzymatic hydrolysis has been found to improve the functionality and bioavailability of proteins, enhancing nutritional and sensory characteristics, as well as antioxidative properties, which help improve the quality of food products (Gasparre et al, 2025, Périé et al, 2025, Vogelsang-O'Dwyer et al, 2022). Similarly, several studies have shown that ultrasound improves the techno-functional, structural, and physicochemical properties of proteins, increasing solubility, emulsifying, foaming properties, and sensory acceptability (Saran et al, 2024). However, such approaches have not been investigated on dry-fractionated ingredients, suggesting space for further research. Additionally, the starch-rich fraction has been largely unexplored, representing an interesting market opportunity for industries operating in the sector.
Therefore, addressing this gap in the research is essential in order to unlock the full potential of these types of ingredients, promoting their use in sustainable food formulations as well as stimulating market growth.

2. PhD Thesis Objectives and Milestones

Within the overall discussion, this PhD thesis project can be framed into 3 main objectives:

- Technological, nutritional and sensory enhancement and optimization of dry-fractionated plant-based ingredients (proteins and starches) via enzymatic, ultrasound, and/or fermentation treatments.
- Valorisation of the starch-rich fraction, representing an unexplored area in literature and of potential interest for industry.
- Development of tailored food formulations using optimized ingredients with enhanced functional, nutritional, and sensory properties.

The resulting workplan has been structured in several main and sub-activities, as shown as follows and in the Gantt diagram given in Table 1:

A1) **Literature review and experimental planning**.

A2) **Technological and biotechnological treatments on dry-fractionated legume-based ingredients to modulate their properties.**
The aim is to improve the techno-functional, nutritional and sensory properties of selected plant-based ingredients through enzymatic hydrolysis (A2.1), ultrasound treatment (A2.2) and controlled fermentation processes (A2.3). Sub-activities involve, to begin with, the selection and collection of raw materials (protein- and starch-rich fractions of dry-fractionated yellow pea, yellow lentil, faba bean and chickpea) and enzymes (Alcalase 2.4 L FG, Protana® Prime and Protana® UBoost, Novozymes Italia S.r.l., Monza, Italy) for hydrolysis setup and optimization. Simultaneously, the ultrasound process (Sonicator UIP1500hdT, Hielscher Ultrasonics GmbH, Teltow, Germany) will be setup and optimized, as well as specific starter microorganisms will be identified and selected, and the subsequent fermentation process will be setup and optimized. All the activities will be accompanied by comprehensive techno-functional, nutritional, and sensory characterization of the ingredients.

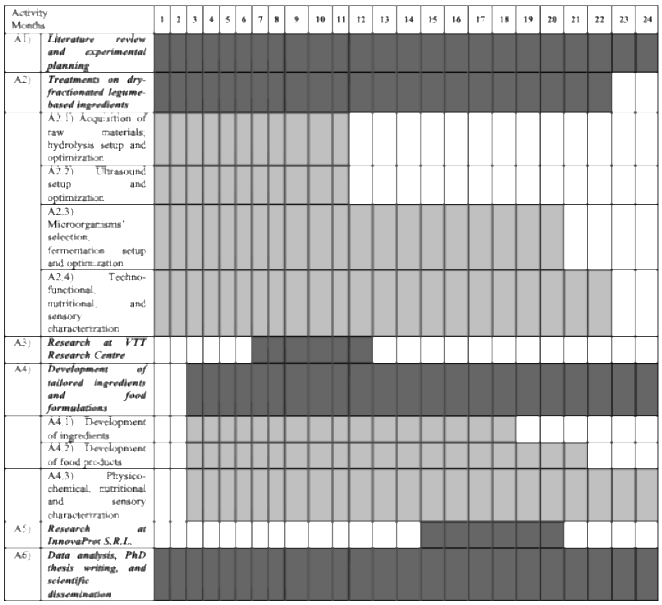
A3) **Experimental research at VTT Research Centre (Espoo, Finland)**.
This activity focuses on acquiring knowledge and skills on fermentation and ingredient characterization.

A4) **Development of tailored ingredients and food formulation**s.
The aim is to produce innovative ingredients with precise techno-functional characteristics (A4.1), tailored for formulation of targeted traditional and innovative foods (A4.2). This activity includes several sub-activities involving, to start with, the development of ingredient formulations with selected functionalities tailored for specific food formulation. These ingredients will be used to develop traditional and/or innovative food products. All the activities will be supported by physico-chemical, nutritional, and sensory analyses (A4.3).

A5) **Experimental research at InnovaProt S.R.L. (Gravina in Puglia, Italy).**
The focus will be on implementing the achieved lab scale results at an industrial level.

A6) **Data analysis, PhD thesis writing, and scientific dissemination.**

Table 1. Gantt diagram for this PhD thesis project.



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Application of non-thermal assisted processing for food stabilization and functionalization (P-I-6)

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This PhD thesis research project is aimed at the application of non-thermal technologies for the modification of food component's structure and functionalities and the improvement of food processing efficiency. In particular, some typical raw materials of the Chinese culture will be subjected to treatment with non-thermal technologies (e.g., Ultrasound, Pulsed Electric Field and Cold Plasma) and characterized in terms of rheological, microstructural and technological properties. The developed innovative raw materials/ingredients will be used in conventional processing and formulation in order to assess their functional behavior in real food systems.

Applicazione di processi non termici per la stabilizzazione e la funzionalizzazione degli alimenti

Questo progetto di tesi di dottorato è finalizzato all'applicazione di tecnologie non termiche per la modifica della struttura e delle funzionalità di componenti alimentari e il miglioramento dell'efficienza di processo. In particolare, alcune materie prime tipiche della cultura cinese saranno sottoposte a trattamento con tecnologie non termiche (ad esempio, ultrasuoni, campi elettrici pulsati e plasma freddo) e caratterizzati in termini di proprietà reologiche, microstrutturali e tecnologiche. Le materie prime/ingredienti innovativi sviluppati saranno utilizzati in processi e formulazioni convenzionali al fine di valutarne il comportamento funzionale in sistemi alimentari reali.

1. State-of-the-Art

In response to the growing focus on health and nutrition, there has been a surge in the demand for high-quality food products. This demand for minimally processed high-quality foods has driven significant advancements in the development of non-thermal processing technologies. These emerging technologies offer several advantages, including reduced energy and water consumption, decreased reliance on synthetic additives, and minimal impact on food nutritional value. Techniques such as Ultrasound (US), short-wave ultraviolet radiation (UVC), ultraviolet light-emitting diodes (UV-LEDs), pulsed light (PL), cold plasma (CP), and pulsed electric fields (PEF) are increasingly recognized as innovative approaches in food processing. These methods have the potential to extend shelf life and preserve food quality more effectively than traditional methods (Arshad *et al*., 2022). Non-thermal techniques are especially relevant in food science due to their ability to alter the functional properties of food components without the adverse effects of heat.

In Chinese cuisine, black tea and rice flour are important raw materials. Non-thermal treatments could be exploited for obtaining specific quality characteristics or inducing some new properties due to modifications promoted in the structure of the matrix and/or improve the efficiency of further processing such as fermentation and drying.

The structural and functional properties of rice starch can influence its gelatinization, pasting behavior, and retrogradation—factors that are crucial in various food applications (Liu *et al*., 2024). Rice is one of the important components of numerous food formulations wherein its composition and cooking quality are mainly related to starch and influenced by genetic, environmental shortcomings, and agronomical factors (Verma *et al*., 2022). In particular, black rice flour is valued for its distinctive flavor, color, and nutritional profile. However, its functional properties might be affected by variety, season and storage, decreasing its economic value. The tailoring of rice starch structure could be a strategy to increase its functionality and increasing its application in food formulation.

Black tea is prized for its health benefits and is commonly used in beverages and baked products. The manufacturing process of black tea mainly includes withering, rolling, fermentation, and drying, where fermentation is considered as the critical step for substance transformation and quality formation. During fermentation, many reactions take place simultaneously, including oxidation of polyphenols catalyzed by polyphenol oxidase (PPO) and peroxidase (POD) (Chen *et al*., 2023). The application of PEF to fresh tea leaves is believed to be able to induce the degradation of cell structure favoring enzymatic oxidation and therefore the development of the healthy antioxidant compounds typical of the product.

Similarly, these techniques can enhance the stability of bioactive compounds in black tea during storage by inhibiting enzymatic and oxidative degradation without the use of heat (Raghunath *et al*., 2023). Understanding how non-thermal processing affects the functionality of rice starch and the storage stability of black tea is essential for developing high-quality, shelf-stable food products.

Cold Plasma (CP) is considered the fourth state of matter and consists in a very reactive mixture with high oxidative power. CP has recently gained significant attention for the modification it can induce in food components, altering

structural and functional properties (Kopuk *et al.,* 2022).
Thus, this PhD project is focused on the evaluation of the effect on these emerging technologies on processing efficiency and on techno-functional properties of Chines raw materials and ingredients for improving the sustainability and increasing the innovation in the food sector.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:
A1) **Literature review and research** on the application of non-thermal technologies for modification of food components and improving process efficiency.
A2) **Application of ultrasound to rice flour** to improve texture and functional properties. Rice flour from two different seasons will be used as fresh and aged rice. Design of Experiment will be used to understand the effect of different factors on the quality, structure and techno-functional properties (A2.1). The optimized ingredient will be used as modified starch in the formulation of a food product (e.g., fat replacer in ice cream) (A2.2).
A3) **Application of PEF to fresh tea leaves** for the improvement of black tea processing. The influence of PEF variables such as electric field strength, pulse width and frequency will be evaluated on the withering, fermentation, and drying stages with the aim of increasing process efficiency (A3.1) and final product quality (A3.2).
A4) **Application of Cold Plasma (CP) for raw material modification**. CP treatments will be applied varying some operative conditions such as energy and duty cycle to obtain different reactive species and treatment time in order to modify rice starch properties (A4.1) and to induce oxidation of tea leaves (A4.2).
A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity / Month		2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1	***Literature review and research***																		
A2	***Application of US to rice flour***																		
	1) Optimization of US treatment																		
	2) Use of modified flour for food formulation																		
A3	***Application of PEF to fresh tea leaves***																		
	1) influence on processing efficiency																		
	2) influence of final product quality																		
A4	***Application of CP for raw materials modification***																		
	1) Effect on starch modification																		
	2) Effect on tea leaves																		
A5	***Thesis and Paper Preparation***																		

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Optimisation of Candy Processing Conditions for Sustainable Confectionery Products with Improved Sensory Properties (P-I-7)

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This PhD thesis research project focuses on the optimisation of the drying process of gelatin-based jelly candies and the effect on the characteristics of the final products. The study will consider the main processing parameters of the drying operation (e.g. wet and dry bulb temperatures, relative humidity, time) and the products will be characterized for physical (texture, thermal), microstructural, chemical (moisture, sugar, and acid content), and sensory attributes along with their physical stability. The goal is to develop a more efficient process that also reduces energy consumption, increasing sustainability, while preserving the product's original sensory profile and overall consumer appeal.

Ottimizzazione delle condizioni di lavorazione delle caramelle per ottenere prodotti dolciari sostenibili con proprietà sensoriali migliorate.

Questo progetto di ricerca per la tesi di dottorato si concentra sull'ottimizzazione del processo di essiccazione di caramelle gelatinose a base di gelatina e sull'effetto sulle caratteristiche dei prodotti finali. Lo studio prenderà in considerazione i principali parametri di processo dell'operazione di essiccazione (ad esempio, le temperature di bulbo umido e secco, l'umidità relativa, il tempo) e i prodotti saranno caratterizzati per gli attributi fisici (consistenza, termicità), microstrutturali, chimici (contenuto di umidità, zuccheri e acidi) e sensoriali, oltre che per la loro stabilità fisica. L'obiettivo è sviluppare un processo più efficiente che riduca anche il consumo di energia, aumentando la sostenibilità e preservando il profilo sensoriale originale del prodotto e l'appeal complessivo per il consumatore.

1. State-of-the-Art

Jelly Candy sunlight drying process (SDP) over time became unsustainable due to weather conditioning (cloudy and rainy days) and capacity scaling (industrial manufacture), giving rise to a mechanical drying (dehydrating) process (MDP). The two techniques are conditioned to preserve the organoleptic-structural properties, color, aroma, texture taste and shape of processed jelly candy[1] Candies process consists of several steps and the most important ones are those related to the mixing of the ingredients, the cooking and the drying that lead to obtaining products and medium-to low moisture content, with different texture and quality properties depending on the initial formulation. Besides cooking, drying is a main step in the candies manufacturing in terms of both the final quality of the product and the energetic impact of the process. Currently, empirical approaches are applied in this sector and, besides the use of new ingredients, there is a scarce investigation on how to improve the sustainability of the process and develop innovative [2], knowledge-based processes After a preliminary analysis of the current industrial drying process and its impact on product quality, the study will continue by testing various drying conditions as a combination of temperature, time, and air relative humidity[3] by exploiting experimental design approaches and response surface methodology[4]. The experimental plan will be performed in a dedicated pilot drying cabinet, and, in the final steps, pre-industrial trials will be performed. Results will increase the understanding of the changes in the candy matrix during the drying process and impact on the quality of the final products, offering the opportunity to increase the efficiency of the process and its sustainability [5] in the candy production system. Project Material: Gelatin-based Gummy candies composed of hydrocolloids, sucrose, sugar syrup, water, acid and flavors under different formulation and cold and hot set fabricated and extruded for stoving will be used for this study operated under optimal stoving conditioning of relative humidity, temperature, air flow rate and mass area, correlatively with formulation variance.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Drying process effects on the quality and stability** (chemical, physical, structural, and physico-chemical properties) concerning its sensory profile impact (A1.1), conventional and innovative drying (NIR-moisture) Analysis (A1.2), correlation of conservative parameters on innovative drying process and product quality.

A2) **Optimization of Drying Processes** to identify the model capable of reconstructing the energy consumption

of the drying methods used in jelly candy production, predictively, with changes in formulation. performances as functions of the main operating variables (T, t, RH) and a Prediction model for oven conditioning at varying formulation latent variable alteration (A2.1) and Optimal Stoving Conditioning independent of formulation (A2.2) reduce the drying time and energy requirements while maintaining or improving product quality.

A3) **The impact of the drying operation using the optimized model** in the pilot plant scale to validate the prediction capability of mathematical models identified during activity A2 in terms of gelling agents (gelatin, gelatin-free formulations), sugar content, and type for an optimal performance and of, drying temperature, airflow, and humidity control.

A4) **Modelling and virtualization of the drying process of jelly candies** A Predictive Mathematical Model to simulate the drying kinetics of confectionery products based on their composition and processing variables (A4.1) and set up to predict moisture diffusion, heat transfer, based on product geometry (A4.2) predict the behavior of the drying process under various conditions, aiding in the design of efficient drying systems conditioning.

A5) **Writing and Editing** of the PhD thesis, scientific papers, and oral and/or poster communications.

Table 1 *:* Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	**Drying process effects on quality and stability**																								
	1) conventional and innovative drying (NIR- moisture) Analysis																								
	2) correlation of conservative parameters on innovative drying process and product quality																								
A2)	**Optimization of Drying Processes**																								
	1) Prediction model for oven conditioning at varying formulation																								
	2) Reduce the drying time and energy requirements																								
A3)	**The impact of the drying operation using the optimized model**																								
	1) varying formulation latent variable																								
	2) time and energy requirements																								
A4)	**Modelling and virtualization of the drying process of jelly candies**																								
	1) set up to predict moisture diffusion, heat transfer based on product geometry																								
	2) predict the behavior of the drying process under various conditions																								
A5)	***Thesis and Paper Preparation***																								

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Use of Robotic Applications for Elevating the Quality and Sustainability of Food Products (P-I-8)

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This PhD thesis research project aims to adapt robotics in food processing applications to enhance the quality, safety and sustainability of the food manufacturing process. To extend the application of robotics, unlike picking and placing food materials, robots are involved in several unit operations in the food industry, including cutting, kneading and so on. By expanding the role of robotics in these critical functions, the research contributes to increased automation and fosters innovation within the industry, paving the way toward the Food 5.0 industrial revolution.

Uso delle Applicazioni Robotiche per Migliorare la Qualità e la Sostenibilità dei Prodotti Alimentari

Questo progetto di ricerca per una tesi di dottorato mira ad adattare l'uso della robotica nelle applicazioni di trasformazione alimentare per migliorare la qualità, la sicurezza e la sostenibilità del processo di produzione degli alimenti. Per estendere l'applicazione della robotica, oltre alle tradizionali operazioni di prelievo e posizionamento dei materiali alimentari, i robot vengono impiegati in diverse operazioni unitarie nell'industria alimentare, come il taglio, la lavorazione dell'impasto e altre ancora. Espandendo il ruolo della robotica in queste funzioni critiche, la ricerca contribuisce ad aumentare l'automazione e a promuovere l'innovazione all'interno del settore, aprendo la strada verso la rivoluzione industriale Food 5.0.

1. State-of-the-Art

The food sector is being rapidly revolutionized by robotics, which is achieved through the implementation of numerous applications that improve efficiency, uniformity, and safety in the production and processing of food. Robots are employed in manufacturing to perform some routine tasks like packaging, sorting, and palletizing, hence enhancing operational efficiency and reducing labor costs (Khan et al., 2018). Automated guided vehicles and autonomous mobile robots are employed in warehouses and distribution facilities to enhance inventory management and logistics (Patricio & Mendes, 2020). Before their implementation, robots in food processing plants can carry out intricate tasks such as cutting, chopping, kneading, cleaning, blending, and cooking with great precision and modular gastronomical applications (Derossi et al., 2023). This ensures consistent results and minimizes avoidable waste, making them suitable for various culinary applications. In addition, robotic arms that are equipped with sensors allow for precise quality control measures by detecting defects and ensuring adherence to food safety standards. These innovations improve efficiency and help maintain hygiene while lowering the danger of infection in food processing operations. With the ongoing advancement of robotics technology, its application in the food industry holds the potential for additional advancements in terms of efficiency, quality assurance, and overall operational excellence.

In the Industry 4.0 evolution of Industry 5.0, robots have a crucial role in technological development, particularly in the food sector. Robots can be integrated with IoT, sensors, AI, and big data, which may enhance this application orientation in food (Dadhaneeya et al., 2023). For instance, integrating robotic technologies in food processing is a significant innovation aimed at boosting quality and sustainability across the industry. Robotics can improve food manufacturing precision and uniformity, ensuring every product satisfies strict quality standards. These technologies automate complex tasks in the food manufacturing line, reducing human error and improving the overall system's efficiency. Furthermore, robotics promotes the implementation of sustainable practices by improving resource use, minimizing waste, and supporting environmentally friendly production processes. Robotic applications in food manufacturing are growing as demand for safe, nutritious, and ethically produced food rises, promising a future of quality and sustainability (Hassoun et al., 2023).

Thus, this PhD thesis project aims to incorporate robotics into some unit operations for food manufacturing, to not only improve productivity and efficiency but also broaden the range of possibilities that may not be accomplished with conventional processing methods. With ongoing progress, it is foreseeable that robotics will assume a progressively crucial function in the forthcoming era of food manufacturing.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Selection, design and development of hardware and software components** Robot-assisted unit operation setup (cutting, kneading, etc.)
A2) **Performance evaluation of robotics arms coupled with unit operation setup** to evaluate the developed system to execute the desired performance.
A3) **Quality evaluation of processed food materials** in the developed system, comparing the processed materials in the developed system with the conventional processed materials
A4) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 *Gantt diagram for this PhD thesis project.*

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A 1)	***Selection, design and development of hardware and software components***																								
A 2)	***Performance evaluation of robotics arms coupled with unit operation setup***																								
A 3)	***Quality evaluation of processed food materials***																								
A 4)	***Thesis and Paper Preparation***																								

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Upcycling pea waste side streams for developing future food ingredients (P-I-9)

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This Ph.D. research, supported by Fondo per il Programma Nazionale di Ricerca e Progetti di Rilevante Interesse Nazionale (PRIN) PNRR M4.C2.1.1 founded by Next Generation EU, aims at investigating the possibility to recover proteins from waste side streams and explore how the effect of different technologies, from extraction to ingredient preparation, might affect the technological and functional properties of the protein ingredient. The main objective of this Ph.D. is to develop an innovative pea protein ingredient for the formulation of plant-based prototypes. More specifically, the aims are: (i) to identify the best extraction technique for obtaining functional food ingredients; (ii) to investigate the effect of chemical and physical techniques on protein functionalities at micro and macroscopical level; (iii) to develop novel food ingredients and food prototypes.

Riutilizzo di sottoprodotti di scarto dei piselli per sviluppare ingredienti alimentari innovativi

Questa ricerca di dottorato, sostenuta dal Fondo per il Programma Nazionale di Ricerca e Progetti di Rilevante Interesse Nazionale (PRIN) PNRR M4.C2.1.1 finanziato da Next Generation EU, mira a indagare la possibilità di recuperare proteine dai flussi di scarto dell'industria dei piselli e a esplorare come l'effetto delle diverse tecnologie, dall'estrazione alla preparazione degli ingredienti, possa influenzare le proprietà tecnico-funzionali di ingredienti derivati proteico. L'obiettivo principale di questo dottorato è sviluppare ingredienti innovativi a base di proteine di piselli per la formulazione di prototipi alimentari. Più specificamente, gli obiettivi sono: (i) identificare la migliore tecnica di estrazione per ottenere ingredienti alimentari funzionali; (ii) indagare l'effetto delle tecniche chimiche e fisiche sulle funzionalità delle proteine a livello microscopico e macroscopico; (iii) sviluppare nuovi ingredienti alimentari e prototipi alimentari.

1. State of the art

With the increasing demand for proteins caused by several factors, such as the growing global population, the rising consumer awareness about nutrition and health together with the gradual shift towards a more sustainable food system, is of outstanding importance to try to recover proteins from food waste side stream, hence also reducing food waste(Aschemann-Witzel *et al.*, 2021; Ratnayake and Naguleswaran, 2022; Yang *et al.*, 2022). Pea (Pisum sativum) is one of the most important crops grown worldwide and across the European Union, with the global production exceeding 35 million tons annually. During industrial processing, up to 15% of peas - often damaged, yellowed, stained, or of sub-standard size - are discarded and directed toward anaerobic digestion. These damaged peas, although excluded from final food products, remain rich in high-value nutrients such as proteins (~5% fresh weight), carbohydrates (~14%), and fibers (~5%), For this reason, their waste led to a loss of both economic and nutritional value. The valorization of this underutilized biomass represents a strategic opportunity to promote sustainability and circularity in food systems. Recent interest has focused on recovering functional ingredients—including flour and protein extracts—from pea waste streams (Kumar and Brooks, 2025). These ingredients have shown promising techno-functional properties (e.g., emulsifying, gelling, and solubility) suitable for a wide range of food applications (Yang *et al.*, 2022; Verkempinck *et al.*, 2024). However, industrial adoption remains limited due to challenges related to raw material variability, processing optimization, and cost-efficiency of scalable biorefinery technologies. In this context, often the reason for difficulty in the application of such ingredients for product development is the loss of techno-functional properties caused by extreme extraction and processing techniques (Angelis *et al.*, 2024). Various physical and chemical modification techniques have been explored to enhance and tailor the performance of recovered proteins (Sarigiannidou *et al.*, 2022; Tanger *et al.*, 2022; Zhang *et al.*, 2025). These treatments, including heat, pH shifting, high-pressure processing, enzymatic hydrolysis, and ultrasonication, can significantly influence protein structure, thereby modifying solubility, surface activity, aggregation behavior, and gelation capacity (Nasrabadi, Doost and Mezzenga, 2021; Zhang *et al.*, 2025). The pre-treatment and treatment conditions applied during protein extraction and stabilization of the resulting powders are expected to highly impact the chemical, technological and sensory properties of pea derived ingredients. These characteristics will be evaluated to facilitate the further application of these ingredients in different food product categories. Fresh and pre-treated pea waste samples, pea flour and pea protein-based ingredients will be characterized for their total composition (dry matter, protein, starch, fiber), their physical and chemical characteristics such as particle size distribution, surface charge, isoelectric point, polyphenolic content and antioxidant activity, protein content and quality. Techno-functional properties such as solubility, viscosity,

gelling and emulsifying properties of the protein ingredients will be measured using standard procedures (Hinderink *et al.*, 2021; Rodriguez and Beyrer, 2023; Angelis *et al.*, 2024). These evaluations will be performed under varying conditions such as temperature, pH, salt concentration, and protein concentration. After this initial screening, different chemical and physical techniques will be tested to improve the most interesting properties recognized and of interest for the possible design of a food model, which mimic the technological functionality of a dairy alternative product. Over the course of three years, the significance at the macroscopic scale of changes occurring at the microscopic level to the systems under consideration will be analyzed, with emphasis on emulsifying and gelling properties of proteins.

2. Ph.D. Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Evaluation of the physico-chemical properties of pea waste and ingredients derived from** with a focus on the change caused by the extraction process selected. The gross composition of the ingredients will be analyzed with a particular focus on protein, carbohydrates and fat and minor compounds (polyphenols, minerals)

A2) **Functional properties of innovative pea ingredients** and examination of how those properties change after physical and chemical modification. Specific focus will be on solubility and the emulsifying and gelling properties of the ingredients under different extrinsic conditions (e.g., pH, ionic strength).

A4) **Prototyping of an innovative dairy alternative**

A5) **Writing and Editing**

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Physico-chemical properties of Pea protein***																								
A2)	***Functional properties characterization***																								
	1) Solubility																								
	2) Emulsifying																								
	3) Gelling																								
A3)	***Prototyping***																								
A4)	***Thesis and Papers preparation***																								

Table 1 Gantt diagram for this PhD thesis project.

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Agronomic and technological strategies to produce Mediterranean cereal and legume-based snacks for sustainable healthy nutrition (P-I-10)

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The present project aims to cultivate Mediterranean cereals and legumes through agronomic strategies that reduce environmental impact, improve soil health, and enhance food quality while minimizing inputs and costs. A field trial will be conducted to investigate the intercropping of durum wheat (including both modern and ancient genotypes) with local legumes inoculated with *Rhizobium* strains. The resulting cereal and legume crops will be processed into flour and used as highly nutritious ingredients for protein-rich snack formulations. These products will be evaluated for their nutritional value, sensory quality, and consumer acceptability.

Strategie agronomiche e tecnologiche per la produzione di snack mediterranei a base di cereali e legumi per un'alimentazione sana e sostenibile

Il presente progetto mira a coltivare cereali e legumi mediterranei attraverso strategie agronomiche che riducono l'impatto ambientale, migliorano la salute del suolo e la qualità degli alimenti, riducendo al minimo gli input e i costi. Verrà condotta una prova in campo per studiare la consociazione tra grano duro (genotipi moderni e antichi), leguminose locali inoculati con ceppi di *Rhizobium*. Le colture di cereali e legumi ottenute saranno trasformate in farine e utilizzate come ingredienti altamente nutritivi per formulazioni di snack ricchi di proteine. Questi prodotti saranno valutati per il loro valore nutrizionale, la qualità sensoriale e l'accettabilità da parte dei consumatori.

1. State-of-the-Art

In agreement with the EU *Green Deal*, there is a pressing need to adopt sustainable agricultural techniques as an alternative to conventional practices, especially in the semi-arid Mediterranean zones of southern Europe, where the irregular rainfall regimes and the low soil fertility pose serious barriers to agricultural activity. Currently, the plant proteins used in the food sector are mainly derived from soy seeds. Unfortunately, soy cultivation is associated with environmental damage much more than any other crop on the planet. On the other hand, Mediterranean grain legumes like chickpeas, peas, and lentils, among others, are well-recognized as sustainable sources of proteins for the human diet, being well adapted also to marginal land in the Mediterranean area and capable of producing protein ingredients to develop innovative food products. Intercropping, the simultaneous cultivation of two or more crop species in the same space (Vandermeer, 1989), offers a promising solution over monocropping, including improved land use efficiency, soil fertility maintenance, reduction of weed and pest incidence, and enhanced yield stability (Bedoussac et al., 2015; Daryanto et al., 2020). Cereal/legume intercropping is able to boost crop yields in low-N input systems and sustainably and efficiently produce high-quality cereal and legume grains (Hauggaard-Nielsen, 2009). A key component of legume-based systems is their symbiosis with *Rhizobium spp.,* which account for approximately 50% of 175 million tons of global biological N_2 fixation annually. Inoculating grain legumes with *R. leguminosarum* strains enhances their agronomic and qualitative performance, providing ecosystem services to soil, environment, and cash crops and a lower need for synthetic fertilizers (Chaechian et al., 2022; Raklami et al., 2021; Sammama et al., 2022).
In recent years, there has been a significant global shift towards plant-based proteins. This change is driven by a greater awareness of the environmental impacts of animal agriculture, concerns about animal welfare, health considerations, and the rising demand for sustainable food choices. Plant-based proteins have gained worldwide popularity and are now being incorporated into diets on a much larger scale. Many studies have also demonstrated the potential of plant proteins to reduce the risk of various metabolic diseases, thereby improving food nutritional quality and human health (Kumar et al., 2022; Karabulut et al., 2024).
The primary goal of this project is to integrate sustainable agronomic practices with technological strategies to promote Mediterranean cereal and legume grains as key ingredients for healthy food, such as cereal-protein snacks. The project aims to reduce the environmental impact, meet agri-food supply chain expectations, improve soil health and food quality, minimize inputs and costs, as well as design and develop rich-protein foods that are both nutritious and healthy.

2. PhD Thesis Objectives and Milestones

The research activities will be developed according to a synergic approach, combining field trials, laboratory experiments, and model applications.
A1) **Field trials o n durum wheat–grain legume intercropping systems**. A field trial will be carried out in the second year to investigate the effect of intercropping and *Rhizobium* inoculation on crop quantitative and qualitative performance. Two to three ancient varieties of durum wheat will also be included to assess their performance in combination with chickpeas, peas, and lentils. The trial will aim to identify optimal cereal-legume combinations for sustainable production in Mediterranean conditions. In the third year, the best-performing legumes and wheat will be established in larger plots, each in triplicate .

A2) **Flour Characterization.** The resulting cereal and legume crops will be processed into flour using a roller mill and subjected to chemical, physico-chemical, and technological characterization. (A2.1) Chemical-physical analyses will be carried out through GC/MS, HPLC/MS, UV, and FT-IR equipment. (A2.2) The water solubility, hydrophobicity, emulsifying and foaming capacity, water and oil absorption capacity, viscosity, and gelatinization ability will be assessed.
A3) **Production and characterization of cereal and protein snacks. (**A.3.1**)** Composite cereal and legume flours will be prepared based on the nutritional and technological properties of each flour and used for developing protein-rich snacks. The design of experiments (DoE) will be applied to optimize formulations by maximizing the nutritional values of the final products. **(**A.3.2**)** The snacks will be characterized by their nutritional value, sensory quality, and consumer acceptability.
A4) **Ecosystem services and environmental impact of cereal/legume intercropping.** (A4.1) Ecosystem services provided by intercropping systems will be categorized and quantified by Provisioning Services, Regulating Services, Supporting Services, and Cultural Services. (A4.2) The Life Cycle Assessment (LCA) methodology will be used to quantify the environmental impact of wheat/legume intercropping compared to monocropping.
A5) **Writing and Editing** of the PhD thesis, scientific papers, and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Field trials on durum wheat–grain legume systems***																								
	1) Experimental field trial																								
	2) Field trial in big plots																								
A2)	***Chemical and technological analyses of wheat and legume grains***																								
	1) Chemical analyses																								
	2) Technological analysis																								
A3)	***Production of cereal and protein snacks***																								
	1) Formulation optimization by DoE																								
	2) Nutritional and sensory quality and consumer acceptability																								
A4)	***Ecosystem services and environmental impact of cereal/legume intercropping***																								
	1) Analysis of ecosystem services																								
	2) Analysis of environmental																								
A5)	***Thesis and Paper Preparation***																								

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Study on the Effect and Mechanism of Action of Chitosan on Fixed Compounds, Polyphenolics, Volatiles Substances and the Colour of Wines and Beers (P-I-11)

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This PhD thesis research project aims to deepen how chitosan (KT) may affect fixed compounds, polyphenolics, and volatiles compounds of wines and beers, influencing colour and antioxidant activity. Through systematic compositional analysis of chitosan-treated wines, the study aims to optimize the industrial application of chitosan as a sustainable processing aid in winemaking.

Studio sugli Effetti e sul Meccanismo d'Azione del Chitosano sui Composti Fissi, Polifenoli, Sostanze Volatili e il Colore di Vini e Birre

Questo progetto di ricerca di dottorato indaga gli effetti del chitosano (KT) sui composti fissi, polifenoli e composti volatili di vini e birre, con particolare attenzione alle sue influenze sul colore e sull'attività antiossidante. Attraverso un'analisi composizionale sistematica dei vini trattati con chitosano, lo studio mira a ottimizzare l'applicazione industriale del chitosano come coadiuvante tecnologico sostenibile in enologia.

1. State-of-the-Art

Constituents of wines can react with significant amounts of oxygen, polyphenols being among the most readily oxidized wine constituents. Wine polyphenolic substances are usually subdivided into two groups: flavonoids and non-flavonoid compounds. Flavonoids are the main determining factors affecting the color, astringency, and bitterness of grapes and wine. The most common wine flavonoid compounds are flavonols, flavan-3-ols, and anthocyanins. Non-flavonoid compounds are mainly derivatives of benzoic acid and of cinnamic acid.
Despite controlled oxidation could be beneficial for wine by enhancing and stabilizing color (Singleton, 1987), unwanted oxidation can lead to a decrease in the phenolic compounds such as (+)-catechin, (−)-epicatechin, quercetin, caffeic acid and anthocyanins, and an increase in red polymeric pigments improving the wine color density (Castellari et al., 2000). During wine oxidation, a sequence of univalent reduction steps settles from oxygen up to water, via a Fe(II)/Fe(III) redox cycle where hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical (HO•) are formed (Danilewicz, 2012; Waterhouse and Laurie, 2006). According to 'thermal' Fenton mechanism (as opposed to light-induced), the reduction of H_2O_2 to HO• is mediated by metals (e.g., by Fe(II)/Fe(III) redox couple) (Marin et al., 2021). In this process wine polyphenols play a major role by redox cycling iron (Oliveira et al., 2011). Fenton-derived HO• will also react with wine carboxylic acids such as tartaric acid, forming a carbon-centered radical derivative which undergoes oxidation and decarboxylation steps to yield glyoxylic acid. Accordingly, reducing the development of aldehydic oxidation intermediates would be a relevant strategy to maintain color and flavor of wines.
Chitosan (KT) is a main derivative of chitin and can be industrially obtained by N-deacetylating chitin through a process involving deproteinization, demineralization, decolorization, and deacetylation (Aranaz et al., 2009). Through deacetylation, free amine groups (-NH_2) are produced along the polysaccharide backbone and result a polycationic character in KT, which depends on the deacetylation degree (DD) and molecule weight (MW) (Friedman and Juneja, 2010). The chemical structure of KT could be functionalized with a vast diversity of ligands by means of reactions such as carbonylation, alkylation, sulfonation, carboxymethylation, and quaternization, which enlarge enormously the potential applicability of modified KT (Brasselet et al., 2019; Higueras et al., 2015; Rocha et al., 2017).
Further, KT is a suitable material for developing drug delivery systems in the form of nanoparticles or nanovesicles due to its gel-forming ability, enabling the controlled release of chemical-active molecules.
In the last decade, KT has been accepted by European Commission as a fining agent for treatment of wine, for different purposes: prevention of iron and copper casses, reduction of heavy metals or possible contaminants, especially ochratoxin A, and inhibition of unwanted microbial growth, particularly Brettanomyces spp.
It has quite recently been proposed that chitosan can complement sulfites in wine or other beverages, thanks to its antimicrobial behavior (Castro Marín et al., 2021). In addition, the intriguing antioxidant features demonstrated by this polymer make it a promising candidate for the production of sulfite-free wines where both microbial and oxidative stability should be conveniently controlled all along the winemaking process and the shelf life. Some research on these subjects has already been carried out, but the exact impact of its utilization on wine composition and the specific mechanisms of action are still to be precisely defined.

2. PhD Thesis Objectives and Milestones

This study will focus on wines and beers, exploring the effects of KT on their compositions in terms of fixed composition, polyphenolic and volatile substances.
The doctoral thesis project can be divided into the following activities, summarized in the Gantt chart shown in Table 1:

A1) **Bibliographic review** about the effects of KT's antioxidant capacity and metal ion adsorption capacity on metal ions and oxidation in a wine-like environment.
A2) **Analysis of the effects of chitosan addition** on the physicochemical properties, fixed compounds, and Fe (II)/Fe (III) oxidation status.
A3) **Evaluation of distinct vinification steps and shelf lives about red/rosè wines** with the addition of KT.
A4) **Analysis of KT nanoparticles**, including the production and characterization of KT nanoparticles, and analysis of white-rosè-red wines with the addition of KT nano particles and its utilization.
A5) **Evaluation of KT's ability in controlling the quality of beer environment**, including the impact on *Brettanomyces* flavour profiles and the control of contamination in beer-like environment.
A6) **Writing and publishing of the doctoral thesis, posters, scientific articles and oral presentation.**

Table 1 Gantt chart of the PhD research activity.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
A1)	Bibliographic review (continued)																																				
A2)	Investigation of Chitosan activity as a function of pH																																				
A3)	Lab Scale production of red/rosè wines (and shelf life)																																				
	Analysis of wines added of KT in distinct vinification steps																																				
A4)	Production and characterization of KT nanoparticles																																				
	Utilization of nanoparticles in white-rosé-red wines																																				
	Analysis of wines added of nanoparticles																																				
A5)	Confirmation of KT ability to control Brett in Beer environment																																				
	Production of craft beers																																				
	Use of KT in intentionally contaminated Beers																																				
A6)	PhD thesys writing																																				

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Composizione fenolica e tipicità in vini Nebbiolo: Influenza del terroir, annata e tecniche di vinificazione (P-I-12)

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Lo scopo principale di questo progetto di dottorato riguarda lo studio di come fattori chiave, come la posizione, annata maturazione dell'uva e tecniche di vinificazione, influenzano la composizione fenolica e di conseguenza l'astringenza, il colore e la tipicità di vini Nebbiolo, con l'obiettivo di preservare la loro identità sensoriale e culturale.

1. State-of-the-Art

Wine typicity refers to the unique characteristics that express a wine's regional and varietal identity, making it both recognizable and distinguishable (Cadot et al., 2012). Geographical origin and grape variety are key contributors to typicity and are closely tied to the concept of authenticity (Santagostini & Guglielmi, 2024). However, authenticity alone does not account for other crucial dimensions of wine, such as its aesthetic value (Malfeito-Ferreira, 2023). Wine is a complex and aesthetic product that engages multiple sensory and emotional responses, including mental imagery (Kosslyn et al., 2001). Qualities such as complexity, harmony, and persistence are central to its appreciation. These holistic attributes cannot be fully assessed through chemical analysis alone (Jraissati & Deroy, 2021) but are essential for classifying wines as "fine wines". In fact, typicity is increasingly sought after by consumers who value regional authenticity (Luomala, 2007). To protect and preserve typicity, many wine regions implement strict regulations through appellation systems (Fernández-Ferrín et al., 2019).

Nebbiolo, a red grape variety from Piedmont, Italy, is an emblem of typicity. It produces prestigious wines such as Barolo and Barbaresco, which fall under Italy's highest classification, DOCG (Denominazione di Origine Controllata e Garantita) (Robinson et al., 2013). These wines are produced under rigorous rules concerning vineyard location, vinification methods, and aging (Pecile et al., 2025). Despite its status, Nebbiolo faces modern challenges, including climate change and evolving market demands (Santagostini & Guglielmi, 2024). Preserving its typicity is therefore essential to preseve its cultural and sensory heritage. Research has shown that both chemical and sensory characterizations are critical for defining typicity. For example, a study on 12 Bordeaux vintages concluded that authenticity arises from a broad chemical profile, not specific molecules (Schartner et al., 2023). Nebbiolo is especially known for its high astringency and low color intensity. A 2021 study of 110 Italian monovarietal wines showed that exhibited one of the highest total phenolic contents (2841 ± 348 mg/L catechin equivalents) and proanthocyanidins (4625 ± 532 mg/L), contributing significantly to its astringency (Giacosa et al., 2021). In contrast, Nebbiolo wine has very low anthocyanin levels (110 ± 17 mg/L malvidin-3-O-glucoside chloride), particularly malvidin-3-O-glucoside (43 ± 16 mg/L), aligning with its characteristic low color intensity. Polyphenolic reactions during winemaking and aging significantly influence wine color and astringency sensation. Studies show that nearly half of a wine's polyphenolic content forms through these reactions, including anthocyanins, flavonols, hydroxycinnamic acids, and flavanols (Somers, 1971). Extraction methods, yeast strain selection, and the addition of enological products also play key roles. Different yeast strains release varying polysaccharide levels, which can reduce perceived astringency. Oak barrel aging introduces gallotannins and ellagitannins, further modulating astringency (Glabasnia & Hofmann, 2006).

Given these factors that affect the Nebbiolo wine composition and their sensory characteritcs, it is crucial to understand how variables such as location, vintage, maturation level, winemaking practices, and aging influence Nebbiolo wines, particularly regarding phenolic compounds and their impact on astringency and color. This understanding could help refine winemaking practices to better preserve the typicity and sensory quality of Nebbiolo wines, ensuring their legacy for future generations.

2. PhD Thesis Objectives and Milestones

The aim of this PhD project is to investigate how different factors influence the phenolic composition of Nebbiolo wines, and consequently their typicity. This will involve integrating chemical and sensory analyses to assess the impact of environmental and winemaking variables.

Completed:

Assessment of the Role of Location and Vintage-A study was conducted on 150 Nebbiolo-based wines from diverse geographical areas, designations, and vintages. It evaluated the impact of these variables on chemical composition and sensory characteristics.

Planned Goals and Experiments:

A1) Evaluation of Nebbiolo Typicity. A sensory analysis will be performed with expert asessors trained on Nebbiolo wines to explore how astringency and color contribute to the perception of Nebbiolo typicity.

A2) Impact of Fermentation Study. A comprehensive study compares Nebbiolo wines from different locations fermented spontaneously vs. with inoculated yeast, focusing on their differences in their polyphenol profiles.

A3) Study of Grape Maturation Effects. This will analyze how grape maturity at harvest influences the detailed phenolic composition, including quercetin and sensory attributes of Nebbiolo wines.
A4) Analysis of Polymeric Compounds in Wines. This investigation will explore the polymeric compounds formed during aging, using mass spectrometry to trace their formation and evolution in Nebbiolo wines of different ages.
A5) Identification of Novel Polymeric Structures. Due to Nebbiolo's unique phenolic profile, a key objective is to identify previously uncharacterized polymeric compounds that may contribute to its distinct astringency and typicity.
A6) Application to Winemaking Practices. In the final phase, insights from all studies will be instrumental to experimental micro scale winemaking. These will be used to test adjustments to winemaking techniques aimed at enhancing typicity, validated through chemical profiling and expert tastings.
A7) Writing and Editing of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1. Gantt diagram for this PhD thesis project.

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A 1)	***Evaluation of Nebbiolo Typicity***																								
A 2)	***Impact of the Fermentation Study***																								
A 3)	***Study of Grape Maturation Effects***																								
A 4)	***Analysis of Polymeric Compounds in Wines***																								
A 5)	***Identification of Novel Polymeric Structures***																								
A 6)	***Application to Winemaking Practices***																								
A 7)	***Writing and Editing***																								

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The PhD project aims to conduct a descriptive study on the chemical and sensory characteristics of monovarietal white wines produced using innovative or unconventional strategies, including consumer perception and study of intercultural differences. This project focuses on analysing the connections between chemical compounds and sensory descriptors to better understand their impact on the wine's sensory performance.

Nuovi approcci sensoriali e chimiche strumentali per la definizione di tipicità dei vini: studio della diversità dei vini bianchi monovarietali

Il progetto di dottorato ha l'obiettivo di condurre uno studio descrittivo sulle caratteristiche chimiche e sensoriali dei vini bianchi monovarietali prodotti con strategie innovative o non convenzionali, includendo l'analisi della percezione da parte dei consumatori e delle differenze interculturali. Questo progetto si concentra sulle relazioni tra composti chimici e descrittori sensoriali, al fine di comprendere in modo più approfondito il loro impatto sull'aroma finale del vino.

1. State-of-the-Art

Typicity (or typicality) is generally defined as "the ability to express a combination of traits characteristic of a distinctive group" (Souza Gonzaga et al., 2020). In the context of wine, most authors refer to the concept of *terroir*, which is defined as a complex of factors, such as soil, climate, landscape, viticultural, and winemaking traditions. Wine quality, as well as typicity, is a multidimensional concept that involves a combination of sensory elements (Basalekou et al., 2023). The typicity of a specific wine is expressed through its chemical composition, which in turn defines its sensory profile. Physicochemical parameters used to characterize wine quality and typicity include basic composition parameters (pH, total acidity, volatile acidity, free and total sulphur dioxide, residual sugar, organic acids), but mainly rely on secondary metabolites, i.e. polyphenols and volatile compounds. The study of correlations among physico-chemical composition and sensory descriptors enhances our understanding of the sensory role of the compounds in wine. Several authors proposed the application of machine learning techniques (Parr et al., 2020) to build predictive models that correlate sensory attributes with chemical data. The sensory perception, olfactory, gustatory and tactile, of singular compounds in wine is a complex process that depends on the individual differences and interactions with wine matrix and each other. Sensory evaluation of wine quality and typicity is further complicated by the conceptual, cultural and linguistic, differences (Ferreira et al., 2021).
New production technologies and the emergence of previously unstudied wine types highlight the need for further research into the chemical and sensory profiles of these innovative products. Winemaking techniques and grape intrinsic differences both contribute to high variability of sensory profiles, often requiring a broader range of attributes, which complicates the application of traditional Descriptive Analysis. To address these challenges, new sensory methods (Check-All-That-Apply, Rate-All-That-Apply on a trained/expert/consumer panel) as well as approaches from social science (i.e., focus groups) are necessary. These methods facilitate consensus on the terminology and enable sensory evaluations to be conducted more efficiently (Meyners et al., 2016), making the process more accessible within academic settings.
For example, little information is available in scientific literature on white wines with maceration (for reviews, see Buican et al., 2023 and dos Santos&Kempka, 2024). This wine category, especially wine produced with fermentative and post-fermentative maceration, lacks an accepted descriptor terminology. Oxidation in white wines is another subject for perceptional and conceptual differences among consumers that can be a typical attribute, fault, or quality marker (Mallard et al., 2025).
By analyzing chemical and sensory characteristics of selected wines, this study aims to:

- provide descriptive information on this wine category including consumer's perception,
- identify possible connection between singular compounds and certain sensory descriptors,
- evaluate the effectiveness of applied sensory analysis techniques and determine the most suitable statistical approach for their interpretation.

2. PhD Thesis Objectives and Milestones

This PhD thesis project can be subdivided into five main activities (A1 - A5) shown on the Gantt diagram (Table 1). These activities are aimed at supporting the following objectives:
Objective 1. Study of the connections between chemical compounds and sensory descriptors
The integration of the results of sensory analysis with the data of physicochemical parameters may allow to find the connections between chemical compounds and sensory descriptors and suggest a role of these compounds in creating aroma profile.
A1) Instrumental-chemical and sensory analysis of commercial monovarietal Italian white wines produced with maceration compared to *non*-macerated control. The former includes determination of physicochemical characteristics (basic wine composition, colour and polyphenol characteristics, volatile compounds). The latter

involves a series of panel trainings, descriptor terminology development (missing in the current literature), colour, aroma and mouth-feel sensory evaluation with the use of Rate-All-That-Apply (RATA) method on a trained panel familiar with the product.

Objective 2. Consumer study of emerging products (A2) The integration of consumer study into chemical and sensory data will show the consumer perception and valorisation of a specific wine group.

Objective 3. Intercultural study of the wine product sensory and hedonic perception (A3) Intercultural study. Based on the received analytical data a smaller set of wines will be selected for the consumer study to investigate the consumer's preferences and market segmentation of wines with maceration and/or other innovative techniques. The selected wines will be analysed both for chemical-instrumental and sensory characteristics. Sensory analysis will be performed on a trained panel familiar with the product, as well as on consumers, and it will be repeated abroad on the same samples on a foreign panel to evaluate intercultural differences in perception and appreciation of the specific group of white wines.

A4) Supplementary activities. Evaluation of quality through sensory analysis of experimental white wines Supplementary activities of this PhD project involve collaboration in several projects aimed at investigating innovative or unconventional strategies, particularly conducting sensory analysis on their experimental wines produced with a standardized protocol. In the 1st year, this activity included sensory analysis for two projects: (1) assessing the impact of maceration duration and oxygenation in white wine, and (2) evaluating different techniques for oxidation management in the first steps of white wine production.

A5) Writing and Editing of the PhD thesis, scientific papers and oral or poster communications, data elaboration.

Table 2 Gantt diagram for this PhD thesis project.

Activity \ Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
A1)	***Screening of commercial white wines produced with maceration***																																				
	1) Physicochemical parameters																																				
	2) Panel training and sensory analysis																																				
	3) Interviewing winemakers																																				
A2)	***Consumer study on macerated wines***																																				
A3)	***Intercultural study***																																				
	1) Instrumental analysis (Italy)																																				
	2) Sensory analysis (Italy)																																				
	3) Consumer study (Italy)																																				
	4) Instrumental analysis (abroad)																																				
	5) Sensory analysis (abroad)																																				
A4)	***Sensory analysis of experimental wines***																																				
	1) Maceration and oxygenation																																				
	2) Oxidation management																																				
A5)	***Thesis and Paper Preparation***																																				
	1) Data elaboration																																				
	2) Paper preparation																																				
	3) Final thesis preparation																																				

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Oenological functions of *Metschnikowia pulcherrima* in co-culture with *Saccharomyces cerevisiae* to improve wine production (P-I-14)

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This PhD project aims to elucidate the mechanisms by which *Metschnikowia pulcherrima* confers enological advantages in multistarter fermentations with *Saccharomyces cerevisiae*. The core focus will be on understanding how *M. pulcherrima* contributes to copper reduction and detoxification in must; pulcherriminic acid production as a natural biocontrol agent; the molecular basis of these traits and their regulation, and the interaction and communication mechanisms with *S. cerevisiae*.

Metschnikowia pulcherrima in co-coltura con *Saccharomyces cerevisiae* per la valorizzazione di vini territoriali

Questo progetto di dottorato mira a chiarire i meccanismi attraverso cui *Metschnikowia pulcherrima* conferisce vantaggi enologici nelle fermentazioni condotte con colture miste insieme a *Saccharomyces cerevisiae*. L'obiettivo principale è comprendere come *M. pulcherrima* contribuisca alla riduzione e detossificazione del rame nel mosto; alla produzione di acido pulcherriminico come agente di biocontrollo; alla base molecolare di questi tratti e alla loro regolazione; nonché ai meccanismi di interazione e comunicazione con *S. cerevisiae*.

1. State-of-the-Art

In the modern enological context, the application of non-*Saccharomyces* yeasts has gained increasing attention due to their potential to modulate wine aroma, improve microbial control, and reduce the need for chemical additives. Among them, *Metschnikowia pulcherrima* stands out as one of the most promising species thanks to its multifaceted role in mixed fermentations (Fazio *et al*., 2023).

One of the most distinctive features of this yeast is its ability to secrete pulcherriminic acid, which chelates iron and leads to the formation of pulcherrimin, a red pigment with antimicrobial properties. This mechanism effectively limits the availability of iron in the medium, inhibiting the growth of spoilage microorganisms such as *Brettanomyces, Hanseniaspora*, and *Pichia*. The result is a natural biocontrol effect that enhances microbiological stability during early fermentation stages, thus offering a potential alternative or complement to sulfur dioxide (SO_2) additions (Puyo *et al*., 2023).

Another key enological trait of *M. pulcherrima* is its ability to reduce the copper content, a heavy metal commonly found in grapes must be due to vineyard treatments. High copper concentrations can inhibit yeast metabolism and compromise the success of alcoholic fermentation, especially for *S. cerevisiae* (Perpetuini *et al*., 2023).

In addition to these traits, *M. pulcherrima* contributes positively to the sensory complexity of wine by producing esters, thiol precursors, and other secondary metabolites. Its low fermentative power and restrained ethanol production make it particularly suitable for use in combination with *S. cerevisiae*, allowing for greater control over fermentation kinetics and final wine composition (Morata *et al*., 2019).

Despite these advantages, the molecular basis of copper resistance and pulcherriminic acid biosynthesis in *M. pulcherrima* remains poorly understood. Furthermore, the dynamics of interspecies communication between *M. pulcherrima* and *S. cerevisiae*—including competition, signaling, and metabolic interactions—are still largely unexplored. Deciphering these mechanisms is essential to optimize multistarter fermentations and fully harness the oenological potential of *M. pulcherrima* in a controlled and reproducible way.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) Identification and typing of *M. pulcherrima* strains isolated from organic musts and fruits
A2) Evaluation of copper resistance in *M. pulcherrima* and identification of genes involved in copper metabolism
A3) Quantification of pulcherriminic acid production and qPCR analysis of genes involved
A4) Evaluation of copper reduction during must fermentation using different strains of *M. pulcherrima* showing diverse copper tolerancein pure culture and in co-culture with *S. cerevisiae*
A5) Transcriptomic and metabolomic investigation of *M. pulcherrima* in copper-rich environments and during co-culture with *S. cerevisiae*.
A6) Assessment of interspecies communication using co-culture systems, and metabolomic profiling
A7) Data analysis, thesis writing, publication of results, and dissemination through conferences.

Table 1 Gantt diagram for this PhD thesis project.

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A 1)	***Bibliographic research***																								
A 1)	***Strains identification and typing***																								
A 2)	***Copper tolerance determination***																								
	1) Assessment of the copper tolerance																								
	2) Genes detection																								
A 3)	***Pulcherriminic acid (PA) production***																								
	1) Quantification of PA																								
	2) qPCR analysis																								
A 4)	***Copper reduction during fermentation***																								
A 5)	***Omics analyses***																								
A 6)	***Interspecies communication***																								
A 7)	***Data analysis***																								
A 8)	***Thesis and Paper Preparation***																								

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Environmental sustainability and impact of grapevine pest control on wine growing (P-I-15)

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Mandatory control of *Scaphoideus titanus*, vector of Flavescence Doré phytoplasma, harms insect biodiversity and wine production in Piedmont vineyards. The proposed project aims at studying the effect of different pest management strategies (organic vs IPM) on insect biodiversity (interspecific, intra-specific, and microbiome composition) and on wine production (in terms of quantity and quality) in various areas of Piedmont, increasing the knowledge on the performance of agricultural practices.

Sostenibilità ambientale e impatto della lotta ai fitofagi della vite sulla viticoltura

Il controllo obbligatorio di *Scaphoideus titanus*, vettore del fitoplasma della flavescenza dorata, compromette la biodiversità degli insetti e la produzione vinicola nei vigneti piemontesi. Il progetto proposto mira a studiare l'effetto di diverse strategie di gestione dei fitofagi (biologica vs. IPM) sulla biodiversità degli insetti (composizione interspecifica, intraspecifica e del microbioma) e sulla produzione vinicola in diverse aree del Piemonte, aumentando le conoscenze sull'efficacia delle pratiche agricole.

1. General and specific objectives

The PhD program aims to evaluate the effects of two pest management strategies - organic farming and integrated pest management (IPM) - on entomological biodiversity and its impact on wine production in different areas of Piedmont. The thesis aims to deepen the knowledge on the relationships between vineyard management, insect communities and environmental parameters, in close connection with the goal of obtaining more sustainable viticulture models.
In particular, three specific objectives are identified:

1. To analyse entomological biodiversity at interspecific and intraspecific levels of the vineyard agroecosystem.
2. To evaluate the variability of the microbiome of the vector *Scaphoideus titanus* (Ball, 1932) and of other leafhopper species that could be reached by *S. titanus*-targeted control: *Zygina rhamni* (Ferrari, 1882); *Erasmoneura vulnerata* (Fitch, 1851); *Hebata vitis* (Göethe, 1875); *Arboridia kakogawana* (Matsumura, 1932).
3. To provide information for wine growers about the impact of applied agricultural practices, integrating ecological, genetic, and agronomic data.

2. State of the art

Entomological biodiversity is recognized as a key element for the resilience of the vineyard agroecosystem (Hatt and Döring, 2023). Beneficial insects, pollinators and natural antagonists contribute to the regulation of phytophagous insects, reducing the dependence on plant protection products (Geppert *et al.*, 2024). However, in Piedmont, the mandatory control (Directive 2009/128/EC) of the vector of Flavescence Dorée (*S. titanus*) requires at least two insecticide treatments per year, regulated at regional level (Gonella *et al.*, 2024). The environmental impact of these practices is still poorly known.
Recent research shows that the use of insecticides can reduce biodiversity, favour secondary outbreaks and alter the insect microbiome (Xiong and Lu, 2022). Furthermore, the organoleptic quality of wine can be influenced by chemical residues and microbiological interactions (Wagner *et al.*, 2023). The project proposes to fill this gap by integrating ecological and molecular approaches (Hawthorne *et al.*, 2024) to evaluate the effects of management strategies at the territorial scale.

3. Timeline of the PhD program

The planned activities, which reflect the objective set, can be grouped into three large macro-areas according to the Gantt diagram given in Table 1.

1. **Analysis of entomological biodiversity in vineyards.**
   Methodology: sampling with DD-VAC aspirator, yellow sticky traps, netting and pan traps.
   Output: species catalogues, measures of richness, abundance and taxonomic composition, statistical analysis.
   Milestone: completion of monitoring in at least 5 Piedmonts wine-growing areas.

2. **Genetic and metagenomic study of grapevine leafhoppers**.
   Techniques: mitochondrial haplotyping, development of nuclear markers, and metabarcoding.
   Output: identification of subpopulations with differential invasiveness (e.g., potential insecticide resistance, extended environmental range…); microbiome profile in relation to provenance, treatment, and year.
   Milestone: completed molecular-genetic analyses on all species of interest.
3. **Integration of results and operational recommendations**.
   Methodology: multivariate analysis of the relationships between management, biodiversity, and productivity.
   Output: List of the most significant parameters affecting insect diversity in vineyard agroecosystems.
   Milestone: submission of the thesis and presentation of results.

Table 1. Gantt diagram for this PhD thesis project.

Activity / Period	2024 Q4	2025 Q1	2025 Q2	2025 Q3	2025 Q4	2026 Q1	2026 Q2	2026 Q3	2026 Q4	2027 Q1	2027 Q2	2027 Q3	2027 Q4
1. *Entomological biodiversity analysis*	●	●	●	●	●	●	●	●					
a) ▯ Methods: vacuum cleaner, traps, etc.	●	●	●	●	●	●	●	●					
b) ▯ Outputs: catalogues, measurements, analyses	-	-	●	●	●	●	●	●					
c) ▯ **Milestone**: Monitoring completed	-	-	-	-	-	-	-	▯					
2. *Genetic and metagenomic study*	-	-	-	-	●	●	●	●	●	●	●		
a) ▯ Techniques: haplotypes, markers, microbiome	-	-	-	-	●	●	●	●	●	●	●		
b) ▯ Output: sub-populations, profiles	-	-	-	-	-	●	●	●	●	●	●		
c) ▯ **Milestone**: molecular analysis completed	-	-	-	-	-	-	-	-	-	-	-	▯	
3. *Integration and recommendations*	-	-	-	-	-	-	-	-	●	●	●	●	●
a) ▯ Methods: multivariate analysis	-	-	-	-	-	-	-	-	●	●	●	●	●
b) ▯ Output: Influential Parameters	-	-	-	-	-	-	-	-	-	●	●	●	●
c) ▯ **Milestone**: thesis delivered	-	-	-	-	-	-	-	-	-	-	-	-	▯

6. Selected literature

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Use of by-products from the olive oil and wine making industries to increase their sustainability and obtain high quality and healthy products (P-I-16)

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This project aims to develop an optimized system for recovering bioactive compounds from by-products of wine and olive oil production, using an innovative pilot-scale extraction process. The extraction process will be optimized to recover polyphenols, organic acids, and sugars from by-products such as pomace, marc, lees, and grape seeds. Recovered bio-extracts will then be characterized to determine compositional profile, antioxidant, and antimicrobial properties. The final stage of the project includes the application of bio-extract in food systems, dietary supplements, or cosmetics at varying concentrations.

Utilizzo di sottoprodotti delle industrie dell'olio e del vino per aumentare la loro sostenibilità e ottenere prodotti di alta qualità e salubrità

L'obiettivo del progetto di ricerca è di sviluppare un sistema per recuperare composti bioattivi dai sottoprodotti generati durante la lavorazione del vino e dell'olio d'oliva utilizzando un impianto pilota di estrazione innovativo. Il processo di estrazione sarà ottimizzato per recuperare polifenoli, acidi organici e zuccheri da sottoprodotti come la vinaccia, la sansa, le fecce e i vinaccioli. I bio-estratti recuperati saranno poi caratterizzati per determinare il profilo compositivo, le proprietà antiossidanti e antimicrobiche. L'ultima fase del progetto prevede l'applicazione del bio-estratto nei sistemi alimentari, integratori alimentari o cosmetici a varie concentrazioni.

1. State-of-the-Art

Olive oil and wine production have a high socioeconomic, cultural, and environmental significance in Italy (Muíño et al., 2017; Rodrigues et al., 2023). However, these agro-industrial processes generate substantial quantities of by-products rich in bioactive compounds, which pose environmental and economic challenges due to their complex composition, high volume, and geographic distribution (Rodrigues et al., 2023).

In wine production, grape pomace is the main by-product, alongside smaller quantities of lees and fining sediments (Coelho et al., 2020). Olive oil extraction yields olive pomace and milling wastewater, with the amount varying depending on the extraction method employed. These by-products are rich in valuable compounds such as polyphenols, organic acids, and sugars. Their composition depends on the raw material (grape or olive), variety, processing method, and many other factors. For instance, grape pomace is typically rich in gallic acid, caffeic acid, catechin, and malvidin (Coelho et al., 2020; Rodrigues et al., 2023) while oleuropein, hydroxytyrosol, and tyrosol are predominant in olive pomaces (Falsini et al., 2024).

Recovering valuable bioactive compounds from by-products represents a strategic step toward enhancing the sustainability of the agri-food sector. A range of conventional and green extraction methods have been utilized, but process optimization is crucial for improving efficiency and minimizing environmental impact (Rodrigues et al., 2023). Key process parameters influencing extraction outcomes include temperature, time, solvent type, liquid-to-solid ratio, and pressure (Rodrigues et al., 2023; Macaluso et al., 2024). Rodrigues et al., (2023) optimized solid-liquid extraction from grape pomace using a central composite experimental design. They identified solvent concentration as the most influential factor affecting total phenol content (TPC), while time, temperature, and liquid-to-solid ratio showed variable effects depending on the solvent used. Additionally, pre-treatment steps applied to by-products are often integrated into optimization studies, as they could influence both the yield and quality of the recovered compounds (Macaluso et al., 2024).

The recovered bioactive compounds or extracts can be incorporated into different matrices for food, nutraceutical, cosmetic, and pharmaceutical applications. In food, olive pomace extracts, containing 10.5% hydroxytyrosol and 1.6% tyrosol, have been used to stabilize colour and delay oxidation in lamb patties (Muíño et al., 2017). Pectin extracted from grape pomace has improved the texture and prebiotic content of low-fat yogurt (Mobasserfar et al., 2024).

In nutraceutical contexts, olive oil enhanced with olive leaf extracts has been shown to reduce oxidative stress and alleviate metabolic disorders caused by high-fat diet in animal models (Macaluso et al., 2020). Moreover, olive oils fortified with oleanolic acid have been shown to lower the risk of developing diabetes in prediabetic individuals (Santos-Lozano et al., 2019).

In cosmetic and insecticide applications, olive mill wastewater extracts improved the UV absorption of sunscreens (Galanakis et al., 2018), while formulations containing hydroxytyrosol, phosphatidylcholine, and olive pomace powders have demonstrated effective insect-repellent properties (Falsini et al., 2024). This project will apply

experimental design methodologies to optimize the recovery of bioactive compounds from by-products of wine and olive oil, using an innovative extraction pilot plant. The resulting extracts will be incorporated into various product formulations for food, nutraceutical, cosmetic, and pharmaceutical applications.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Perform a comprehensive characterization** of the wine and olive oil by-products, focusing on the determination of total phenolic content (TPC), total antioxidant activity, moisture content, carbohydrate content, total flavonoid content, and organic acid content (A1.1) and the quantification of individual phenolic compounds using appropriate analytical techniques (A1.2).

A2) **Optimization of bioactive compound recovery at a pilot scale** with the aim of identifying the optimal process conditions (e.g., solvent concentration, time, temperature, and pressure) that will yield the highest recovery of target compounds (A2.1). Characterization of the functional properties of the bio-extracts obtained after optimization (A2.2).

A3) **Formulation development and functional evaluation:** to obtain a range of formulations incorporating the recovered bioactive extracts (A3.1), and to evaluate their effects on the physicochemical, sensory, and functional properties of selected application matrices (A3.2). The objective is to identify the most effective and stable formulation.

A4) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Characterization of By-products***																								
	1) Quantification of total phenolic content and antioxidant activity																								
	2) Profiling of individual phenolics																								
A2)	***Optimization of Extraction Process***																								
	1) Identify optimal process conditions																								
	2) Characterization of bio-extracts obtained after optimization																								
A3)	***Formulations and Evaluation***																								
	1) Development of Formulations																								
	2) Functional evaluation of formulations																								
A4)	***Writing and Editing of PhD Thesis***																								

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Innovative oenological practices to adapt the winemaking and wine preservation process to the different grape composition resulting from climate change (P-I-17)

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Cotutor: Dr. Antonella Bosso

New oenological strategies are required to mitigate climate change effects on grapes. This PhD project will deepen the existing knowledge on innovative solutions to preserve quality and typicality of different wines. In particular, this PhD project will investigate approaches to increase wine acidity and to predict calcium precipitation in wine.

Pratiche enologiche innovative per adattare il processo di vinificazione e conservazione dei vini alla diversa composizione delle uve dovuta al cambiamento climatico

La definizione di nuove strategie di vinificazione è necessaria per mitigare gli effetti del cambiamento climatico sulle uve. Questo progetto di tesi di dottorato si pone come obiettivo l'arricchimento delle conoscenze esistenti sulle possibili soluzioni innovative per preservare la qualità e la tipicità dei vini. In particolare, questo progetto di tesi sarà focalizzato sui possibili approcci per aumentare l'acidità del vino e predire le precipitazioni di calcio nel vino.

1. State-of-the-Art

Climate change is dramatically affecting the wine market. Global warming and drought are causing an extensive yield reduction and the alteration of phenological timing (Kuhn *et al*., 2013). Climate change modifies grape and wine composition, in particular it causes an accumulation of sugars, an increase in alcohol content and conversely a decrease in total acidity, it alters aromas and colour, in particular anthocyanins content (van Leeuwen & Darriet, 2016). Therefore, the result of the climatic change equation is an unbalanced wine that is no longer coupled with quality and legal parameters, consumer sensory expectations and typical characteristics. In recent years, in order to mitigate climate change effects, different agronomical strategies have attracted the interest of the scientific community (Droulia & Charalampopoulos, 2021) but few studies addressed climate change issue from an oenological point of view. This PhD project aims at investigating innovative oenological approaches to mitigate the effect of climate change on grape composition. The aim of this PhD project will be to be reached by addressing four major challenges: decreased acidity and calcium precipitation.

1.1 Acidity

Fumaric acid is an authorised stabiliser which is added to prevent malolactic fermentation (OIV-OENO 581A-2021). It is an interesting alternative to common acidifiers for its affordability, higher acidifying power, antibacterial and antifungal activity (Payan *et al*., 2023) and persistent sour and "fruit" taste (Das *et al*., 2016). Moreover, when added to must or during alcoholic fermentation, it is converted into malic acid, therefore it could be potentially used to restore the malic acid loss caused by malic acid respiration in CC-grapes. In this PhD project different acidification practices will be carried out on climate change-wines (CC-wines) and their effect will be investigated. In particular, two organic acids, tartaric acid and fumaric acid will be used to acidify both musts and wines obtained from white and red grapes. The two acids will be compared taking into consideration their effect on the pH, on the colour and its stability and on the evolution of polyphenols during aging.

1.2 Calcium precipitation

Besides known climatic change effects, in recent years calcium precipitation was hypothesized to be a consequence of climate change (Fioschi *et al*., 2024). To our knowledge, until today this topic was mainly addressed by private companies, whereas in literature latest research dates back to 80s and 90s decades. The most problematic aspect relies in the difficulty in calcium precipitation prediction (Payan *et al*., 2023). This PhD project will focus on the study of the variability of calcium content in wines of different vintages and varieties, in order to assess the presence of possible differences related to the different climatic conditions of vintages. In particular, calcium content will be measured together with other parameters that affect calcium stability. Meanwhile, calcium stability will be checked with conductivity tests. Conductivity test will also be applied to investigate chemical parameters that influence calcium stability and finally their results will be compared observing the occurrence of calcium

precipitation in wines stored for almost two years.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 2:

A1) **Acidification trials on white wine:** acidification trials on white wine were carried out during first year of the project, in particular tartaric and fumaric acid were applied and their effect on pH, colour and polyphenol content was evaluated. During the second year the statistical analysis will be performed on the data collected.
A2) **Acidification trials on white and red must and red wines:** white and red must and a red wine will be acidified with tartaric and fumaric acid. After bottling, chemical analysis will be carried out to evaluate the effect of the two acidifiers on the pH, on the colour and its stability and on the evolution of polyphenols. Chemical analysis will be followed by statistical interpretation of data.
A3) **Variability of calcium precipitation between vintages:** samples of wine of different vintages and varieties will be collected, calcium content will be measured, conductivity tests will be performed, and statistical analysis will be performed on collected data.
A4) **Factors of calcium precipitation**: samples will be prepared with wines with specific chemical parameters, conductivity tests will be performed, and finally statistical analysis will be performed on observed data.
A5) **Comparison of conductivity tests with observed precipitations during storage**: conductivity tests will be performed on wines of vintage 2025. The same wines will be stored for almost two years and precipitation will be monitored. Finally, statistical analysis will be performed.
A6) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Acidification trials on wine***																								
	1) Statistical analysis																								
A2)	***Acidification trials on must***																								
	1) Chemical analysis on bottled wine																								
	2) Statistical analysis																								
A3)	***Variability between vintages***																								
	1) Samples collection																								
	2) Chemical analysis																								
	3) Statistical analysis																								
A4)	***Factors of calcium precipitation***																								
	1) Sample preparation																								
	2) Chemical analysis and conductivity tests																								
	3) Statistical analysis																								
A5)	***Comparison of conductivity tests***																								
	1) Conductivity tests																								
	2) Monitoring of precipitation																								
	3) Statistical analysis																								
A6)	***Writing and editing***																								

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Grape-based fermented beverages distinct from wine (P-I-18)

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This PhD thesis research project is aimed at developing innovative fermented beverages based on grapes and winemaking by-products, using microbial strains that differ from those conventionally employed in wine production. Specifically, the study explores the fermentation potential of microbial consortia derived from Kombucha to ferment matrices such as grape pomace, grape must, and plant infusions. The ultimate goal is to obtain novel, sustainable, and functional beverages clearly distinct from traditional wine in terms of alcohol content aromatic profile, and nutritional properties.

Bevande fermentate a base d'uva alternative al vino

Questo progetto di tesi di dottorato mira a sviluppare nuove bevande fermentate a base d'uva e sottoprodotti dell'industria enologica, utilizzando microrganismi alternativi a quelli vinari convenzionali. In particolare, vengono esplorati lieviti e batteri del consorzio Kombucha per fermentare matrici come vinacce, mosto d'uva e infusi vegetali, al fine di ottenere prodotti innovativi, funzionali e sostenibili, differenti dal vino per contenuto alcolico, profilo aromatico e valenza nutrizionale.

1. State-of-the-Art

The demand for functional, low-alcohol fermented beverages is increasing globally. Among these, Kombucha has gained scientific interest due to its complex microbial ecosystem and its versatility in fermenting various plant-based substrates. At the same time, the winemaking industry generates large amounts of by-products (grape pomace, musts) with high nutritional potential, which are often underutilized.
Previous studies have shown that kombucha-derived microbes can adapt to different substrates, producing bioactive metabolites, modifying the aromatic profile, and contributing to microbial stability. These effects are due to microbial metabolism, acidification, enzymatic activity, and the transformation of native compounds into more active forms.

Table 1 Reported functional effects of kombucha fermentation on plant-based substrateds

Substrate	Observed effects on fermentation	Key References
Black tea	□ Glucuronic acid, □ acetic/lactic acids	Villareal-Soto et al., 2018
Green tea	□ Antioxidant activity, □ Catechins bioavailability	Greenwalt et al., 2000
Herbal infusions (e.g., lemon balm)	□ Total phenolics, □ Formation of new polyphenol derivatives	Di Cagno et al., 2021
Apple or grape juice	□ Antioxidant activity, □ Organic acids	Coton et al., 2017
Plant extracts (e.g., ginger, turmeric)	□ Bioactive metabolites, □ Antimicrobial properties	Di Cagno et al., 2021

While these examples highlight the functional potential of Kombucha, research applying this fermentation model to grape-based by-products remains limited. Key gaps in knowledge include:

- Fermentative behavior in grape-based substrates;
- Microbial dynamics and population evolution during fermentation;
- Impact on phenolic compounds and antioxidant activity;
- Sensory acceptance of the final products.

This project aims to bridge these gaps through a multidisciplinary approach combining microbiological, chemical, and sensory analysis.

2. PhD Thesis Objectives and Milestones

Within the overall goal of developing new grape-based fermented beverages distinct from wine, the project is structured into the following activities:

A1) **Characterization of the fermentation substrates** (grape pomace, must, and herbal infusions), and assessment of optimal inoculation conditions.

A2) **Isolation, selection, and characterization of kombucha-derived microbial strains with high fermentation potential**.

A3) **Controlled fermentation of the selected matrices, with monitoring of**:
- pH, acidity, sugar content;
- Microbial growth (plate counts and molecular methods);
- Phenolic composition and antioxidant activity (DPPH, Folin-Ciocalteu);
- Volatile metabolite production.

A4) **Preliminary sensory evaluation of the resulting beverages and assessment of consumer acceptability**.

A5) **Thesis writing, scientific publications, and oral/poster presentations**.

Table 2 Gantt diagram for this PhD thesis project.

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Characterization of the fermentation substrates***																								
A2)	***Isolation, selection, and characterization of kombucha-derived microbial strains***																								
A3)	***Controlled fermentation of the selected matrices***																								
A4)	***Preliminary sensory evaluation of the resulting beverages and assessment of consumer acceptability***																								
A5)	***Thesis and Paper Preparation***																								

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Biotechnological solutions for limiting climate change consequences, and maintaining high-quality standards in wine production (P-I-19)

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Nowadays, in the context of climate change, one of the goals in the wine industry is the development of sustainable production practices that reduce negative impacts on the environment and health, also driven by increasing attention from producers and consumers. This PhD project, carried out in collaboration with the French company BioLaffort, aims to identify biotechnological solutions, such as the use of selected microorganisms, as a viable alternative to conventional chemical and physical methods, that can improve and/or correct the quality standards of wines, on which the effects of the current climate crisis are weighing heavily by the action of several consequences from there derived.

Soluzioni biotecnologiche per limitare le conseguenze del cambiamento climatico e mantenere standards di alta qualità nella produzione di vino

Nel contesto del cambiamento climatico, la tendenza ad oggi nel settore vinicolo è lo sviluppo di pratiche di produzione sostenibili che riducano gli impatti negativi sull'ambiente e la salute, spinta anche dalla crescente attenzione da parte di produttori e consumatori. Questo progetto di dottorato, realizzato in collaborazione con l'azienda francese BioLaffort, ha l'obiettivo di individuare soluzioni biotecnologiche, quali l'impiego di microrganismi selezionati, come valida alternativa ai metodi convenzionali chimici e fisici, che possano migliorare e/o correggere gli standards qualitativi dei vini, su cui gravano gli effetti derivanti dalla crisi climatica attuale.

1. State-of-the-Art

Climate change has become one of the most important topics discussed in recent years (Costa et al., 2023; Gerbi et al., 2025) due to its significant impact on various sectors and systems, with disastrous consequences, including the wine industry, where climate is a key factor influencing vine cultivation, grape quality and wine sensory attributes (Gutiérrez-Gamboa et al., 2021; Sgubin et al., 2022). This has transformed traditional wine-growing regions that are less suitable for viticulture, such as France and Italy, where there has been an 11% drop in wine production over the last 10 years (OIV, International Organization of Vine and Wine, 2024); at the same time, other areas in Northern Europe, where grape growing was once unthinkable, are now benefiting from new, more favorable climatic conditions, making them potential new wine-growing regions (Fraga et al, 2013; Vion et al., 2024). Therefore, the concept of terroir also plays a crucial role in this context, due to the loss of the typical characteristics of each vineyard area. The alteration of ripening due to current climatic conditions causes changes in the composition of grape berries, characterized by a drastic decrease in the concentration of organic acids, affects the appearance, microbial and chemical stability of wines and has a direct impact on taste attributes, especially acidity, which masks sweetness (Gutiérrez-Gamboa et al., 2021). The main organic acids in grapes are dihydroxybutanedioic acid and hydroxybutanedioic acid, more commonly known as tartaric acid and malic acid, respectively. These two acids account for up to 90% of the total organic acids (Plantevin et al., 2024; Carbonneau et al., 2015), while others, such as acetic, citric, lactic and succinic acids, are present in lower concentrations (Gutiérrez-Gamboa et al., 2021). This raises concerns about both consumer health and the quality of the final product (Peltier et al., 2021). The OIV allows various acidification methods, including chemical procedures, the most common, by adding acidifying agents such as tartaric, malic, citric, lactic or fumaric acid; physical acidification by means of cation exchange resins and electrodialysis; and microbiological acidification (Payan et al., 2023). However, modern winemaking tends to minimize additives in wines, favoring biological approaches to manage acidity through the activity of wine-related microorganisms (Vion et al., 2024). The selection of industrial starters is particularly important because yeast strongly influences the final quality of wine (Rainieri et al., 2000; Marullo et al., 2010). This has led to the development of yeast selection programs that provide winegrowers with specialized strains for specific oenological applications (Vion et al., 2021; Vion, Muro, et al., 2023). Therefore, the application of yeast selection and breeding programs would be effective in improving wine quality standards.

2. PhD Thesis Objectives and Milestones

In accordance with the general objective described above, this PhD thesis project can be broken down into the following activities, as illustrated in the Gantt diagram shown in Table 1:

A1) **Characterization and evaluation of oenological properties** of wild *S. cerevisiae* and non-*Saccharomyces* yeasts from apple and grape must (A1).

A2) **Microvinification trials** through which the biocontrol activity of selected yeasts against plant pathogens will be evaluated (A2.1), and small-scale vinification trials with acidifying yeast strains provided by the company

BioLaffort (A2.2).

A3) **Chemical and sensory analysis** aimed at assessing the oenological parameters, volatile composition (A3.1) and overall balance of the wines produced. In parallel, the metabolic flux of organic acids (A3.2) during vinification will be determined, describing how they influence wine acidity parameters (pH, volatile acidity).

A4) **Non-Destructive analysis** (NIR, E-Nose) (A4.1) to support conventional analytical methods in fermentation monitoring and wine quality control and development of predictive models (A4.2) by correlating data from destructive and non-destructive sensor technologies.

A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Characterization and evaluation of oenological properties***																								
	Molecular characterization of wild *S. cerevisiae* and non-*Saccharomyces* yeasts from apple and grape must and evaluation of oenological properties																								
A2)	***Microvinification activities***																								
	1) Microvinification trials for evaluation of enological properties and the biocontrol activity of the selected yeast																								
	2)Small-scale vinification trials with acidifying yeast strains provided by the company BioLaffort																								
A3)	***Chemical and sensory analysis***																								
	1) Evaluation of oenological parameters and volatile composition																								
	2) Metabolic flux determination of organic acids																								
A4)	***Non-Destructive analysis***																								
	1) Use of non-destructive technologies (NIR, E-Nose)																								
	2) Predictive model development																								
A5)	***Thesis and Paper Preparation***																								

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Innovative approaches to wine health and sustainability in hot climates (P-I-20)

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Climate change and consumer demands have required an evolution in winemaking management. For this reason, the present PhD project aims to use different technologies approaches for adapting to climate change. Four approaches will be taken into account: 1. Pulsed electric field for preservation/biostimulation and aging treatment, 2. Pre-fermentative cryogenic treatments on crashed grapes, 3. Non-*Saccharomyces* yeasts for improving sensorial profile and bioprotection 4. Study of effect of potassium poliaspartate on calcium stability.

Approcci innovativi per la salubrità e la sostenibilità dei vini nei climi caldi

I cambiamenti climatici e le esigenze dei consumatori hanno richiesto un'evoluzione nella gestione della vinificazione. Per questo motivo, il presente progetto di dottorato mira a utilizzare diversi approcci tecnologici per l'adattamento ai cambiamenti climatici. Saranno presi in considerazione 4 approcci: 1. Impiego di campi elettrici pulsati per la preservazione/biostimolazione e l'affinamento dei vini, 2. Trattamenti criogenici pre-fermentativi sulle uve pigiate, 3. Lieviti Non-*Saccharomyces* per il miglioramento del profilo sensoriale e la bioprotezione, 4. Effetto del poliaspartato di potassio sulla stabilità dei tartrati di calcio.

1. State-of-the-Art

Several points have already been addressed in scientific literature. Pulsed electric field (PEF) technology is a technique that has established itself in recent years. It uses electrical pulses to destroy microbial and plant cells by creating pores in the membrane (Akdemir Evrendilek, 2022) leading to potential control of spoiling. It reports that fermentation assisted by PEF can help to further reduce the sugar-to-ethanol conversion ratio and when it applied at sub-lethal doses, it has been shown to have stimulatory effects on microbial growth and metabolism (El Kantar & Koubaa, 2022). PEF treatments, moreover, applied to heavy lees from must clarification, rich in grape solids, can help to increase the extraction efficiency of the liquid phase from the lees and it accelerates release of mannoproteins from *Saccharomyces cerevisiae* during aging on fine lees (Martínez *et al.,* 2019).

Pre-fermentative cryomaceration involves placing wine grapes directly in contact with a cryogen to achieve temperatures below 10 °C, preventing the onset of undesired alcoholic fermentation (OIV, 2023) and improved the recovery of aroma-related compounds such as alcohols, terpenes, ethyl esters and acetate esters (Giametta *et al.,* 2025). This technique has been widely applied in white wine production to enhance the contact between grape skins and juice, thereby maximizing the extraction of aromas and their precursors present in the skins. Cryomaceration can also increase the phenolic compound content, particularly in white wine production. However, it must be carefully controlled as it can impact on both the sensory profile and the stability of the final products (Cheynier *et al*., 1990).

Non-*Saccharomyces* Yeasts (NSYs) are increasingly used in winemaking due to their metabolic peculiarities compared to *Saccharomyces cerevisiae*. Indeed, NSYs show lower sugar-to-ethanol conversion rates and, in some cases, determine biological acidification, through the production of lactic acid, which can mitigate the undesirable effects (low titratable acidity, high pH) of global warming (García *et al.,* 2016). Furthermore, sensory enrichment/differentiation can be achieved thanks to their different volatilome (Paradiso *et al.,* 2022). White winemaking generally requires pre-fermentative clarification of the must. The most adopted clarification technique is low-temperature sedimentation, which requires the use of cold and sulphites. Research is focused on the development of alternative strategies to avoid deterioration during must sedimentation. Bioprotection fits well into this new concept of deterioration prevention, and several microorganisms (mainly NSYs) have already been identified as bioprotection agents (Lebleux *et al.,* 2023), directly colonizing the environment and preventing the development of deterioration microorganisms. Their use, also combined with other tools, such as oenological tannins, therefore allows improving the sustainability of must clarification (Puyo *et al.,* 2023).

Potassium polyaspartate (KPA), whose use was approved by OIV in 2016 and regulated by European Union (Commission Delegated Regulation (EU) 2017/1961, 2017), is a polymer constituted by monomeric L-aspartic acids, synthesized by fermentation process and used to prevent undesired potassium bitartrate (KHT) precipitation in bottled wines. Calcium L-tartrate (CaT) precipitation also recently affects bottled wines as an effect of compositional changes due to climate change. Studies of the effect of KPA on it are limited.

2. PhD Thesis Objectives and Milestones

The PhD project can be subdivided into the following activities:

2.1 **PEF treatment for preservation/biostimulation and aging on lees**

- *PEF treatment for preservation/biostimulation*. A study will be conducted with the objective of reducing

or stimulating yeasts concentration (CFU * ml^{-1}, glucose-fructose (G/F, g $^{L-1}$) consume rate) in induced model broths/musts of fermentation, in a process of scaling up. It will be cultivated both *Saccharomyces* and Non-*Saccharomyces* yeasts. Fermentation will be monitored with principal parameters (G/F, acetic acid). It will define PEF electric field strengths, frequencies, time of impulses and time of treatments.

- *PEF treatment for aging lees*. White wine will be tested using PEF at different times of treatment on lees performing in a pilot plant of 50L. Wine samples were characterized along a 3-month aging from a chemical and sensorial points of view. It will be evaluated as the potential release of mannoprotein, aroma-related compound.

2.2 **Pre-fermentative cryomaceration**

- Two cooling methods were tested on crushed grapes obtained from Bombino Nero grapes. The first method utilized a concentric tube heat exchanger cooled by water. The second method consisted of a pre-fermentative cryogenic treatment, in a protective atmosphere, by means of heat exchange through direct contact between the crushed grapes and liquid CO_2. On samples will be determined particularly phenolic and volatile compounds and it will be assessing the sensorial analysis on treated and untreated wines.

2.3 NSY for **improving sensorial profile and bioprotection**

- *Sensorial profile*. Different NSY strains will be selected for their fermentative/metabolomic performances. Preliminary tests will be conducted on model musts (Dennis *et al.,* 2012) to evaluate yeast performance, monitoring the main parameters (sugars, ethanol, lactic acid) at regular intervals. Strains with acidifying potential and/or sensorial impact will be selected for micro-fermentations on real wine samples in laboratory scale and subsequential scaling up in a semi-industrial scale pilot bioreactor.
- *Bioprotection***.** The aim is to reduce the energy cost needed to maintain the cold chain during the clarification phase of the white must using bioprotective agents (NSYs) with and without the combination with other protective agents. An experimental plan will be developed with sedimentation tests with and without inoculation, with or without the use of SO_2, (this aspect is motivated by reducing the use of SO_2 during clarification) with the aim of increasing the decantation temperatures without compromising the quality of the must. Microbiological evaluation of potential microbiological pests. Also in this case, tests will be conducted with the use of PEF for the biostimulation of microorganisms in the clarification phase.

2.4 **Effect of potassium poliaspartate on calcium stability in wines**

- It'll be evaluated temperature of saturation of both potassium and calcium, it will be conducted cold tests analysing Ca^{2+} and K^+ ions (mg L^{-1}) as variation: $\Delta Ca^{2+}/K^+$ = mg L^{-1} $Ca^{2+}/K^+_{initial}$ - Ca^{2+}/K^+_{finale}. It will be analysed crystals' surface and morphology. Determination of chemical composition of principal parameters.

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Valorization of Brewing By-Products as an Alternative Protein Source (P-I-21)

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This PhD project aims to develop a comprehensive strategy for the valorization of brewing by-products - primarily brewer's spent grain, spent yeast, and spent hop - as alternative protein sources for food applications. The project involves the investigation and optimization of extraction methods, functional characterization of protein-rich fractions, and assessment of their potential uses. The goal is to contribute to the sustainable valorization of low-value by-products from the brewing sector into high-value functional ingredients, in line with circular economy and green transition goals, for the development of plant-based alternative products.

Valorizzazione dei sottoprodotti della birrificazione come fonte proteica alternativa

Questo progetto di dottorato ha l'obiettivo di sviluppare una strategia completa per la valorizzazione dei sottoprodotti della birrificazione - principalmente trebbie esauste, lievito esausto e luppolo esausto - come fonti proteiche alternative per applicazioni alimentari. Il progetto prevede lo studio e l'ottimizzazione dei metodi di estrazione, la caratterizzazione funzionale delle frazioni proteiche e la valutazione dei loro possibili impieghi. L'obiettivo finale è contribuire alla trasformazione sostenibile di sottoprodotti del settore birrario in ingredienti funzionali ad alto valore aggiunto, in linea con i principi dell'economia circolare e della transizione verde, per la produzione di alimenti ad alto contenuto di proteine da fonti alternative.

1. State of the Art

With annual global beer production exceeding 1.9 billion hectoliters (Statista, 2023), the brewing industry generates vast quantities of underutilized by-products. The most abundant by-product is brewer's spent grain (BSG), which is generated at a rate of ~20 kg per 100 liters of beer and contains 15–30% protein. Other by-products include brewer's spent yeast (BSY), which is produced at a rate of 2.6 kg per hectoliter and contains up to 78% protein, and spent hop (SH), which is produced at a rate of 1.4 kg per hectoliter and contains up to 70% denatured proteins (Belardi et al., 2025). Currently, these materials are often landfilled or used as low-value animal feed. However, due to their high amount of protein, they could be used as sustainable plant-based alternative protein sources. Despite their potential, the direct use of these by-products in food systems presents several challenges. Firstly, their microbial safety and stability must be ensured as they are prone to spoilage or contamination if not properly stabilized. Secondly, the presence of bitter compounds, off-flavors, or excessive fiber content may negatively affect sensory properties and consumer acceptability (Ahuja et al., 2024). BSG (85% of brewing by-products) proteins are mainly composed of prolamins, glutenins, glutelins, and albumins. Glutamine and proline are the most abundant amino acids in BSG protein extracts, accounting for 30–38.5% (w/w) of the total. These extracts also contain essential amino acids, with leucine, phenylalanine, and valine each contributing around 4–7% (w/w) of total protein (Devnani et al., 2023). BSG is more extensively studied than BSY and SH, but given their characteristics, they may represent interesting sources of food-grade proteins. BSY protein extracts, mainly mannoproteins, contain high levels of flavor-enhancing amino acids, such as glutamic acid, aspartic acid, glycine and alanine. Essential amino acids account for around 38% of the total amino acid content (Brassesco et al., 2025). Prolamins and glutelins are predominantly present in hot trub, where essential amino acids account for around 40% (Santos & Martins, 2024). The techno-functional properties of these proteins, which derive from their physicochemical characteristics, depend strongly on the extraction and purification methods used (Chin et al., 2024). These properties are fundamental to the formulation of various food structures, such as emulsions, foams and gels. Current literature on food applications of protein extracts from brewing by-products is limited, with only a few studies on enriched bakery products (Brassesco et al., 2025) and pasta (Sahin et al., 2021). These by-products offer threefold potential: reducing food industry waste; replacing animal proteins with locally sourced, renewable alternatives; and developing functional ingredients for new sustainable food products.

2. PhD Thesis Objectives and Milestones

In line with the overall objective outlined above, the activities of this PhD thesis project are structured as follows, as detailed in the Gantt diagram in Table 1:

A1) Characterization of brewing by-products (A1.1) Physicochemical characterization of BSG, BSY, and SH: protein, fiber, lipid, ash content, and moisture, antioxidant compounds. (**A1.2)** Assessment of variability due to raw material (e.g., malt type, yeast strain), brewing conditions, and storage, relevant for industrial scalability and consistency in plant-protein sourcing.

A2) Optimization of protein extraction methods (A2.1) Comparative assessment of extraction methods (alkaline, enzymatic, green solvents) suitable for food-grade applications. **(A2.2)** Optimization using Design

of Experiments (DoE), aiming to maximize protein yield and purity while minimizing environmental impact. **(A2.3)** Analysis of green metrics (e.g., E-factor, energy use, solvent recovery) to ensure eco-compatibility and align with the sustainability goals of plant-based protein production.

A3) Functional and nutritional characterization of extracted proteins (A3.1) Evaluation of techno-functional properties essential for plant-based product development: solubility, water holding capacity (WHC), oil holding capacity (OHC), emulsifying and foaming capacities, gel formation. **(A3.2)** Determination of amino acid profiles and molecular distribution. **(A3.3)** In vitro screening of bioactivities (antioxidant capacity, DPP-IV inhibition) to identify health-promoting protein ingredients.

A4) Food prototype development (A4.1) Development of model **plant-based alternative food prototypes** (e.g., meat analogs, protein-rich beverages, protein-rich snacks) integrating brewing-derived proteins. **(A4.2)** Preliminary **techno-economic analysis** and environmental assessment (e.g., carbon footprint, water use) to position the ingredients as viable and competitive protein alternatives.

A5) Dissemination and thesis writing (A5.1) Publication of research findings in peer-reviewed journals in food science, sustainability, and bioeconomy. **(A5.2)** Participation in international conferences focused on plant-based innovation and circular food systems. **(A5.3)** Completion and defense of the doctoral dissertation.

Table 1 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Characterization of Brewing By-Products***																								
	1) Physicochemical characterization																								
	2) Assessment of variability due to raw material																								
A2)	***Optimization of Protein Extraction Methods***																								
	1) Comparative assessment of extraction methods																								
	2) Optimization using Design of Experiments																								
	3) Analysis of green metrics																								
A3)	***Functional and Nutritional Characterization of Extracted Proteins***																								
	1) Evaluation of techno-functional properties																								
	2) Determination of amino acid profiles and molecular characterization (MW, Mn...)																								
	3) In vitro screening of bioactivities																								
A4)	***Food Prototype Development***																								
	1) Development of model plant-based food prototypes																								
	2) Preliminary techno-economic analysis and environmental assessment																								
A5)	***Dissemination and Thesis Writing***																								
	1) Publication of research findings																								
	2) Participation in international conferences																								
	3)Completion and defense of the doctoral dissertation																								

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Valorization of algal biomass through enzymatic biotechnology for the development of new food products (P-I-22)

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The aim of the project is to valorize algal biomass through enzymatic biotechnologies for the extraction and modification of high-value nutritional protein fractions. Promising microalgae species will be selected, sustainable extraction methods will be developed, and the techno-functional properties of the extracts will be characterized. This approach seeks to optimize the use of algal proteins in the formulation of novel functional foods. The project promotes food innovation in the context of sustainability and nutritional security.

Green Biotechnological Strategy for the Development of Microalgae-Based Functional Foods

The strategy involves selecting and characterizing protein-rich microalgae, followed by enzymatic extraction of protein fractions. These extracts will be optimized for key techno-functional properties using green technologies. The optimized fractions will be incorporated into prototype food formulations. Final products will be evaluated for sensory, nutritional, and functional performance.

1. State-of-the-Art

With the global population expected to reach around 9.5 billion by 2050, it is increasingly important to ensure food security by developing sustainable and high-quality food sources (Williamson et al., 2024). The global protein demand is anticipated to rise significantly between 32% and 78% to meet nutritional needs and align with the United Nations' Sustainable Development Goals (Kumar et al., 2022). Traditional protein sources, particularly animal-derived proteins, are associated with high environmental costs, including greenhouse gas emissions, land and water overuse, and biodiversity loss (Kusmayadi et al., 2021). In this context, microalgae have emerged as an attractive alternative due to their rapid growth rates, minimal resource requirements, and rich composition of proteins, polyunsaturated fatty acids, and bioactive compounds (Bartek et al., 2021; García-encinas et al., 2025).
Despite their potential, several challenges hinder the widespread integration of microalgae into the food sector. The most critical barriers include the difficulty of disrupting their rigid cell walls and undesirable sensory properties such as intense pigmentation and "fishy" odor (Lucakova et al., 2022). Additionally, many microalgae species remain underexplored in terms of their techno-functional properties, which are essential for their incorporation into food formulations (Nunes et al., 2024). Currently, conventional extraction techniques, such as mechanical and chemical methods, often result structural damage to proteins, and generation of toxic by-products, with a significant environmental impact (Phong et al., 2018). These limitations restrict the functional use of microalgal biomass in developing viable food products.
To address these issues, green extraction technologies have gained attention and in particular enzyme-assisted extraction methods since they offer selective, mild, and eco-friendly solutions to isolate high-quality protein fractions while preserving their functional properties (Iqbal et al., 2021). In this context, enzymatic treatment has the potential to enhance extraction efficiency and to reduce nutrient loss, aligning with industry needs for sustainability and regulatory compliance (Akbarbaglu et al., 2022). Furthermore, proteins derived from microalgae exhibit promising techno-functional characteristics such as solubility, emulsification, and foaming which are crucial for food applications like beverages, soft food products and high-protein snacks (Bleakley & Hayes, 2021; Silva et al., 2022).
The goal of this project is to explore the biodiversity of selected microalgal species, optimize enzymatic extraction methods for protein recovery, and enhance the functional performance of protein fractions through sustainable biotechnological approaches. Ultimately, the aim is to develop innovative, environmentally sustainable functional foods that meet the nutritional demands of modern consumers, particularly within the context of plant-based dietary trends.

2. PhD Thesis Objectives and Milestones

WP1 – Definition and Planning of Project Activities:
This work package involves the review of current literature on micro-algal protein extraction and techno-functional properties to establish a sustainable research framework. It also includes planning and coordination activities to ensure timely progress and collaboration across all project phases.

WP2 – Selection of Algal Biomass and Application of Extraction Methods
This phase focuses on selecting and biochemically characterizing promising microalgal species for protein extraction. Conventional and enzyme-assisted extraction methods will be tested and optimized to maximize

yield and sustainability.

WP3 – Study and Modification of Techno-Functional Properties

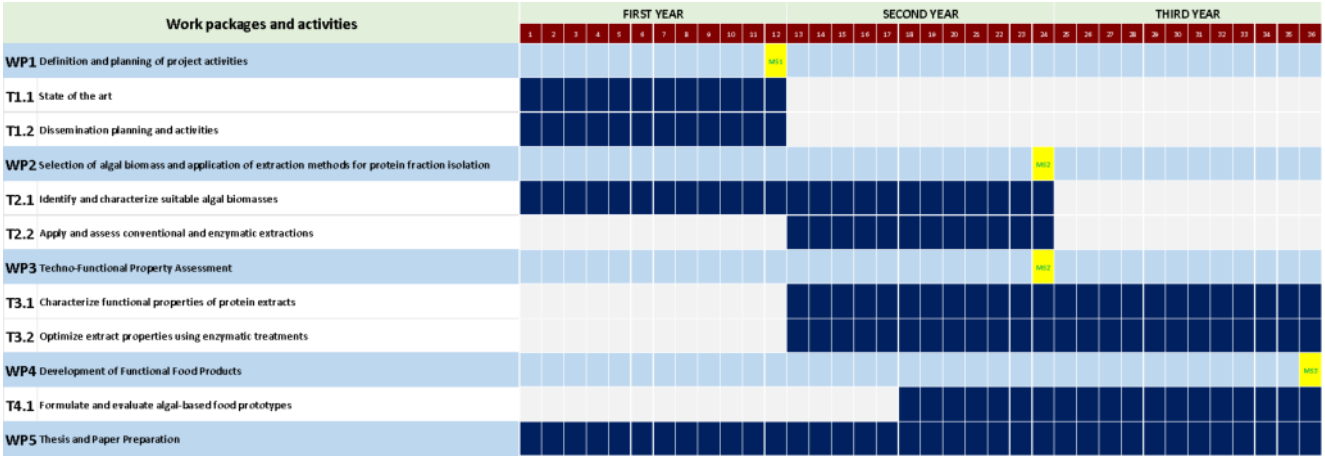
Extracted protein fractions will be evaluated for functional properties such as solubility, emulsification, and foaming capacity. Enzymatic treatments will be used to enhance these attributes for better application in food formulations.

WP4 – Development of Innovative Functional Foods

Optimized protein extracts will be incorporated into prototype food products, addressing sensory and stability challenges. This step validates the practical use of microalgal ingredients in real food matrices aligned with plant-based nutrition trends.

WP5-Writing and Editing of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.



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Valorization of bioactive compounds from Citrus by-products for food applications (P-I-23)

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This PhD research project aims to evaluate strategies for the recovery and encapsulation of the bioactive fraction present in residues from the citrus industry, with particular attention to the bergamot pomace (*Citrus Bergamia* Risso et Poiteau). The recovered and encapsulated bioactive components will be added to foods; in addition, coatings will be developed to be applied to plants to preserve and improve their quality. The aim is to extend the shelf life of food and obtain enriched foods.

Valorizzazione di composti bioattivi da sottoprodotti agrumari per applicazioni alimentari

Questo progetto di ricerca di tesi di dottorato mira a valorizzare strategie per il recupero e l'incapsulamento della frazione bioattiva presente nei residui dell'industria agrumaria, con particolare attenzione al pastazzo di bergamotto (*Citrus Bergamia* Risso et Poiteau). I componenti bioattivi recuperati e incapsulati saranno aggiunti agli alimenti, inoltre, verranno sviluppati dei coating edibili da applicare a vegetali per preservare e per migliorarne la qualità. L'obiettivo è estendere la durata di conservazione dei prodotti alimentari e ottenere alimenti arricchiti.

1. State-of-the-Art

Citrus fruits are one of the most widespread industrial crops in the world, with annual production expected to exceed 150 million tonnes in 2023 (FAO 2023). The citrus processing industry generates large volumes of by-products, which, under circular economy and sustainability frameworks, represent a valuable source of bioactive compounds. This study focuses on the valorisation of residues from bergamot (*Citrus bergamia* Risso et Poiteau). Industrial transformation primarily aims at essential oil extraction, leaving a residue enriched in polyphenols, pectin, essential oils, and D-limonene. These components confer antimicrobial activity, complicating conventional waste management and underscoring the need for alternative recovery strategies (Sharma et al., 2022). Recent studies have explored different techniques for the extraction of bioactive compounds from bergamot pomace, including food-grade solvent extraction, microwave, and ultrasound-assisted methods, with a focus on optimising processing time and temperature parameters (Gattuso et al., 2023). Although the phytochemical richness of bergamot processing waste is well-established, the application of advanced extraction technologies remains limited. This evidence shows a notable gap in current valorisation strategies. The aim of the research will be to enhance the extraction efficiency and recovery of bioactive compounds from bergamot pomace. Microencapsulation is an effective technique to protect and stabilise bioactive compounds. The main technologies used are freeze-drying and spray-drying. Comparative studies show that spray-drying guarantees greater retention of anthocyanins and better colour stability, compared to freeze-drying (Hay et al., 2025). The incorporation of citrus by-products into bakery products and beverages has demonstrated beneficial effects, including enhanced antioxidant activity. For instance, the addition of bergamot flour into biscuits led to increased antioxidant activity and reduced baking time (Laganà et al., 2022). Building on these findings, this project aims to investigate whether microencapsulated bergamot-derived compounds offer comparable functional benefits. In particular, the study will assess their antioxidant efficacy and evaluate improvements in sensory acceptability. The bioactive compounds from citrus by-products are also used in active packaging, improving food preservation. Compared to the direct addition of antioxidants, the packaging allows a controlled and targeted release. Demircan and Velioglu (2024) showed that applying edible coatings enriched with bergamot extracts to fresh fruit salads effectively prolonged shelf life while enhancing their sensory attributes. This study uniquely integrates extraction with microencapsulation techniques to valorise bergamot pomace-derived phenolics, aiming to address existing gaps in their application for food fortification. The effects of microencapsulation on the nutritional, technological, and sensory properties of fortified products will be assessed in comparison with free extracts and citrus pomace flour, focusing on yield, stability, and functional performance. The objective is to develop and test a variety of food products: a baked item, a moist leavened product, and a vegetable-based beverage, all enriched with microencapsulated ingredients to evaluate their technological stability and nutraceutical value. Additionally, edible coatings formulated with bergamot extracts will be applied to vegetable products to extend shelf life through improved antimicrobial protection.

2. PhD Thesis Objectives and Milestones

To achieve the objectives mentioned in the previous paragraph, the following is the breakdown of activities as shown in the Gantt chart in Table 1.

A1) Recovery of bioactive compounds from bergamot by-products. Optimisation of extraction parameters processes.

A2) Microencapsulation of bioactive compounds. Use of different wall materials for the formulation of feed to be encapsulated and evaluation of yield and efficiency of encapsulation. Comparison of spray drying and freeze-drying techniques (A2.1). Methods of particle preservation will then be evaluated to select the most ideal one for preserving the characteristics of the product obtained (A2.2).

A3) Application of microencapsulates in food systems and shelf-life evaluation. The objective will be to incorporate the microencapsulates obtained in A2 into selected food and beverage matrices, to enhance their nutraceutical properties and assess their impact on product shelf-life. The study will evaluate the stability, bioactivity, and sensory performance of the fortified products.

A4) Application of bioactive fractions recovered from bergamot pomace in food packaging systems, including the formulation of edible coatings and subsequent evaluation of their effectiveness in extending shelf life and maintaining sensory acceptability.

A5) Writing and editing of the doctoral thesis, scientific reports, and oral and/or poster presentations.

Table 1: Gantt diagram for this PhD thesis project.

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Recovery of Bioactive Compounds***																								
	1) Process parameter optimization																								
A2)	***Microencapsulation***																								
	1) Microencapsulate characterization																								
	2) Evaluation of Microencapsulate shelf life																								
A3)	***Application of microencapsulation***																								
A4)	***Packaging application***																								
	1)Food packaging development																								
A5)	***Thesis and Paper Preparation***																								

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Fava bean (Vicia faba L.) proteins: allergens identification, extraction methods, and technological modulation of biological and techno-functional properties (P-I-24)

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This PhD research project aims to mitigate the allergenic potential of fava bean proteins while improving their techno-functional properties. The study will initially focus on the identification of potential allergens present in fava beans. Subsequently, a range of technological and biological treatments will be employed, including pulsed electric fields and enzymatic activity. These treatments will be designed not only to decrease allergenic potential but also to preserve, or even enhance, the techno-functional properties of fava bean proteins.

Proteine della fava (*Vicia faba* L.): identificazione degli allergeni, metodi di estrazione e modulazione tecnologica delle proprietà biologiche e tecno-funzionali

Questo progetto di ricerca di dottorato mira a mitigare il potenziale allergenico delle proteine della fava e allo stesso tempo migliorarne le proprietà tecno-funzionali. Lo studio si concentrerà inizialmente sull'identificazione dei potenziali allergeni presenti nelle fave. Successivamente, verranno impiegati una serie di trattamenti tecnologici e biologici, tra cui campi elettrici pulsati e attività enzimatiche. Questi interventi saranno utilizzati non solo per ridurre il potenziale allergenico, ma potenzialmente anche per migliorare le proprietà tecno-funzionali delle proteine di fava.

1. State-of-the-Art

The growing consumer demand for plant-based products induced a wide interest in plant protein ingredients. Some legume proteins have been used as ingredient to replace meat, eggs, milk, and cheese. It is essential to understand how food processing conditions modulate the functionality of individual or food matrix proteins. Fava beans (Vicia faba L.) are poorly explored legumes despite their high protein content, which is approximately 20–35%, and their better amino acid profile (higher lysine content) if compared to other common legumes (Gulzar et al., 2024). In addition, fava bean proteins showed higher solubility, foaming stability, oil and water holding capacities, and good foaming capacity, but lower emulsion activity and stability when compared to other legumes. Multiple methodologies have been studied to extract and enhance the functionality of legume proteins, including alkaline extraction, ultrasound, microwave, pulsed electric field, and enzymatic extraction (Náthia-Neves et al., 2025). The application of enzymes extraction has been demonstrated to result in a substantial enhancement of protein yield compared to alkaline method. This is attributable to the capacity of enzymes such as pectinase, cellulase, and hemicellulase to degrade structural polysaccharides within the cell wall, thereby facilitating the release of intracellular proteins. The combined use of multiple enzymes could have a synergistic effect, further improving extraction efficiency. However, at present, there are no specific studies on the application of enzyme treatments targeting cell wall degradation in fava beans. Another promising strategy that has yet to be extensively explored is the application of pulsed electric fields (PEF) to enhance enzyme activity. PEF treatment has been shown to induce electroporation phenomena, which refers to the formation of micropores in cell membranes. This increase in membrane permeability facilitates access to intracellular substrates by enzymes. This approach has the potential to enhance the efficiency of protein extraction. However, it should be considered that, at high intensities, electric fields have the capacity to alter intra- and intermolecular interactions between peptides, with the potential to exert a negative effect on the quality of the final product (Zhang et al., 2021). Therefore, the identification of optimal operating conditions becomes essential to maximize the extraction yield while maintaining the protein's techno-functional features. Currently, the combination of enzymatic treatments and PEF has not yet been applied to fava beans, thus representing an area of research to explore. Despite the potential inherent in these innovations, it is crucial to consider other implications. Indeed, a high consumption of legumes plays a critical role in the scenario of food allergies. Food allergies are being recognized as a growing public health burden, and they have been referred to as the "second wave" of the allergy epidemic. Current evidence suggests that food allergies affect up to 10% of infants in some countries and have increased in prevalence over the last few decades (Loh and Tang, 2018). Even though the estimated incidence of legumes is low, there is growing concern that the frequency of legumes allergies may increase due to changes in consumer behavior and in the increasing availability of products containing it. Despite the absence of fava beans in the World Health Organization's (WHO) list of allergens, the literature suggests a potential for cross-reactivity with other legumes allergens, thereby classifying them as possible allergens (Smits et al., 2023). Literature offers promising solutions for reducing allergenicity. Studies have shown that common technologies such as thermal, physical, enzymatic, and fermentation treatments can be effective. These treatments have the potential to induce a series of structural modifications (reversible or irreversible) in allergenic proteins. These modifications may result in denaturation, aggregation, or gelation, which

could alter the conformational epitopes that are recognized by IgE. Consequently, this could lead to a reduction in protein allergenicity. PEF is an excellent nonthermal technique for food preservation that employs short pulses of electricity for the permeabilization and disintegration of microbial cells. PEF can also be used to reduce allergenicity, indeed there is evidence that the exposure of proteins to intense PEFs causes a conformational structural change both directly or indirectly affecting the secondary and tertiary structures (Takaki et al., 2021). Moreover, the use of enzymatic hydrolysis in combination with other techniques, such as thermal treatment or high-pressure processing, has yielded promising results in the reduction of allergenicity. Nevertheless, the impact of these treatments on the structure of the proteins could affect the techno-functional properties, indeed, an increase in temperature or changes in protein conformation may induce denaturation, resulting in poorer technological performance. There is a lack of research examining the impact of these treatments on both techno-functional and allergenicity properties, specifically regarding the application of pulsed electric fields and enzymatic hydrolysis to fava beans. This study aims to identify optimized extraction and treatment conditions to obtain fava bean proteins with enhanced techno-functional properties and reduced allergenicity, making them suitable for food applications.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Protein Allergens Identification** through biostatistics analysis (A1.1) in order to find possible cross-reactivity with already known legumes allergen (pea, soy, lentil, bean and lupin), and subsequently evaluation of the fava bean protein allergenicity with analytic methods (SDS-page and western blot techniques) (A2.2)

A2) **Protein Enzymatic Extractions** to identify the best performing enzyme (A2.1) between pectinase, cellulase, hemicellulase and amylases in terms of protein extraction yield and techno-functional properties of protein powder. The best performer enzyme, or possibly the best combination, will be used to enhance the protein extraction yield and the techno-functional properties through Pulsed Electric Field (PEF)-assisted extraction (A2.2).

A3) **Allergenicity and Techno-functional Properties Evaluation** of extracted protein (A2) after treatment with different technologies: Enzymatic hydrolysis by protease enzyme (A3.1), Pulsed Electric Field (A3.2), and High-Pressure Processing (HPP) (A3.3). The allergenicity reduction will be assessed by SDS-page and western blot techniques; techno-functional properties will be also evaluated in order to select the best condition for the next step.

A4) **Protein Application** on model food matrices to assess the impact of the above treatment on a food system on both biological activity and quality properties.

A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity	Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Protein Allergen Identification***																								
	1) Biostatistic Analysis																								
	2) Experimental Validation																								
A2)	***Protein Enzymatic Extraction***																								
	2) Enzymatic Extraction																								
	3) PEF-assisted Enzymatic Extraction																								
A3)	***Allergenicity and Techno-functional Properties Evaluation***																								
	1) Enzymatic hydrolysis																								
	2) PEF treatment																								
	3) HPP treatment																								
A4)	***Application***																								
	1) Application on food model system																								
A5)	***Thesis and Paper Preparation***																								

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Designing next-generation hybrid foods by structuring biopolymers from food waste (P-I-25)

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This PhD project focuses on the understanding of the structure-function relationships in gelled hybrid systems composed of plant proteins in combination with other biopolymers, eventually deriving from waste. Innovation in the field of gelled systems (i.e., hydrogel, oleogel, emulsiogel) is today highly demanded to unlock consumer acceptance of plant-based foods. Great attention will be paid to the understanding of the structural organization at the macro, micro, and nano scales of the hybrid systems, as well as to the relationship with gel functionalities. This approach will finally provide a solid basis for incorporating hybrid structures into food prototypes with tailored functionalities.

Design di alimenti ibridi di nuova generazione strutturando biopolimeri da scarti alimentari

Questo progetto di dottorato si concentra sulla comprensione della relazione struttura-funzionalità in gel ibridi composti da proteine vegetali in combinazione con altri biopolimeri, anche derivanti da scarti alimentari. L'innovazione nel campo dei sistemi gelificati (idrogel, oleogel, emulsiogel) è oggi molto richiesta per aumentare l'accettabilità degli alimenti a base vegetale da parte dei consumatori. Grande attenzione sarà dedicata alla comprensione dell'organizzazione strutturale dei sistemi ibridi su scala macro, micro e nanoscopica in relazione alle loro funzionalità. Questo approccio fornirà una solida base per incorporare strutture ibride in prototipi alimentari con funzionalità personalizzate.

1. State-of-the-art

Nowadays, feeding the increasing global population while guaranteeing the sustainability of the food supply chain is a worldwide priority. In this context, the rebalancing between animal and alternative proteins in the diet represents a promising strategy to improve global sustainability (European Commission, 2020). For these reasons, the possible exploitation of plant proteins in novel food products has gained great attention in recent years, as confirmed by the increasing number of research papers in the literature on plant-based alternatives. Moreover, a more plant-based diet is in line with the global dietary guidelines. Despite this situation, plant protein-based products are still not well accepted by consumers due to their scarce appeal, mouthfeel, and sensory performance in comparison to their animal counterparts. It is well known that plant proteins are characterized by their lack of ability to form supramolecular structural organizations, such as gels, emulsions, and foams (Zhang *et al*., 2021). Moreover, these proteins often present off-flavors and are described as bitter and astringent by consumers (Sarkar, 2024). To solve these problems, a promising strategy is represented by the co-structuring of plant proteins together with other biopolymers also derived from waste (i.e., carbohydrates, proteins, lipids, fibers) to generate hybrid structures able to better perform in the final products. Increased functionalities are due not only to the food composition but also to the generated food structure. Thus, it is essential to understand the relationship between food structure and food functionalities. Among food structures, the world of "gels" is particularly intriguing and interesting, being gels the building block of many food products. Gels are two-phase colloidal structures, consisting of a predominant liquid dispersed in a solid phase that forms a stable 3D network (Hou *et al.,* 2024). Thus, when dealing with gels, different systems can be formed, moving from hydrogels, oleogels, bigels, and gelled emulsions depending on the nature of dispersed phases or the consideration of multiphase systems. In gel systems, the polymers' structural organization at the microscopic level is pivotal to delivering the desired macroscopic functionalities perceived by the consumers (Aguilera, 2019). Only by an in-depth understanding of the gel structure at different lengths of scales and the interactions occurring in the matrix among biopolymers, it would be possible to design the next generation of plant-based food products. Up to now, despite research efforts progressively increasing on this topic, there is an urgent need to reinforce the understanding of the relations among food structure, processing, and functionalities to unlock consumer acceptability through food structure design. Design refers to the deliberate manipulation of the physical and molecular structure of foods to achieve specific sensory, nutritional, and technological properties.

2. PhD Thesis Objectives and Milestones

The proposed project collocates within this context, aiming to design hybrid gel structures exploiting the potentialities of the combination of plant proteins and other biopolymers, eventually deriving from food waste, for the delivery of target functionalities. Firstly, the activity will focus on selecting biopolymers to be

considered in the project. Then, hybrid gels (e.g., hydrogel, oleogel, gelled emulsions) made by different biopolymer combinations will be obtained and characterized at different length scales to generate an in-depth understanding of supramolecular organization and interactions. To this aim, different analytical techniques will be considered, from rheological, mechanical, and calorimetric to microscopy. This analysis will allow to select the best-performing hybrid gel that will be used to design food prototypes. The latter will be characterized by its structure, technological properties, and sensory profile. All the research will be conducted based on accurate literature research. Article writing will be performed during all three years.
Within the overall objective mentioned above, this PhD project can be subdivided into the following activities according to the Gantt diagram given in Table 1.

A1) **Selection of biopolymers:** selection of plant proteins and biopolymers deriving from food waste (A1.1)
A2) **Design and characterization of hybrid gels** (A2.1) gel characterization at different length scales (macro, micro, nano) (e.g., rheological, mechanical, calorimetric, and microscopy) and (A2.2) study of the interactions occurring among biopolymers.
A3) **Design and characterization of a food prototype** (A3.1) including hybrid gels previously designed, (A3.2) quality and sensory characteristics will be evaluated.
A4) **Writing and Editing** of the PhD thesis, scientific papers, and oral and/or poster communications.
A5) **Bibliographic research** will be carried on throughout the whole research period to update available literature. The information acquired during the experimental activities will be properly merged with producing scientific papers.

Table 1. The Gantt diagram for the activity of this PhD thesis project.

Activity months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Selection of biopolymers***																								
	1) Animal and plant sources																								
A2)	***Study of hybrid sistem***																								
	1) Study of interactions																								
	2) Technological properties																								
A2)	***Development of food prototype***																								
	1) Production of food prototype																								
	2) Technological and sensory properties																								
A3)	***Writing and Editing***																								
A4)	***Bibliographic research***																								

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Agronomic and technological solutions for achieving innovative food ingredients from legume crops (P-I-26)

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Legumes are a valuable resource as a plant-derived raw material to promote a more sustainable food system. Despite their good nutritional profile legumes contain several antinutritional factors that decrease their biological value. The objectives of PhD project are to study the effect of genotype and environmental conditions on the content of bioactive compounds and antinutritional factors in soybean and chickpea, considering innovative cultivars rich in phytochemicals and to verify the role of new combination of grain technological processes (soaking and roasting in combination with enzymatic treatments) on the occurrence of positive and negative metabolite.

Soluzioni agronomiche e tecnologiche per ottenere ingredienti innovativi da legumi

I legumi sono materie prime di origine vegetale preziose per promuovere un sistema alimentare più sostenibile.
A discapito del loro buon profilo nutrizionale, i legumi contengono diversi fattori antinutrizionali che ne diminuiscono il valore biologico. Gli obiettivi del progetto di Dottorato sono studiare l'effetto del genotipo e delle condizioni ambientali sul contenuto di composti bioattivi e fattori antinutrizionali in soia e cece, considerando cultivar innovative ricche di *sostanze bioattive* e verificare il ruolo della nuova combinazione di processi tecnologici della granella (macerazione e torrefazione in combinazione con trattamenti enzimatici) sulla presenza di metaboliti positivi e negativi.

1. State of Art

In terms of environmental pressures and of human health, meat and dairy have substantially higher impacts than plant-based foods. Among plants, legumes are considered a crucial source of protein, that can replace the consumption of animal origin products (Goldstein & Reifen, 2022). Legumes are a valuable food, that provide 20-45% of proteins, including essential amino acids, about 60% of carbohydrates and 5–37% of dietary fiber. In general, they are low in fat content and afford vitamins, minerals and several bioactive compounds (Messina, 1999). The main barrier to the adoption of legumes in the diet is linked to their poor digestibility and off-flavors. The presence of saponins contributes to the undesirable bitter, astringent, and metallic taste in pea and soybean (Heng et al., 2006). To promote the consumption of pulses, it is important to adopt technologies (germination, heat processing, enzymatic treatments) to improve the flavor and select cultivar with a good organoleptic profile (Roland et al., 2017). Unfortunately, the presence of several antinutritional factors (ANF), such as trypsin inhibitors, lectins, raffinose family oligosaccharides (RFO), saponins and ⟨-amylase inhibitors, affects the legumes digestibility and decreases the nutritional value, despite their high protein content and good amino acid profile (Messina, 1999). Trypsin inhibitors can certainly interfere with protein digestion, by inhibiting the capacity of trypsin enzyme to break peptide bonds. Although it has potential health benefits, phytic acid may inhibit the digestion action of some proteins, such as pepsin, trypsin, and α-amylase (Isanga & Zhang, 2008). The consumption of pulses, such as soybean, is also correlated to flatulence due to a high abundance of RFO (Huang et al., 2023). ANF can be classified in two categories based on their heat sensitivity. Trypsin inhibitors, ⟨-amylase inhibitors and lectins, considered heat-labile, have a low resistance to high temperature, whereas RFO, phytic acid and saponins are heat-stable, showing relatively high resistance to heat treatments (Affrifah et al., 2023). The evidence on the health benefits of legume consumption is directing research towards the study of agronomic practices and technological solutions that can reduce the presence of ANF, improve the taste, and facilitate the introduction of legumes in food preparations. Regarding field factors, it was demonstrated that the legume seed composition varies significantly in function of environmental factors (Medic et al., 2014). Genetic selection has played a crucial role in the development of new varieties with lower concentration of antinutrients. Soybean breeders, for example, have developed new lines with reduced quantity of nondigestible oligosaccharides in the seeds (Medic et al., 2014). Moreover, the pigmented legumes, which are rich in bioactive compounds, are very interesting because they attract consumers for their coloring, just like black chickpea and soybean varieties, which contain anthocyanins (Yang et al., 2022). Considering food processing, as well as they can improve legumes digestibility, at the same time food processing aids in the removal or reduction of antinutrients. Such techniques (soaking, dehulling, and germination) are adopted as an initial preparation of legumes, and they are already effective in reducing the antinutrient content. Heat treatment, such as steaming, boiling, or roasting, is necessary to achieve the right texture and reduce some ANF (King et al., 2024). The first goal of this research is to assess the agronomic performance (growth and harvest indexes) and quality traits (protein quantity, bioactive compounds and ANF content, taste) of three soybean genotypes grown in two geographical areas and under different sowing dates (Table 1). Subsequently, the samples from the agronomic test will be subjected to a technological process that includes a preliminary stage of soaking and a roasting phase that makes the seeds edible and improve the taste. The innovation in the project lies in the addiction of a -galactosidase enzyme during the soaking process to reduce

the content of RFO (heat-stable).

Table 1. *Agronomic study plan for three soybean genotypes.*

Factor	Variation	Descriprion
Year	2025	-
	2026	-
Geographic area	Carmagnola (TO), Italy	High soil fertility
	Borgo d'Ale (VC), Italy	Low soil fertility
Sowing time	First-crop	Sowing in mid-May
	Second-crop	Sowing in mid-June
Genotype	Black soybean	Maturity group 1, good taste
	Yellow soybean, white hilum	Maturity group 0+, high protein
	Yellow soybean, brown hilum	Maturity group 1, low RFO

Several analyses will then be carried out using the ionic HPLC and LC-MS methods to determine the content of ANF and bioactive compounds in both raw and processed soybean. As in the case of soybeans, the same tests will be carried out on a specific selection of varieties of chickpeas, by considering kabuli and desi genotype, with different pericarp color.

2. PhD Thesis Objectives and Milestones

According to the Gantt chart in Table 2, the PhD thesis project can be divided into the following activities:

A1) **Development of protocol analysis** to determine detect and quantify ANF in soybean and chickpeas.

A2) **Soybean agronomic study** experiment set up according to scheme reported in Table 1 in 2025 and 2026 growing season. Field measurements (height of plant, crop vigor, yield and yield components).

A3) **Enzymatic treatment study** includes a preliminary phase to assess the efficacy of ⟨-galactosidase enzyme. The second stage involves commercial samples of soybean and chickpeas and samples from the agronomic study (A2).

A4) **Analysis of samples** from tests A2 and A3 for compounds of interest: proximal composition, ANF and bioactive compounds, taste.

A5) **Writing and editing** scientific publications, PhD thesis, and oral and poster presentations.

Table 2. *Gantt diagram for the PhD thesis project.*

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Development ANF protocol analysis***																								
	Heat-labile ANF																								
	Heat-stable ANF																								
A2)	***Soybean Agronomic study***																								
	2025 season																								
	2026 season																								
A3)	***Enzymatic treatment study***																								
	Preliminary tests																								
	Tests on samples of A2 and A3 studies																								
A4)	***Analysis on A2 and A3 samples***																								
	ANF																								
	Bioactive compounds																								
A5)	***Paper and thesis preparation***																								

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Effect of soilless culture on the physiochemical and sensory attributes of strawberry (P-I-27)

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In this study, strawberries grown in peat based soilless systems will be characterized and compared to traditional soil-grown counterparts. With the increasing global demand for high-quality, health-enhancing fruits, techniques like hydroponic and aeroponic systems have become more popular. These techniques provide enhanced control over nutritional and environmental factors, which could result in better flavor, texture, shelf life, and bioactive properties of strawberries. The study will concentrate on describing strawberries cultivated in soilless systems and contrasting them with those grown in traditional soil. Focus will be directed toward examining soluble solids content, acidity, firmness, color, phenolic content, antioxidant activity, firmness and volatile aroma compounds. A mix of analytical methods, such as High-Performance Liquid Chromatography, will be employed together with sensory panels to assess consumer perception. This multidisciplinary research seeks to determine cultivation conditions that enhance strawberry quality, support sustainable horticultural practices, and provide data-driven guidance to growers. Results will foster innovation in fruit cultivation and enhance our comprehension of how growing systems affect nutritional and sensory traits.

Effetto della coltivazione senza suolo sulle caratteristiche fisico-chimiche e sensoriali della fragola

In questo studio, le fragole coltivate in un sistema senza suolo a base di torba verranno caratterizzate e confrontate con quelle coltivate in suolo tradizionale. Con la crescente domanda globale di frutta di alta qualità e con proprietà salutistiche, tecniche come i sistemi idroponici e aeroponici stanno diventando sempre più popolari. Queste tecniche offrono un maggiore controllo sui fattori nutrizionali e ambientali, il che potrebbe tradursi in un miglioramento del sapore, della consistenza, della conservabilità e delle proprietà bioattive delle fragole. Lo studio si concentrerà sulla descrizione delle fragole coltivate in sistemi senza suolo e sul loro confronto con quelle coltivate in suolo. L'attenzione sarà rivolta all'analisi del contenuto in solidi solubili, acidità, consistenza, colore, contenuto fenolico, attività antiossidante, consistenza e composti aromatici volatili. Saranno impiegati metodi analitici, come la Cromatografia Liquida ad Alte Prestazioni (HPLC), insieme a panel sensoriali per valutare la percezione da parte dei consumatori. Questa ricerca multidisciplinare mira a individuare le condizioni di coltivazione che migliorano la qualità delle fragole, supportano pratiche orticole sostenibili e forniscono indicazioni basate su dati concreti ai produttori. I risultati favoriranno l'innovazione nella coltivazione dei frutti e miglioreranno la nostra comprensione di come i sistemi colturali influenzano le caratteristiche nutrizionali e sensoriali.

1. State-of-the-Art

The strawberry *(Fragaria × ananassa Duch*.) is among the most valued fruits globally, known for its outstanding taste, bright color, and rich nutritional content (Crespo et al., 2010). Strawberries, being a plentiful source of vitamin C, phenolics, flavonoids, and anthocyanins, offer numerous health advantages, such as anti-inflammatory, antioxidant, and cardioprotective properties (Meyers et al., 2003). The demand for strawberries among consumers is influenced not just by their nutritional value but also by their sensory attributes, including sweetness, acidity, scent, texture, and visual appeal (Gunness et al., 2009; Recamales et al., 2007). These characteristics are, in turn, significantly affected by genetic, environmental, and agronomic elements.

Traditionally, strawberries are cultivated in soil in either open-field or sheltered environments. Traditional farming systems are facing greater challenges from climate variability, soil-borne illnesses, nutrient loss, inefficient water utilization, and the constraints of seasonal production cycles. Soilless cultivation methods—such as hydroponics, aeroponics, and substrate techniques—have developed into a sustainable and effective alternative to traditional soil farming (Recamales et al., 2007). These systems facilitate accurate management of nutrient supply, water consumption, and root-zone environment, promoting enhanced plant growth and fruit production (Gruda, 2009). Utilizing inert or organic materials like peat, coconut coir, or perlite supports year-round growing, improves moisture and nutrient retention, and decreases the occurrence of soil-borne diseases.

Numerous studies show insufficient integration of sensory, physicochemical and biochemical data, and only a limited number have utilized strong multivariate to analyze the intricate relationships between cultivation techniques and fruit quality.

Additionally, there is less information regarding how certain characteristics of soilless systems—like substrate type, nutrient management, and microclimate—together affect the complete quality profile of strawberries.

A comprehensive and interdisciplinary method to assess strawberry quality across various cultivation systems continues to be a significant void in existing research.

This research intends to fill these gaps by examining the influence of soilless cultivation, especially peat based on the sensory and physiochemical analysis of strawberries, utilizing soil-grown fruits as a reference.

This study will offer thorough insights into how cultivation methods influence fruit quality by combining chemical analyses (e.g., High Performance Liquid Chromatography, spectrophotometry), sensory evaluation, and sophisticated statistical modeling. Factors including total phenolics, anthocyanin levels, sugar and organic acid compositions, color, texture and volatile compounds be analyzed in conjunction with sensory characteristics such as sweetness, acidity, and aroma. In conclusion, this project aims to provide significant insights into the areas of sustainable horticulture, functional food innovation, and sensory science.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 2:

1. Characterization of Strawberry Physicochemical Properties
2. Volatile Profile and Sensory Evaluation
3. Data Integration and Statistical Modelling
4. Thesis and Dissemination Activities.

Table 2 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	**Characterization of Strawberry Physicochemical Properties**																								
	1) Physical Property Assessment																								
	2) Chemical Composition Analysis																								
A2)	**Volatile Profile and Sensory Evaluation**																								
	1) Sensory Evaluation																								
	2) Volatile Profiling																								
A3)	**Statistical Integration**																								
	1) Application of multivariate statistical methods																								
A5)	***Thesis and Paper Preparation***																								

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Ingredients and foods containing alternative protein sources: evaluation of sensory quality and acceptability (P-I-28)

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This PhD thesis research project aims to evaluate the sensory quality and the composition of food products formulated with ingredients from alternative protein sources, with the ultimate goal of identifying the drivers and barriers that influence consumer acceptance of these products.

Ingredienti ed alimenti contenenti fonti proteiche alternative: valutazione della qualità sensoriale e dell'accettabilità

Questo progetto di tesi di dottorato si propone di valutare la qualità sensoriale e la composizione di prodotti alimentari formulati con ingredienti derivanti da fonti proteiche alternative, con l'obiettivo ultimo di determinare le leve e le barriere che influiscono sull'accettabilità di questi prodotti.

1. State-of-the-Art

Global demand for food is expected to nearly double by 2050. Alternative proteins have been proposed as a sustainable solution to provide food security, as natural resources become more depleted. Given a wide variety of alternative proteins accompanied by an increase in data about food, the formulation of protein blends can significantly enhance nutritional quality, serving the nutritional demands of many (Malila *et al.*, 2024). Oil seeds and vegetable byproducts, including legume, cereal and pseudocereal proteins derived from food manufacturing can be upcycled to produce protein ingredients. Such practices not only help support food security but also the goal of having a sustainable global food supply system (Malila *et al*., 2024). Novel sources such as algae, insects, and fungi are increasingly considered for their protein content. These ingredients are available in different forms such as flour, isolates, concentrates, hydrolysates, and extrudate, allowing for diverse applications in food formulation (Kurek *et al.*, 2022).

However, alternative proteins are not widely appealing to many consumers; this is mainly due to their sensory characteristics, relatively high prices, consumer unfamiliarity, feelings of disgust, and unassured health consequences. Based on different biomolecules present, alternative proteins inherently have their own taste and flavor profiles (Malila *et al.*, 2024). Taste, appearance, flavor, odor, and texture are among the properties that co-construct a product's sensory properties (Günden *et al.*, 2024). Consumer acceptance is evaluated through hedonic tests to assess the overall liking and degree of liking for individual sensory attributes. It is important to identify which product characteristics are drivers of product liking, while also taking into consideration differences between person-related factors. A combination of hedonic testing and descriptive analysis provides a more holistic understanding and an ideal approach to evaluate the sensory profile of these products. When combined with instrumental measures, such as color and volatile profile, can be advantageous and help to improve the final product, providing a more accurate description of the physiochemical and sensory properties of these products (Fiorentini *et al.*, 2020). For example, fishy odor and flavor have been identified as negative determinants of consumer acceptance, and these attributes are correlated with the production of specific volatile organic compounds (VOCs). Among the available analytical techniques, gas chromatography (GC) is the most widely used for the identification of VOCs and may be helpful in detecting marker substances that could be involved in determining product quality (Colonia *et al.,* 2023).

The role of sensory profile in the acceptance of novel food is undeniable, yet it is not sufficient. The combination of psychological, social, and cultural factors influences how consumers perceive, process, and respond to information about food and, ultimately, their food purchase and eating behavior (Günden *et al*., 2024).

Research on a wide range of specific alternative proteins is necessary to gain better insight into the acceptance of consumers regarding a wide range of alternative protein products. The comparisons between various products of a specific alternative protein, or across categories of alternative proteins in a comparable way (e.g., a burger from various alternative proteins), are especially crucial to gaining a better understanding of why some specific products are accepted and others are not. Future investigations may consider placing a stronger focus on comparisons: comparisons across multiple drivers; comparisons across multiple alternative proteins; comparisons across countries; and comparisons across consumer segments (Onwezen *et al*., 2021).

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Bibliographic research** on methodologies for investigating consumer eating habits and attitudes, and on sensory analysis techniques, both descriptive and affective, aimed at evaluating novel food products formulated with alternative proteins; on chemical and instrumental analysis methods used to evaluate compositional characteristics affecting both the sensory and nutritional profiles of such products.

A2) **Development and implementation** of interviews, questionnaires, and focus groups to assess eating habits and consumer attitudes.
A3) **Evaluation of formulations and selection of samples**
A4) **Analytical and instrumental evaluations** including volatile compound profiling, protein and lipid profiling, in order to identify key molecules (e.g., olfactory markers) influencing sensory perception and macronutrient content relevant to these food products.
A5) **Panel training and sensory descriptive analysis** using conventional methods (*Quantitative Descriptive Analysis®*) and/or rapid methods (*Flash Profile, Free Choice Profile*), depending on the specific features of the products being evaluated.
A6) **Consumer test**, including both descriptive methods (*Check-All-That-Apply*) and hedonic/affective tests, with particular focus on the assessment of liking, expectations, and emotional responses (in both blind and/or informed conditions), as well as purchase intention. These will be integrated with psychological and physiological assessments such as food neophobia, disgust sensitivity, risk perception, and emotional responses.
A7) **Ongoing data processing and analysis**
A8) **Writing and Editing of the PhD thesis, scientific papers, oral and poster communications.**

Table 1 Gantt diagram for this PhD thesis project.

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Bibliographic research***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
A2)	***Development and implementation of questionnaires***	■	■	■	■	■	■																		
A3)	***Evaluation of formulations and selection of samples***	■	■																						
A4)	***Analytical and instrumental evaluations***	■	■	■				■	■	■	■				■	■	■	■							
A5)	***Panel training and sensory descriptive analysis***	■	■	■	■	■				■	■	■	■				■	■	■						
A6)	***Consumer test***			■	■	■	■	■				■	■	■	■				■	■	■				
A7)	***Ongoing data processing and analysis***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		
A8)	***Writing and Editing of the PhD thesis, scientific papers, oral and poster communications.***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

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The role of grape microbiome in wine origin traceability (P-I-29)

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This PhD project aims to elucidate the role grape microbiome can play in Nebbiolo wine origin traceability and assess its potential to enable discrimination between single varietal Nebbiolo wines originating from different vineyards and produced over two consecutive vintages. On that basis, it will focus on deciphering grape microbiome's composition, dynamics, as well as correlation networks with plant and soil microbiota, over the course of the annual growing season. Ultimately, it aspires to promote the integration of grape & vineyard (grapevine & soil) microbiome – based technology in Nebbiolo wine authenticity assessments and thereby strengthen integrity in the authenticity status of Nebbiolo wines.

Il ruolo del microbioma dell'uva nella tracciabilità dell'origine del vino

Questo progetto di dottorato mira a chiarire il ruolo che il microbioma dell'uva può svolgere nella tracciabilità dell'origine del vino Nebbiolo e a valutarne il potenziale per consentire la distinzione tra vini Nebbiolo monovarietali provenienti da vigneti diversi e prodotti in due annate consecutive. Su questa base, si concentrerà sulla decifrazione della composizione, delle dinamiche e delle reti di correlazione del microbioma dell'uva con il microbiota della pianta e del suolo, nel corso della stagione vegetativa annuale. In definitiva, aspira a promuovere l'integrazione di tecnologie basate sul microbioma dell'uva e del vigneto (vite e suolo) nelle valutazioni dell'autenticità del vino Nebbiolo, rafforzando così l'integrità dello stato di autenticità dei vini Nebbiolo.

1. State-of-the-Art

Wine adulteration has been the "bread & butter" of fraudsters, already since the 19th century. Nowadays, major global challenges such as climate change and rising production costs, are taking a toll on the wine producers' ability to balance wine supply with demand while maintaining a proper cost balance, thereby making the wine industry highly prone to different types of wine adulteration.

Grape and vineyard microbiomes, host a plethora of microorganisms, such as filamentous fungi, yeasts and bacteria, that can have a great impact on the winemaking process and wine quality (Martins et al., 2023). Their composition is affected by weather and climatic conditions, geographical location of the vineyard, soil, environmental stresses, grape cultivar, as well as vineyard management and viticultural practices followed (Aires et al., 2025). The total sum of these microbial communities signifies microbial *terroir*, which has been shown to correlate positively with wine *terroir* (Bokulich et al., 2016). More specifically, Bokulich and co-workers (2016) demonstrated that grape and wine microbiota regional patterns correlate positively with wine chemical composition, thereby setting the basis for the association of microbial *terroir* with wine *terroir*. Mezzasalma and co-workers (2017) demonstrated that grape microbiome is the main source of microbial diversity found in grape musts upon vinification of Cannonau musts, under controlled conditions which took place in the same winery, and without the addition of a starter culture. Martins and co-workers (2023) found that microbial biodiversity on grapes is primarily associated with the profile of flavonoids, amino acids and wax compounds of the grape berries, which may differ in composition not only between different varieties, but also within the same grape variety depending on vineyard geographical location and vineyard treatments. Bekris and co-workers (2025) have recently identified TU (Terroir Units) and vintage as the two main factors having a stronger effect on the composition of the epiphytic grape bacterial and fungal communities, over vineyard geographic location and cultivar. Differences in the composition of certain grape berry metabolites, over the course of the annual growth cycle, correlate with differences in the abundance of certain members of the grape epiphytic microbial communities. According to Martins and co-workers (2023) targeted metabolomics integrated with metabarcoding revealed such positive or negative correlations between grape berries' OTUs (Operational Taxonomic Units) and certain grape berry metabolites, for grape berries of three different Douro wine region cultivars.

Understanding the role grape microbiome can play in wine origin traceability, by evaluating its potential influence on shaping wine regional distinctiveness, as manifested by grape variety and geographical origin of the wine, is essential to develop microbiome-based wine '*fingerprinting*' technology that could be used to authenticate the origin of the bottled wine. Research towards this direction should be focused on: 1) deciphering its composition, 2) understanding the influence of vineyard (grapevine & soil) microbiome on the grape microbial community assemblages, and 3) monitoring their diversity and microbial community dynamics throughout the annual growth cycle, and during different grapevine developmental stages (flowering, fruit set, véraison, harvest). Furthermore, research should also be focused on bringing to light correlations linking core microbiome microbial and metabolic '*fingerprint*s' with wine characteristic chemical traits, indigenous microbiota of wine fermentation, and wine metabolome, all together conferring a potential set of distinctive wine characteristic traits synonymous to the origin of the wine.

2. PhD Thesis Project Objectives and Milestones

This PhD project will attempt to address the afore-mentioned research required to unravel the role of grape microbiome in wine origin traceability by carrying through investigation capable of providing distinction between single varietal Nebbiolo wines originating from:

- Two vineyards located in the same geographical region, bearing a spatial distance between them, and vinified in the same winery, over a period of two consecutive vintages.
- Two vineyards located in different geographical regions, and vinified at two different wineries, over a period of two consecutive vintages.

With this model approach, this PhD project aspires to identify unique grape microbiome '*fingerprints*', associated with the grape variety, the vineyard, and the vintage year, which could help us distinguish Nebbiolo wines originating from different vineyards located at the same or different geographical regions, and produced over two consecutive vintage years.

The overall research objectives of this PhD project, and the respective activities that need to be completed for their fulfilment, are listed below:

Objectives

A1) Deciphering the composition of grape &vineyard (grapevine & soil) microbiome associated with the Nebbiolo grape variety.

A2) Monitoring grape & vineyard (grapevine & soil) microbiome diversity and microbial community dynamics throughout the annual growth cycle, and during different grapevine developmental stages (flowering, fruit set, véraison, harvest).

A3) Understanding the influence of vineyard (grapevine & soil) microbiome on grape microbiome community assemblages.

A4) Unravelling the correlations linking core microbiome microbial and metabolic '*fingerprint*s' with wine regional characteristic traits, indigenous microbiota of wine fermentation, and wine metabolome conferring wine regional distinctiveness.

A5) Dissemination of scientific knowledge effectively, through means of publications, conference presentations, contributions and collaborations with other research groups, actively working in the same research field.

Activities

B(1) Vineyard sampling (bulk & rhizosphere soil, roots, leaves, flowers, fruits), **B(2)** Metabarcoding, **B(3)** Metagenomics analysis**, B(4)** Targeted metabolomics analysis**, B(5)** Bioinformatics analysis**, B(6)** Scientific knowledge dissemination activities

Table 1 Gantt chart outlining the tasks and timeline for completion of this PhD project.

ACTIVITY	PLAN START (month)	PLAN DURATION (week)	YEAR 2026 / MONTHS												YEAR 2027 / MONTHS											
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
B (1) *Vineyard sampling*	3 & 15	24																								
B (2) *Metabarcoding*	4 & 16	28																								
B (3) *Metagenomics analysis*	6 & 18	16																								
B (4) *Untargeted Metabolomics analysis*	9 & 21	8																								
B (5) *Bioinformatics analysis*	11 & 23	8																								
B (6) *Scientific knowledge dissemination*	1 & 13	48																								

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The selection of wood for the production of quality wines (P-I-30)

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Tutor: Prof. Franco Battistutta and Prof. Piergiorgio Comuzzo
Project co-financed by PNRR funds and G. & P. Garbellotto S.p.A

This PhD thesis research project is aimed at developing an online evaluation protocol based on advanced near-infrared spectroscopy, integrated with analytical and sensory techniques, to identify the key chemical and physical properties of individual oak staves that govern wine and spirits aging, in order to accurately predict their impact on flavor development and provide a scientific basis for the optimal selection, design, and management of wooden barrels at industrial scale.

La selezione del legno per la produzione di vini di qualità

Questo progetto di tesi di dottorato mira a sviluppare un protocollo di valutazione in linea basato su spettroscopia nel vicino infrarosso, integrata con tecniche analitiche e sensoriali, per identificare le proprietà chimiche e fisiche chiave delle singole doghe di quercia che influenzano l'invecchiamento di vini e distillati, al fine di prevederne accuratamente l'impatto sullo sviluppo aromatico e fornire una base scientifica per la selezione, progettazione e gestione ottimale delle botti su scala industriale.

1. State-of-the-Art

The wood staves used to assemble barrels are the primary source of variability in wood-aged beverages. Each tree differs genetically and, over decades of growth, experiences a unique combination of environmental and biological factors. This diversity yields staves with distinct structural and chemical properties (Mosedale et al., 1996). All staves share a common biopolymer framework, cellulose, hemicellulose, and lignin, and contain a range of extractable phenolic and aromatic compounds.

Oak staves are produced by splitting mature logs along the natural grain to ensure watertightness and to control how spirit and oxygen interact with the wood. After splitting, the sapwood is removed to prevent leaks and undesirable "green" flavours, leaving only heartwood for seasoning and drying. During natural seasoning, which can last up to three years, staves lose a significant portion of their phenolics and fresh, herbaceous aromas, while developing more delicate, mature scents (Doussot et al., 2002).

To form the barrel, trapezoidal staves are fitted into a head ring and held together with a tightening cable, and is then heated over an open flame, which softens the wood and allows it to bend into shape. Once bent, barrels destined for wine or spirits undergo toasting over oak wood scraps at temperatures between roughly 170 °C - 240 °C for 30–60 minutes, depending on the desired toast level. As heat penetrates the staves, hemicelluloses degrade first, followed by cellulose, and, at higher intensities, portions of lignin break down. These thermal reactions produce key aroma compounds that will later be extracted into the aging liquid. The exact volatile profile depends not only on toasting temperature and duration but also on stave moisture, ash content, inherent biochemical composition, and the cooper's control of flame intensity and airflow (Le Floch et al., 2015).

Traditionally, cooperages have selected oak by forest origin and grain tightness (i.e., growth ring width), with little emphasis on predicting or controlling volatile profiles. More recently, near-infrared (NIR) spectroscopy has been introduced as a tool to classify staves according to total polyphenolic content (Michel et al., 2013). Although useful, this approach provides only partial insight into wood's aromatic potential.
As noted earlier, the wood matrix profoundly influences the formation of volatile compounds, and NIR spectroscopy has shown promise for predicting matrix composition across different species (Jones et al., 2006). Therefore, applying NIR techniques to select staves with targeted aromatic characteristics appears feasible.

Accordingly, this PhD thesis has the objective to (1) verify how wood macro-components influence the aroma profile of oak staves, (2) evaluate the impact of these components on the aroma of wines and spirits through chemical and sensory analysis, (3) determine the suitability of NIR spectroscopy for predicting relevant compounds, and (4) develop a novel classification system based on these parameters.

Given the co-financing by PNRR and G. & P. Garbellotto S.p.A., six months will be dedicated to on-site activities at the cooperage, learning the barrel production process and supporting the industrialization of the new classification method, and six months will be dedicated to research abroad to strengthen academic expertise.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities

according to the Gantt diagram given in Table 1:

A1) **Verification of the influence of wood macro-components on oak stave aroma:** quantification of cellulose, hemicellulose, lignin and extractable phenolic fractions in selected staves (A1.1) and analysis of the resulting volatile profile produced during controlled toasting (A1.2**).**

A2) **Assessment Evaluation of aroma contribution to aged beverages through chemical and sensory analysis:** extraction and quantification of key aroma compounds from stave–spirit contact under standardized conditions (A2.1) and trained sensory tastings on model wines and spirits to correlate chemical data with sensory descriptors (A2.2).

A3) **Assessment of NIR spectroscopy as a prediction tool:** acquisition of near-infrared spectra for all characterized staves (A3.1) and development of chemometric models to predict both wood macro-constituent levels and aroma-precursor concentrations from NIR data (A3.2).

A4) **Development and validation of a novel classification system:** integration of chemical, sensory and NIR results to define objective classification parameters (A4.1) and testing of the system on an independent set of oak staves to assess its robustness and predictive accuracy (A4.2).

A5) **Industrial placement and international research:** six-month on-site collaboration with G. & P. Garbellotto S.p.A. to learn barrel production and assist in the industrialisation of the new classification method (A5.1); six months of research abroad to deepen methodological expertise (A5.2).

A6) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Wood macro-components role***																								
	1) Macro-components quantification																								
	2) Toast-induced aroma profiling																								
A2)	***Aroma contribution assessment***																								
	1) Compound extraction-quantification																								
	2) Sensory correlation																								
A3)	***NIR spectroscopy calibration***																								
	1) Spectra acquisition																								
	2) Chemometric modelling																								
A4)	***Classification system development***																								
	1) Parameter definition																								
	2) System validation																								
A5)	***Industry & international experience***																								
	1) Cooperage collaboration																								
	2) Research period abroad																								
A6)	***Thesis and Paper Preparation***																								

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Development and Optimization of Malt Extracts for Beer Production (P-I-31)

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The PhD thesis research project focuses on the development of malt extracts suitable for traditional beer as well as NOLO (No or Low-Alcohol) beer production, aimed at increasing process efficiency, improving quality, and enhancing sustainability in brewing. It is funded in collaboration with industrial partners Malteurop (FRANCIA) and Department of Agricultural, Food and Environmental Science (DSA3, University of Perugia, Italy) and Italian Brewing Research Centre (CERB) (University of Perugia).

Sviluppo e Ottimizzazione degli Estratti di Malto per la Produzione di Birra

Il progetto di ricerca della tesi di dottorato è incentrato sullo sviluppo di estratti di malto idonei sia alla produzione di birra tradizionale che di birra analcoolica o a basso contenuto alcolico, con l'obiettivo di aumentare l'efficienza del processo, migliorare la qualità e potenziare la sostenibilità nella produzione birraria. Il progetto è finanziato in collaborazione con partner industriali quali Malteurop (Francia), il Dipartimento di Scienze Agrarie, Alimentari e Ambientali dell'Università degli Studi di Perugia e il Centro di Eccellenza per la Ricerca sulla Birra (CERB) dell'Università degli Studi di Perugia.

1. State of the Art

The global brewing industry is increasingly driven by innovation, sustainability, and the demand for flexible production of diverse beer types, including NOLO beers. Malt extract is a concentrated syrup or powder derived from malted barley through enzymatic hydrolysis. The production of malt extract begins with controlled germination of barley grains, where endogenous hydrolytic enzymes including α-amylase, β-amylase and endo-proteases are activated to degrade endosperm-stored starch and proteins (gluten, hordein) and then the malted grains then undergo temperature-programmed mashing around 45-72°C of temperature at pH 5.2-5.6 to optimize enzymatic conversion of these components into fermentable sugars and soluble proteins. The resulting wort is subsequently processed and concentrated to produce malt extract, achieving 90-95% starch conversion efficiency while preserving thermolabile nutrients and bioactive compounds. This standardized extraction methodology ensures consistent quality and functionality of the final malt extract. (Ahmed, 2022)
Malt extract is rich in fermentable sugars primarily maltose alongside glucose, maltotriose, and longer-chain carbohydrates. Additionally, it contains proteins, amino acids, B vitamins (notably niacin), and essential minerals such as magnesium and potassium. The extract also boasts significant levels of phenolic compounds, including ferulic acid and flavan-3-ols like (+)-catechin and (−)-epicatechin, contributing to its antioxidant properties and flavor enhancement, making it valuable for both brewing and food industries.(MacGregor & Bhatty, 1993)(Safonova et al., 2018) Extensive research has demonstrated that malt extracts enhance brewing efficiency through multiple mechanisms. They significantly reduce water and energy consumption by 25-30% compared to conventional mashing processes. (Mussatto, 2014) Second, they allow for precise modulation of fermentable sugar profiles, enabling brewers to achieve targeted alcohol content with greater accuracy. (Brányik et al., 2012) Current industrial practices face challenges in balancing production efficiency with extract quality. Traditional methods often result in suboptimal yields due to incomplete starch conversion or inconsistent enzyme activation. (Safonova et al., 2018) Malt extracts play a vital role in brewing, with diastatic and non-diastatic varieties serving distinct functions. Diastatic malt provides active enzymes (α-amylase, β-amylase) for starch conversion into fermentable sugars, crucial for alcohol production. Non-diastatic malt, kilned at higher temperatures, contributes to flavor, color, and body without enzymatic activity. Understanding these differences is key for optimizing beer production. Recent studies have also demonstrated that optimizing the blend of malt types can significantly enhance the biological value of the extract by increasing phenolic content and antioxidant capacity. For instance, darker malts contribute more phenolic compounds (like ferulic acid and catechins) but may reduce extract yield. Therefore, malt composition must be balanced to preserve both functional and nutritional quality of the extract. (Shopska et al., 2022) This research proposes the comprehensive evaluation and development of malt extract formulations both diastatic and non-diastatic to enhance brewing efficiency and flexibility. The study specifically targets their application in traditional and NOLO beer production to support innovation, sustainability, and product quality in modern brewing.

2. PhD Thesis Objectives and Milestones

A1) Characterization of Existing Malt Extracts:
The activity involves an investigation of the products already in the catalog at Diamalteria by deepening the chemical-physical composition with reference to the essential requirements to produce a beer with a low alcohol

content, i.e. reduced fermentability, a pH below 4.6, light color.
The tests will be carried out on a Congress scale by simulating an extract/water ratio in line with what happens on a production plant. The musts will be prepared with the aim of having a Plato degree between 3 and 8 in accordance with the Italian law of "non-alcoholic beer" and between Plato degree between 5 and 10.5 in accordance with the definition of "light beer" (alcohol between 1.2 and 3.5% v/v).
The obtained worts will be compared to commercial products existing on the market. The obtained worts will be characterized by: Plato degree, fermentability, color, boiled wort color, pH, FAN, viscosity, DMS.

A2) Product Development:
Elaboration of Product recipes dedicated to the creation of No / Low alcohol beer and / or beer-like drinks. This development can take place based on the range of products already marketed by Diamalteria or by creating ex-novo products.
Based on the results obtained in point 1, recipes for non-alcoholic beers and light beers produced with extract of Diamalteria will be developed. The recipes will focus on lager beers, an appropriate style to better understand the chemical-physical and organoleptic characteristics of the extracted raw material.
It is planned to develop 2 non-alcoholic beers with 2 different extracts of Diamalteria and comparison with 1 non-alcoholic beer produced with the traditional method and 1 non-alcoholic beer produced with commercial extract of competitors.
It is expected to produce 2 light beers with alcohol, about 2.5 % v/v with 2 different extracts of Diamalteria and comparison with 1 light beer produced with the traditional method and 1 light beer produced with commercial extract of competitors.
All beers produced with extract will be compared with a beer produced with traditional malts to bring out strengths and weaknesses compared to traditional production methods.

A3) Product characterization:
All beers obtained will be analyzed for the following analyses: attenuation, alcohol content, pH, color, foam hold, turbidity, sensory profile, volatile compounds (in particular aldehydes). The results will be elaborated by means of statistical analysis.

A4) Dissemination:
The results of the project will be shared by a monthly meeting with Diamalteria work team using Microsoft Teams platform.

A5) Literature Review, Research papers, Bibliography and Thesis writing
The overall objective mentioned above this PhD thesis project is divided into the following activities according to this Gantt diagram table 1.

ACTIVITY [WPs]		*Duration of activity*																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1	*Characterization of Existing Malt Extracts*																								
A2	*Product Development*																								
A3	*Product characterization*																								
A4	*Dissemination*																								
A5	*Literature Review, Research papers, Bibliography and Thesis writing*																								

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The Contribution of Soil Microbiome to Grapevine Resilience Against Drought, Fruit Quality and Terroir and Carbon Stock in the Vineyard (P-I-32)

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This PhD project explores the multifaceted role of soil-originating plant growth promoting endophytic bacteria in i) supporting grapevine response to drought stress, ii) promoting grape quality and defining the *Microbial Terroir* and iii) favoring carbon sequestration in the vineyard. Beneficial microbes mitigate drought stress by regulating plant hormone levels and, simultaneously, they stimulate plant net photosynthetic rate and carbon sequestration. By moving through the plant sap vessels, they reach different plant compartments contributing to define the fruit *Microbial Terroir*. The use of targeted microbial inoculants and selected rootstocks is a potential tool to preserve ecological service and viticulture sustainability.

Il contributo del microbioma del suolo alla resistenza della vite alla siccità, alla qualità del frutto e al terroir e al sequestro del carbonio nel vigneto

Questo progetto esplora il ruolo dei batteri endofiti originati dal suolo e promotori della crescita della vite, in i) supportare la risposta allo stress idrico, ii) promuovere la qualità dell'uva e definire il Terroir Microbico e iii) favorire il sequestro di carbonio nel vigneto. Gli endofiti benefici mitigano lo stress idrico regolando i livelli ormonali vegetali e stimolano il tasso fotosintetico netto e il sequestro di carbonio. Attraverso i vasi linfatici, gli endofiti raggiungono diversi comparti vegetali, contribuendo a definire il Terroir Microbico del frutto. Microrganismi e portinnesti selezionati contribuiscono a preservare i servizi ecologici e la sostenibilità nel vigneto.

1. State-of-the-Art

Climate change is a significant threat to agricultural systems across the globe. Among all the climate related challenges, drought stands out as one of most serious threats to crop yield, quality and food security, especially to fruit crops which are sensitive to water availability. Prolonged drought stress not only reduces crops yield and quality, but it also results in soil degradation and reduces soil organic carbon stocks (IPCC 2023). Plants have evolved various mechanisms to manage drought independently such as drought escape, avoidance and tolerance (Zhang et al., 2022). Additionally, they form partnerships with beneficial plant growth promoting (PGP) microorganisms which provide ecosystem services to mitigate the impact of various abiotic stresses (Rolli et al., 2017). Beneficial microbes e.g. PGPR and endophytes help to mitigate drought stress by regulating plant hormones levels, increasing antioxidant activities, producing osmolytes and exopolysaccharides (Egamberdieva et al., 2017, Wang et al., 2012, Rossi et al., 2012). Bacterial endophytes are reported to enhance the expression of vacuolar ATPase in roots which promotes accumulation of compatible solutes in the vacuole, thereby enhancing water uptake and turgor retention (Vigani et al., 2018).Drought protecting PGPR are reported to enhance the photosynthesis rate in host plants and helps to sustain plant growth under water scarcity (Vigani et al., 2018). Rising temperature and global climate change are also increasing plant-soil respiration which may contribute to enhance carbon release from soil (Bond-Lamberty et al., 2018). However, by stimulating net photosynthetic rate while protecting plants from drought stress, root associated microorganisms help plants in building carbon stock. Further, carbon metabolism of soil microbes plays a key role in soil organic carbon (SOC) dynamics as microbial activities are involved in regulation of biomass and necromass production in microbes which contributes to SOC stocks (Liang et al., 2017). Moreover, plant associated microbial communities not only develop resilience in plants against stress but also serve various ecological functions that enhance plants fitness and quality (Rosenberg & Zilber-Rosenberg, 2016). The role of plant associated microbiomes has not been extensively researched for their impact on crop's terroir, apart from fermented products such as wine, coffee, and chocolate. Diverse microbes in different plant organs can alter phytochemistry by modifying plant metabolism, such as boosting nutrient absorption by the roots and subsequently their accumulation in leaves, seeds and fruits. Plant associated endophytes can break down plant molecules, depleting them form the metabolome or they can add their own distinct smells and flavors (Johnston-Monje et al., 2023).For instance, in study of spontaneously fermented Cabernet Sauvignon grapes in China (Wei et al., 2022) it was found that microbiome of fruit and must were primarily responsible for wine flavor and aroma. Collectively, root associated microbes support drought tolerance through complex and interdependent mechanisms. These mechanisms not only combat drought stress but also enhance carbon sequestration in trees and improve fruit quality and flavor. This highlights the key role of microbes in plant resilience, offering a path for developing innovative strategies to utilize microbial potential in sustainable agriculture and managing water scarcity. Thus, this PhD thesis project will be directed to isolate and inoculate PGPR and endophytes which will develop drought tolerance in grapevines, improve the fruit quality and enhance carbon stock in the vineyard. Experiments will be carried out in greenhouses and vineyards by performing various analysis e.g. soil microbiome analysis, plant physiological and fruit quality analysis, carbon pool analysis and

biomass carbon measurements.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Analysis of soil microbiomes diversity and function** in grapevine orchards.

A2) **Isolation of collection of grapevine microbial endophytes** with plant growth promoting competences and **assessment of influence of soil microorganisms for promoting resilience** in grapevine against drought stress (Physiological and morphological analysis).

A3) **Evaluation of impact of soil microbial communities on fruit quality and terroir** (Sugar, acid, phenolic, flavor)**.**

A4) **Assessment of role of soil microbiomes in carbon stock dynamics** and carbon sequestration in grapevine orchards.

A5) **Writing and editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity \ Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Soil Microbial Analysis***																								
	1) DNA Extraction																								
	2) 16S rRNA Amplicon Sequencing																								
A2)	***Plant Inoculation and Physiology***																								
	1)Plant Inoculation Experiments																								
	2) Photosynthesis and Transpiration Rate Measurement, Stomatal Conductance																								
A3)	***Fruit Quality Analysis***																								
	1) Brix, pH and Titratable Acidity (TA)																								
	2) Phenolic Compounds (HPLC-MS, Spectrophotometry)																								
A4)	***Carbon Stock Analysis***																								
	1) Soil Organic Measurement																								
	2) Biomass and Carbon Stock Estimation in grapevines (C Fraction analysis, C stock evaluation)																								
A5)	***Thesis and Paper Preparation***																								

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Study of the effects of climate change combined with biostimulant application on grapevine physiology and the consequences for nutraceutical quality of grapes (P-I-33)

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The research project aims to explore the physiological responses of grapevines to key abiotic stresses associated with climate change, particularly heat waves and water deficit, with a specific focus on alterations in primary and secondary metabolisms. Experimental trials will be carried out under both controlled conditions and vineyard settings. A biostimulant will be applied to evaluate its effectiveness in enhancing plant resilience, particularly in terms of carbon assimilation, water use efficiency, and metabolite accumulation. The goal is to preserve grape quality, maintain or improve the concentration of health-promoting compounds (polyphenols, carotenoids, stilbenes) and support the development of sustainable and climate-adaptive practices.

Analisi degli effetti del cambiamento climatico, in combinazione con l'applicazione di biostimolanti, sulla fisiologia della vite e sulle principali ripercussioni sulla qualità dell'uva

Il progetto mira a studiare le risposte fisiologiche della vite a stress abiotici legati al cambiamento climatico, come ondate di calore e siccità, con particolare attenzione ai cambiamenti del metabolismo primario e secondario. Le prove si svolgono in condizioni controllate e in vigneto. Verrà testato un biostimolante per valutarne l'efficacia nel migliorare la resilienza della pianta, soprattutto in termini di assimilazione del carbonio, uso efficiente dell'acqua e accumulo di metaboliti. L'obiettivo è preservare la qualità dell'uva, mantenere o aumentare l'accumulo di metaboliti secondari (polifenoli, carotenoidi, stilbenoidi) e favorire pratiche viticole sostenibili e adatte alle nuove sfide del cambiamento climatico.

1. State-of-the-Art

Viticulture is a key sector within the global agri-food economy, ranking third in terms of economic importance. However, the sustainability of wine production is increasingly threatened by climate change. According to leading predictive models, numerous traditional wine-growing regions are expected to experience substantial climatic shifts over the coming decades (Sgubin *et al.*, 2023). Among the most critical threats are rising temperatures, prolonged droughts, and an increase in the frequency and intensity of extreme weather events. In particular, heatwaves have emerged as one of the most pressing climate-related stressors for viticulture. The World Meteorological Organization (WMO) defines a heatwave as a period of five or more consecutive days during which the daily maximum temperature exceeds the average maximum by at least 5 °C (9 °F) (Marx *et al*., 2024). The increasing prevalence of such events, often coinciding with sensitive phenological stages in grapevines, has raised serious concerns about long-term vineyard productivity and grape quality. Despite the wide climatic adaptability of *Vitis vinifera*, the specific thresholds beyond which heat and drought induce irreversible damage to fruit and wine quality remain poorly understood. This knowledge gap is particularly significant when considering how these thresholds may vary according to cultivar, rootstock and vineyard management practices (Van Leeuwen *et al*., 2024). One of the most important indicators of grape quality lies in its nutraceutical profile, especially the concentration of flavonoid and non-flavonoid polyphenols. These compounds are highly responsive to environmental stimuli, agronomic techniques, and soil characteristics. Elevated temperatures have been consistently linked to reduced accumulation of anthocyanins in grape berries. For example, a recent study on the Nebbiolo cultivar demonstrated that exposure to extreme heat conditions (>40 °C) correlates with significantly diminished flavonol content (Wilson *et al*., 2024).
Furthermore, grapevine metabolism is intricately influenced by water availability, reinforcing the multifaceted nature of abiotic stress. Since the 1980s, researchers have explored how drought conditions affect the synthesis of secondary metabolites in grapes with a focus on the polyphenolic content (Kennedy *et al*., 2002; Ojeda *et al*., 2002; Koundouras *et al.*, 2006; Poni *et al*., 2007) and on volatile organic compounds (Oliveira *et al*., 2003; Koundouras *et al*., 2006). In light of these challenges, recent research has explored the use of biostimulants as a strategy to improve plant resilience under stress conditions. However, the market communication related to the biostimulants is still unsatisfying and users can be easily confused. The market offers a wide range of different products, even not clearly identified ones. Further research is needed to gain a deeper understanding of the biostimulant application in viticulture.

2. PhD Thesis Objectives and Milestones

The observations will be conducted on grapevine plants under both controlled conditions (CC) and field conditions (vineyard – FC) over a two-year period, spanning 2025 and 2026 (table 1). The experimental trial is conducted on

a cv of international interest, Merlot, and on a cv of local interest, Nebbiolo.
Within the overall objective of evaluating grapevine physiological and metabolic responses to abiotic stress under climate change, this PhD thesis project is subdivided into the following activities according to the Gantt diagram:

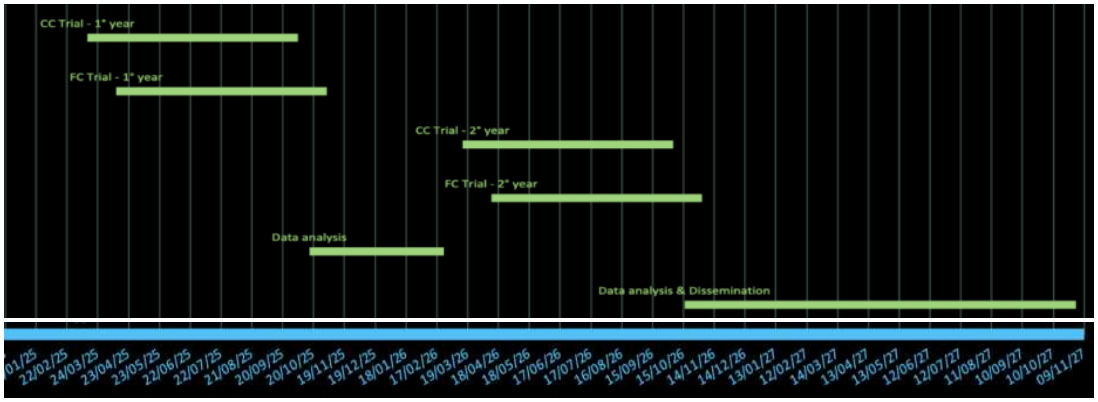


Table 1 Gantt diagram for this PhD thesis project.

A1) Experimental trials in controlled conditions (CC): abiotic stresses, heat waves – HW – and water stress – WS, will be simulated in CC on potted grapevine plants (Merlot and Nebbiolo). Biostimulant treatments will be applied before stress induction. Stress duration will be 10 days during pre-veraison. Climatic parameters will be monitored during trials.
A1.1: HW & WS simulation and physiological response evaluation trough gas exchange measurements and stem water potential.
A1.2: Biostimulant pre-treatment and comparative assessment.
A2) Experimental trials in field conditions (FC): Monitoring of Merlot and Nebbiolo cultivars in vineyards located in Diano d'Alba and Serralunga d'Alba, differing by microclimate and water availability. Biostimulants will be applied before stress periods.
A2.1: *In situ* monitoring phenological stages, carbon assimilation and water use efficiency through gas exchange measurements and stem water potential.
A2.2: Evaluation of vegetative and productive performance under different stress regimes.
A3) DATA Analysis: metabolomic analyses of leaves and berries focusing on primary and secondary metabolites to assess stress and treatment effects.
A3.1: Quantification of total solids, osmolytes and polyphenols (Folin-Ciocalteu method).
A3.2: Identification of polyphenolic classes (HPLC-DAD) in Merlot and Nebbiolo.
A4) Integration and analysis of physiological and metabolic data to assess treatment efficacy and understand grapevine resilience mechanisms.
A4.1: Data integration across trials and seasons.
A4.2: Interpretation of the results of data analysis × environment × treatment interactions.
A5) Writing and editing of the PhD thesis, scientific publications and dissemination: preparation of manuscripts, conference communications (poster/oral) and thesis compilation.

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Development of an innovative omic-based method to monitor Alternaria mycotoxins in tomato and assess their persistence (P-I-34)

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Tutor: Prof. Pasquale Ferranti

This PhD project aims to develop a quick and easy-to-apply analytical method for the quantification of *Alternaria* toxins in tomatoes and tomato-derived products at different steps of the production process. The goal is to enable in-situ detection of the toxins to support timely corrective actions. The project will cover the development of monitoring strategies, ranging from biosensors to HPLC coupled with various detectors, to be applied throughout the entire transformation process.

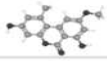
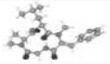
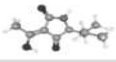
Sviluppo di un metodo innovativo basato su tecniche omiche per il monitoraggio delle micotossine di Alternaria e la valutazione della loro persistenza nei prodotti a base di pomodoro

Questo progetto di tesi di dottorato mira a sviluppare un metodo analitico rapido e di facile applicazione per la quantificazione delle tossine di *Alternaria* durante il processo di produzione di derivati di pomodoro. Lo scopo è permettere la rilevazione in situ delle tossine, per supportare interventi correttivi tempestivi. Il progetto comprenderà lo sviluppo di strategie di monitoraggio, dai biosensori all'HPLC accoppiata a diversi rivelatori, da applicare lungo l'intero processo di trasformazione.

1. State-of-the-Art

Fungi of the genus *Alternaria* (about 300 species), among which *Alternaria alternata* is the predominant, can infect various crops and consequently food products, including wheat, sorghum, barley, tomato, citrus, apples, olives among others. They are capable of producing more than 70 secondary metabolites, but only a few have been structurally classified and reported as relevant mycotoxins due to their genotoxic effects in vitro and/or fetotoxicity in rats, with potentially serious implications for human health. Tomatoes were found to be the most contaminated products, and the main source of exposure for these toxins in the population. To date there is a lack of specific regulations addressing Alternaria toxins in food. In 2022 the European Commission issued a recommendation for monitoring these contaminants in food, with limits in tomato-based products for alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TeA), which are the toxins most frequently found to contaminate foods at 10, 5 and 500 µg/kg respectively (European commission, 2022).

***Table 1**- Chemical properties and analytical parameters of main Alternaria mycotoxins*

	Name	Formula	Class	m/z	Ionization	FLD	DAD
	Alternariol (AOH)	$C_{14}H_{10}O_5$	Dibenzo-α-Pyrones	257.3	[M−H]-	Excitation: 258 nm Emission: 440 nm	280 nm
	Alternariol monomethyl ether (AME)	$C_{15}H_{12}O_5$	Dibenzo-α-Pyrones	271.3	[M−H]-		
	Altenuene (ALT)	$C_{15}H_{16}O_6$	Dibenzo-α-Pyrones	292.28	$[M+H]_+$		
	Tentoxin (TEN)	$C_{22}H_{30}O_4N_4$	Tetrapeptide	413.3	[M−H]-		
	Tenuazionic Acid (TeA)	$C_{10}H_{15}O_3N$	Tetramic acid derivative	196.3	[M−H]-	Not detected	

Due to lack of systematic research studies on the metabolism of Alternaria toxins in humans the Committee on the toxicity of Chemicals in Food, Consumer Products and Environment (EFSA Panel (CONTAM), 2011), has chosen to use the toxicological concern threshold (TTC) approach to assess the relative level of risks for human health.
Regarding the stability of toxins, several studies have monitored their degradation during production processes, under different conditions. The toxins show heat-resistance during and are stable during the shelf life of the food products.
Due to the structural heterogeneity of the molecules and the goal of developing a multiplex method capable of monitoring all toxins simultaneously, the extraction and purification protocol must be versatile, yet effective in solubilizing all target compounds. Nowadays, several analytical methods are available, each differing in

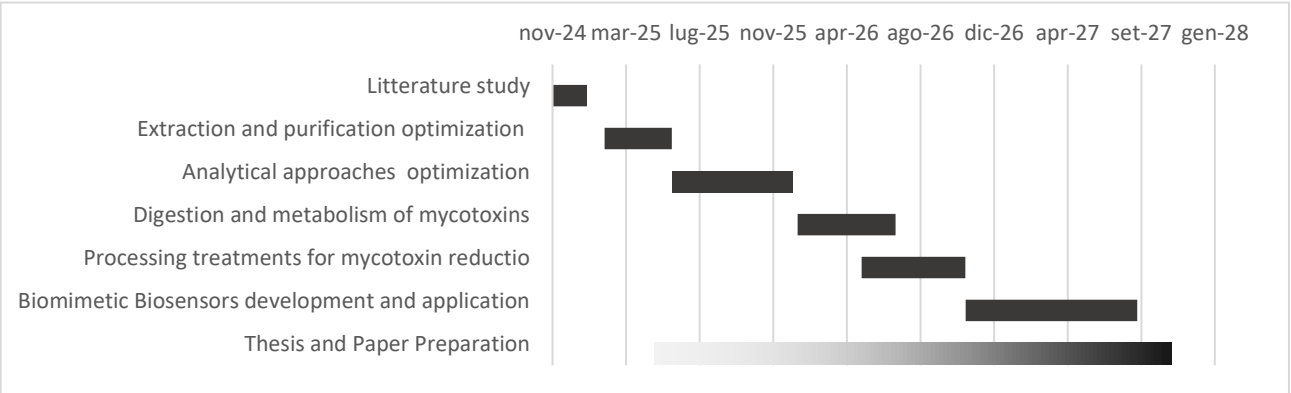
sensitivity, selectivity, complexity, and cost. For preliminary screening Thin Layer Chromatography (TLC) can be used as qualitative method. Liquid chromatography (LC) coupled with different detectors offers higher sensibility and can be used as quali-quantifier method. Electrochemical methods (such as ELISA, PCR…) are highly specific and portable, making them suitable for in situ use. To date, the golden standard for the quantitative assessment of contamination by *Alternaria* in food products is LC-MS analysis, presenting the highest sensitivity and selectivity.

2. PhD Thesis Objectives and Milestones

This PhD thesis project, according to the Gantt diagram given in Table 2, can be subdivided into the following activities:

a) **Literature study**: Study of literature for a comprehensive analysis of the state of the art in order to optimize the project and introduce innovations.
b) **Extraction and purification optimization** aims at a multiplex method. Following the parameters of exaction, using organic solvents will be optimised defining the optimal conditions in terms of type of solvent, ratio, time, temperature through a screening followed by an experimental design approach. Additionally, the solid-phase extraction (SPE), will be optimised to select the best performing type of SPE column stationary phase (e.g., C18, HLB, etc.). Under optimised conditions the recoveries will be determined.
c) **Analytical approaches optimization**: optimization of the operational parameters for HPLC based detection of the toxins using systems coupled with different detectors including the most common and sensitive ones, such as Diode Array Detection (DAD), Fluorescence Detection (FLD), and Mass Spectrometry (MS).
d) **In vitro gastrointestinal Digestion and metabolism of mycotoxins**: optimisation of an analytical pipeline to monitor the fate of the Alternaria toxins, thought gastrointestinal digestion system using an *in vitro* INFOGEST static model of digestion. Validation of monitoring analytical tools for the toxins in the different simulated compartments.
e) **Study of the effect of processing treatments on the mycotoxin:** different cooking methods, including among others, microwave, UV, pressure cooking will be applied to contaminated samples applying different time, to identify the most effective conditions for toxin reduction or elimination from the food matrix.
f) **Biomimetic Biosensors development and application:** Development of in field-deployable biosensors designed to detect by interacting with the target toxins and convert them into measurable signals, potentially allowing real-time results monitored by remote devices. The implementation of all optimized methodologies will be carried out in collaboration with La Doria.
g) **Writing and editing** of research papers, posters and oral presentation, culminating in the final semester dedicated to the completion of the PhD thesis

Table 2. Gantt diagram for this PhD thesis project.



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Food Chemistry: Development and application of untargeted methods and omics approaches for the characterization of raw materials and semi-finished products in the confectionery industry (P-I-35)

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Tutor: Prof.ssa Augusta Caligiani[1] Company tutor: Franco Rosso[2]

This PhD thesis aims to apply untargeted methods and omics approaches for quality control in the confectionery industry. This research uses analytical techniques such as Ultra-High Performance Liquid Chromatography (UHPLC) and High-Resolution Mass Spectrometry (HRMS). The research activities include the analysis of beet sugar to identify contaminants containing sulfonic groups and to evaluate their effect on pH and the partial inversion of sucrose during sugar syrup production. Moreover, the project includes the development of LC methods with pre-column derivatization for the characterization of carbonyl compounds in milk and hazelnuts.

Chimica degli alimenti: Sviluppo e applicazione di metodi untarget e approcci omics per la caratterizzazione di materie prime e semilavorati dell'industria dolciaria

Questa tesi di dottorato ha l'obiettivo di applicare metodi untarget e approcci omics per il controllo qualità nell'industria dolciaria. Le tecniche analitiche utilizzate in questi studi includono la Cromatografia Liquida a Ultra-Alte Prestazioni (UHPLC) e la Spettrometria di Massa ad Alta Risoluzione (HRMS). Le attività di ricerca comprendono l'analisi dello zucchero da barbabietola per individuare contaminanti contenenti gruppi solfonici e valutarne l'effetto sul pH e sulla parziale inversione del saccarosio durante la produzione di sciroppi zuccherini. Inoltre, il progetto prevede lo sviluppo di metodi LC con derivatizzazione pre-colonna per la caratterizzazione di composti carbonilici nel latte e nelle nocciole.

1. State-of-the-Art

In recent years, there has been a shift towards developing untargeted methods, particularly through the application of omics sciences. The integration of omics sciences with the growing focus on food quality, authenticity, and safety has given birth to the term 'Foodomics', which explores food composition, quality, and functional properties (Cifuentes, 2009). This PhD thesis project will focus on applying omics sciences in the confectionery industry. One of the first projects undertaken concerns chemical contaminants in beet sugar. Sugar beet (*Beta vulgaris* L.), a plant from the Chenopodiaceae family, is rich in sucrose and widely grown in moist regions to produce beet sugar. It provides about 25% of the world's sugar and supports global supply together with cane sugar. Because it is an important raw material in the confectionery industry, its quality may be evaluated by measuring organic acids and inorganic ions (De Bruijn & Bout, 1999). Few studies have investigated organic and inorganic acids in sugar production, typically using ion chromatography, ion-selective electrodes, capillary isotachophoresis, and gas chromatography. These analyses identified compounds such as lactic, acetic, formic, and malic acids, as well as inorganic ions like chloride, nitrate, nitrite, phosphate, and sulphate (Wojtczak, 2013). Acids may influence the inversion of sugar. Sucrose inversion happens when the bond between the two sugars in sucrose is hydrolyzed, releasing the individual monosaccharides. Partial inversion of sugar solutions makes it possible to create syrups that do not crystallize at room temperature, even though they have a lot of dry solids (P.W. van der Poel, 1998). The reaction depends on the pH, temperature, sugar concentration, and the hydrolyzing agent (Wienen & Shallenberger, 1988). As part of the PhD project, these effects will be further investigated at different pH levels, concentrations, temperatures, and times using experimental design. The main part of this research originated from an initial observation: different batches of beet sugar, when processed under the same conditions of temperature, concentration, and time, can lead to the acidification of a sugar syrup and the partial inversion of the sugar. In many applications, slight hydrolysis of sucrose into glucose and fructose does not significantly affect its functional performance. However, in other cases, even low levels of inversion (from around 2%) can reduce sucrose's ability to crystallize (P.W. van der Poel, 1998). Preliminary analyses revealed a high concentration of acids, particularly sulfonic acid, which are known to be significantly lower pH (Pakostova, 2019). The aim is to investigate whether sulfonic esters, sulfones, and sulfonamides could, under certain process conditions, contribute to acidification and partial inversion of the sugar syrup. Another activity of the PhD project concerns the qualitative (fingerprinting) profiling and quantitative individual determination of carbonyl compounds (aldehydes and ketones), regardless of their volatility, in confectionery raw materials like milk and hazelnuts, using LC separation techniques with pre-column derivatization. Aldehydes and ketones are naturally contained in foods such as fruits and vegetables, dairy products, and various beverages (Laghrib, 2019). This work aims to improve current methods for detecting carbonyl compounds, especially those responsible for off-notes due to aging or rancidity, as well as those that positively influence the quality of raw materials and semi-finished products. Moreover, several aldehydes are highly toxic compounds: for example, acrolein, detected in various foods such as cheese and fried products

(Laghrib, 2019). Quantification of carbonyl compounds is commonly performed using GC or HPLC (Syukri, et al., 2018). The polar nature and variable volatility of carbonyl compounds, combined with the absence of characteristic functional groups, complicate their direct detection. For this reason, derivatization is commonly used, as it makes them detectable by analytical instruments through their reaction with specific reagents. Various types of derivatization methods are described in the food field literature, such as dansylhydrazine (DH) or DNPH (Syukri, et al., 2018). In conclusion, the aim is to develop a derivatization method to evaluate these compounds in food matrices such as milk and hazelnuts, in order to obtain an immediate overview of the aldehydes and ketones present.

2. PhD Thesis Objectives and Milestones

Based on the overall objective, the PhD thesis project can be divided into the following activities according to the Gantt diagram shown in Table 1:

A1) Process Effects on Sugar: Study, using a factorial experimental design, how pH, temperature, concentration, and time influence a sugar mixture, in order to better understand the relationships between process variables and the evolution of the related chemical transformations, including sucrose inversion. Milestones: Completion of the experimental study and comprehensive analysis of how process parameters affect sucrose inversion dynamics.

A2) Sucrose Inversion by Impurity Concentrate: Prepare a concentrate of impurities from a defective sugar batch (A2.1), add it to high-purity sugar to evaluate whether it induces partial sucrose inversion (A2.2). Milestones: Experimental confirmation of sucrose inversion caused by impurity concentrate, compared to untreated samples.

A3) Determination of Sulfo-Organic Compounds: Untargeted UHPLC-HRMS approach for screening potential sulfo-organic contaminants in beet sugar samples and evaluating their correlation with pH decrease and sucrose inversion (A3.1); MS^n experiments for structural elucidation of the most relevant sulfo-organic compounds (A3.2) Milestones: Relevant sulfo-organic compounds identified and structurally characterized using MS^n

A4) Carbonyl Profiling: Develop and optimize LC-based methods with pre-column derivatization for the detection of carbonyl compounds in raw materials such as milk and hazelnuts (A4.1), this will begin with the screening of derivatizing agents (A4.2). Milestones: Validated analytical protocol and tested application on selected food matrices.

A5) PhD thesis preparation and other activities: writing the PhD thesis. Writing scientific papers, poster communication and sector conferences.

Table 1*- Gantt diagram for this PhD thesis project.*

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Process Effects on Sugar***																								
	Test of Sugar Mixtures (pH, T, time, conc.)																								
A2)	***Sucrose Inversion by Impurity concentrate***																								
	1) Preparation of Impurity Concentrate																								
	2) Concentrate Addition																								
A3)	***Determination of Sulfo-Organic Compounds***																								
	1) Untargeted UHPLC-HRMS: Screening of Sulfo-Organic Compounds																								
	2) MS^n: Qualitative Analysis																								
A4)	***Carbonyl Profiling***																								
	1) Optimizing LC Derivatization Methods																								
	2) Screening Derivatization Agents																								
A5)	***PhD thesis preparation and other activities***																								

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Bridging Analytical Chemistry and Regulation in Dietary Fiber Research: From Molecular Characterization to Novel Food Authorization (P-I-36)

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Tutor: Prof. Augusta Caligiani, Dr. Ginevra Rosso, Dr. Franco Rosso

This PhD thesis research project is aimed at developing and validating advanced analytical methodologies for the structural characterization and quantification of dietary fibers. The research includes the optimization of a GC-based method for comprehensive monosaccharide analysis, the establishment of a novel workflow for polysaccharide structural characterization, based on enzymatic hydrolysis, high-resolution ion mobility mass spectrometry profiling of resulting oligosaccharides, and AI-assisted data interpretation, and the application of these methods to support the preparation of regulatory dossier for novel fiber-based ingredients.

Approcci Analitici e Regolatori nello Studio delle Fibre Alimentari: dalla Struttura Molecolare all'autorizzazione come Novel Food

Questo progetto di tesi di dottorato è finalizzato allo sviluppo e alla validazione di metodologie analitiche avanzate per la caratterizzazione strutturale e la quantificazione delle fibre alimentari. L'attività di ricerca comprende l'ottimizzazione di un metodo basato su GC per l'analisi dettagliata dei monosaccaridi, la definizione di un nuovo flusso di lavoro per la caratterizzazione strutturale dei polisaccaridi, basato su idrolisi enzimatica, profilazione tramite spettrometria di massa ad alta risoluzione con mobilità ionica degli oligosaccaridi ottenuti e interpretazione dei dati assistita da intelligenza artificiale. Le metodologie sviluppate saranno applicate anche a supporto della redazione di dossier regolatori per nuovi ingredienti a base di fibre.

1. State-of-the-Art

Carbohydrates, the most abundant macromolecules in plant-based foods, are a primary energy source. However, their biological relevance extends far beyond this traditional view. Many carbohydrates, especially those that resist digestion in the upper gastrointestinal tract, exert diverse physiological effects, including modulation of the gut microbiota, immune regulation, and metabolic health benefits (Anderson JW; et al. 2019; Bindels LB; et al. 2015). These compounds, collectively referred to as dietary fibers, constitute a highly heterogeneous group, with structures differing in monosaccharide composition, glycosidic linkages, molecular weight, and branching.

Despite their significance, the term *dietary fiber* is often employed in a generic sense, without adequately reflecting this structural and functional diversity. Such ambiguity in definition hampers our ability to establish robust structure-function relationships and limits the development of targeted nutritional interventions or health claims (Tungland BC; et al. 2002). A deeper, chemically-informed characterization is therefore essential to unlock their full biological potential.

However, this goal presents considerable analytical challenges. Protein analysis is simplified by universal enzymes (e.g., trypsin) and established sequence databases, the structural analysis of carbohydrates remains far more complex. This is largely due to the lack of standardized enzymatic tools and the absence of comprehensive, curated reference libraries for glycan structures. Their structural complexity, stemming from isomerism, branching patterns, and the absence of a simple linear backbone, makes their analysis significantly more difficult (Haytowitz DB; et al. 2019). Bridging this gap is essential to enable reliable, high-throughput carbohydrate characterization, particularly for the study of dietary fibers and the development of fiber-based functional ingredients Currently, carbohydrate characterization often requires the use of multiple, time and resource intensive techniques: acid hydrolysis followed by GC for monosaccharide profiling, high-performance anion-exchange chromatography (HPAEC) for oligosaccharide separation, MALDI-MS for mass fingerprinting, and LC-MS/MS for structural elucidation, each providing only partial information.

Considering these challenges, there is a growing need for integrated and streamlined analytical workflows capable of providing both qualitative and quantitative data on dietary fiber components. In particular, the development of methods that allow structural reconstruction of polysaccharides from oligosaccharide fragments—similarly to how peptides are used to infer protein sequences—represents a major research goal. Such workflows could be significantly enhanced by artificial intelligence (AI) tools for pattern recognition and fragmentation prediction, enabling faster and more reproducible identification of glycosidic linkages and structural patterns. This PhD project is framed within this context, aiming to explore complementary analytical strategies, including gas chromatography coupled to mass spectrometry and liquid chromatography coupled with ion mobility and high-resolution mass spectrometry (e.g., UHPLC-IM-QTOF-MS), for comprehensive and scalable dietary fiber characterization.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Development of an advanced GC-MS based method for the qualitative and quantitative analysis** of monosaccharides derived from dietary fiber hydrolysis. This activity will focus on method optimization, validation, and application to selected fiber-rich samples. Milestone: a validated method for simultaneous determination of neutral and acidic monosaccharides by GC-MS

A2) **Establishment of a novel workflow for the structural profiling of fiber-derived oligosaccharides** using liquid chromatography coupled with ion mobility and high-resolution mass spectrometry. The activity includes the optimization of enzymatic hydrolysis protocols to generate defined and informative oligosaccharide fragments (A2.1) and the characterization of fragmentation patterns, integration of AI-assisted annotation, and creation of a reference database for structural identification (A2.2). Milestone: validated enzymatic workflow and searchable AI-supported database for oligosaccharide profiling

A3) **Definition of a robust analytical workflow supporting the preparation of novel food dossier related to dietary fibers**. This includes the analytical data compilation (A3.1) and the drafting of compositional and safety sections (A3.2) in accordance with regulatory requirements. Milestone: EFSA dossier sections (composition and safety) drafted based on validated analytical results.

A4) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Tabella 1 *Gantt diagram for this PhD thesis project.*

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Monosaccharide Analysis***																								
	1) GC method development																								
A2)	***Oligosaccharide Profiling***																								
	1) Enzymatic hydrolisis protocol																								
	2) Fragmentation, AI interpretation																								
A3)	***EFSA Novel Food Dossier***																								
	1) Analytical data compilation																								
	2) Regulatory dossier																								
A4)	***Thesis and Paper Preparation***																								

2.1. Risk analysis

Given the innovative nature of structural oligosaccharide work, possible risks include insufficient fragmentation reproducibility or limitations in current AI-models. Since oligosaccharides are typically not present in significant amounts in native fiber matrices, enzymatic hydrolysis is a necessary step to generate them prior to analysis. In case of analytical limitations, complementary strategies such as hybrid NMR-MS workflows will be considered.

3. Expected Outcomes

-A validated GC-MS protocol for the simultaneous quantification of neutral and acidic monosaccharides.
-Optimized protocols for enzymatic hydrolysis of fibers, using commercial enzymes with multiple activities
- The building of an open access database, in which each oligosaccharide structure will be directly associated with its corresponding analytical descriptors
-A proof-of-concept method for rapid oligosaccharide profiling aimed at reconstructing the polysaccharide structure, with potential AI support.
-A complete set of analytical data supporting novel food dossier on dietary fibers.

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Isolation and Characterization of Antioxidant Compounds from Maillard Reaction Products in Cooked Foods Using Advanced Spectroscopic Techniques (P-I-37)

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This thesis investigates antioxidant-active Maillard Reaction Products (MRPs) formed during the cooking of various foods. The Maillard Reaction, a non-enzymatic browning process, not only enhances flavor and appearance but also produces MRPs with potential health and preservative benefits due to their antioxidant properties. The study aims to isolate, characterize, and assess these compounds, focusing on how factors like temperature, time, pH, and food composition affect their formation. Techniques such as HPLC and NMR spectroscopy are used to purify MRPs and determine their structures and antioxidant activities. The research correlates structural features with bioactivity and explores differences across food types. Ultimately, it seeks to inform food processing strategies that optimize both health benefits and sensory qualities.

Strategia sperimentale per la progettazione ottimale di unità di ultrafiltrazione per il recupero di biopolimeri di interesse alimentare

Questa tesi analizza i Prodotti della Reazione di Maillard (MRPs) con attività antiossidante, formati durante la cottura di diversi alimenti. La Reazione di Maillard, un processo di imbrunimento non enzimatico, non solo migliora il sapore e l'aspetto dei cibi, ma produce anche composti con potenziali benefici per la salute e proprietà conservanti grazie alla loro attività antiossidante. Lo studio si propone di isolare, caratterizzare e valutare questi composti, esaminando l'influenza di fattori come temperatura, tempo, pH e composizione degli alimenti sulla loro formazione. Tecniche come la cromatografia liquida ad alte prestazioni (HPLC) e la spettroscopia NMR vengono utilizzate per purificare i MRPs e determinarne la struttura e l'attività antiossidante. La ricerca mette in relazione le caratteristiche strutturali con la bioattività e analizza le differenze tra diverse matrici alimentari. L'obiettivo è fornire indicazioni utili per lo sviluppo di metodi di cottura e prodotti alimentari trasformati che mantengano o migliorino le proprietà antiossidanti benefiche, preservando al contempo le qualità sensoriali desiderate.

1. State-of-the-Art

The Maillard Reaction (MR), first described by Louis Camille Maillard in 1912, is a nonenzymatic browning process involving amino acids and reducing sugars under heat, leading to the development of flavor, aroma, and color in various foods (Murata, 2021; Sun et al., 2022). Beyond sensory enhancement, MR contributes to food preservation and may offer nutritional and health benefits (Starowicz et al., 2019; Sun et al., 2010; Sun et al., 2023).

MR produces a diverse range of Maillard Reaction Products (MRPs), including melanoidins, Amadori products, and α-dicarbonyl compounds, which have demonstrated antioxidant and antimicrobial activities (Nooshkam et al., 2019; Feng et al., 2022; Hafsa et al., 2021; Wang et al., 2011). These MRPs can scavenge radicals, chelate metals, and inhibit lipid oxidation, contributing to both shelf life and potential health protection (Sun et al., 2022; Zhang et al., 2021; Kanzler et al., 2016). Particularly, melanoidins—late-stage, high molecular weight MRPs—show strong antioxidant potential similar to ascorbic acid through redox activity and metal ion chelation (Tamanna & Mahmood, 2015; Feng et al., 2022; Han et al., 2022). Their antioxidant behavior is also linked to umami flavor development (Habinshuti et al., 2019; M. Yu et al., 2018).

Conventional antioxidant assays (e.g., TBARS, β-carotene bleaching, DPPH) report MRPs' capacity in terms of concentration (Foti, 2015; Asma et al., 2024), but recent studies stress the importance of kinetic behavior—how quickly MRPs react with radicals—as a more accurate measure of efficacy (Amorati et al., 2018; Angeli et al., 2021; Sohail et al., 2022).

MRPs' dual role in flavor and oxidative protection is attracting interest in 'clean label' food products, as natural preservatives that meet consumer demand for minimally processed ingredients (Monti et al., 1999; Jia et al., 2023). Their antimicrobial activity further enhances food safety and quality (Mildner-Szkudlarz et al., 2023; Han et al., 2022).

Nonetheless, gaps remain in understanding the molecular mechanisms behind MRP formation and activity. Nuclear Magnetic Resonance (NMR) spectroscopy has been pivotal in recent studies to elucidate MRP structures

and relate them to antioxidant functions. For instance, NMR, alongside FT-IR and GC-MS, was used to identify 3-hydroxy-4-methyl-2(5H)-furanone (3H4MFN), a potent antioxidant comparable to vitamins C and B6 (Zhang et al., 2024). Another study on glucose-glycine systems using NMR identified key MRPs like methylpyridin-3-ol (MP) and DDMP, which showed electron-donating capabilities and time-dependent increases in antioxidant activity (Bolchini et al., 2025). Thus, NMR remains a critical tool in linking MRP molecular structures to their bioactivities, especially antioxidant functions, advancing both fundamental and applied food science.

2. PhD Thesis Objectives and Milestones

Year 1: Foundation and Method Setup

- **Term I–III (M1.1):** Literature Review, Framework Development
- **Term II–IV (M2.1):** Experimental Design, Method Development
- **Term IV (M3.1):** Pilot Studies, Instrument Calibration, 1st Paper

Year 2: Full Experiments and Data Generation

- **Term I (M1.2):** Literature Update, Model Refinement
- **Term I–II (M2.2):** Sample Collection, Full Experiments
- **Term I–III (M3.2):** NMR Structural Analysis, Correlation, 2nd Paper

Year 3: Comparative Study & Thesis Completion

- **Term I–II (M1.3):** Theoretical Integration
- **Term I–II (M2.3):** Comparative Study, Application Work
- **Term II–IV (M3.3):** Final Analysis, Thesis Writing, 3rd Paper, Defense

Milestones	1st year				2nd year				3rd year			
Terms	I	II	III	IV	I	II	III	IV	I	II	III	IV
M1 –												
M2 –												
M3 –												

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Enhancing Antioxidant Activity in Breadcrumbs Through Controlled Maillard Reaction (P-I-38)

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The Maillard reaction is a non-enzymatic process that occurs during food processing at high temperatures. It involves interactions between amine groups—typically from amino acids and proteins—and carbonyl compounds, primarily from reducing sugars. This reaction plays a key role in the browning and flavor development of foods while yielding bioactive compounds with antioxidant potential. Using a combination of flow injection analysis (FIA), DPPH kinetic assays, and total phenolic content (TPC) measurements, we evaluated the effects of thermal processing, specifically time and temperature heating, on the phenolic content, antioxidant activity and safety profile of breadcrumbs pre- and post-Maillard reaction. High-performance liquid chromatography-mass spectrometry (HPLC-MS) was employed to characterize the most effective Maillard reaction products (MRPs) contributing to antioxidant enhancement. Furthermore, isothermal calorimetry was used to evaluate the oxidative stability of thermally treated breadcrumbs when incorporated into oil-in-water emulsions. Results demonstrate that optimized Maillard reaction conditions significantly improve the antioxidant capacity of breadcrumbs, supporting their potential application as functional ingredients in food systems.

Miglioramento dell'Attività Antiossidante nei Pangrattati. Attraverso una Reazione di Maillard Controllata

La reazione di Maillard è un processo non enzimatico che si verifica durante la trasformazione degli alimenti ad alte temperature. Essa coinvolge interazioni tra gruppi amminici—provenienti tipicamente da amminoacidi e proteine—e composti carbonilici, principalmente zuccheri riducenti. Questa reazione svolge un ruolo fondamentale nella formazione del colore e dello sviluppo del sapore degli alimenti, producendo al contempo composti bioattivi con potenziale antiossidante. In questo studio, abbiamo valutato gli effetti del trattamento termico—con particolare attenzione al tempo e alla temperatura di riscaldamento—sul contenuto fenolico, sull'attività antiossidante e sul profilo funzionale dei pangrattati prima e dopo la reazione di Maillard, utilizzando una combinazione di analisi a iniezione di flusso (FIA), saggi cinetici DPPH e misurazioni del contenuto fenolico totale (TPC). La cromatografia liquida ad alte prestazioni accoppiata alla spettrometria di massa (HPLC-MS) è stata utilizzata per caratterizzare i prodotti della reazione di Maillard (MRPs) più efficaci nel contribuire al miglioramento dell'attività antiossidante. Inoltre, la calorimetria isotermica è stata impiegata per valutare la stabilità ossidativa dei pangrattati trattati termicamente quando integrati in emulsioni olio-in-acqua. I risultati dimostrano che condizioni ottimizzate della reazione di Maillard migliorano significativamente la capacità antiossidante dei pangrattati, supportando il loro potenziale impiego come ingredienti funzionali nei sistemi alimentari.

1. State-of-the-Art

The Maillard Reaction is non-enzymatic chemical process in food science that contributes to desirable characteristics, such as the toasted aroma of baked bread. This reaction was first discovered by Louis-Camille Maillard in 1912 [1]. It involves interactions between amine groups—typically from amino acids and proteins—and carbonyl compounds, primarily from reducing sugars. Beyond enhancing sensory quality, Maillard reaction products (MRPs) have been found to exhibit significant antioxidant properties. These compounds can inhibit lipid oxidation, scavenge free radicals, thereby enhancing food stability and preserving nutritional quality. It also contributes to off-flavors, nutrient loss, and reduced shelf-life. As a result, recent interest in MRPs has grown due to the antioxidant potential [2,3,4]. Despite significant progress in characterizing the sensory benefits of MRPs, the mechanisms underlying their antioxidant reactivity remain poorly understood.

Identifying food by-products that can be transformed into antioxidant-rich MRPs is increasingly important. Bread is an essential part of our daily diet and Processing bread wastes constitutes a major problem for most bakeries. They are generally processed into "breadcrumbs" and used in breading formulations or extensively used for making a crisp and crunchy coating in breading formulations for baked foods. Breadcrumbs are a rich substrate for MR due to their high carbohydrate and protein content. Therefore, it can be associated positively with antioxidant activity by changing the thermal processing parameters, particularly temperature and baking time. Although a few studies have been reported regarding MRPs and antioxidant activity in bakery products, the growing interest in sustainable food processing and waste recovery makes breadcrumbs a promising material for further investigation [5,6,7]. Additionally, integrating thermally treated breadcrumbs into emulsions could provide

insight into their role in enhancing oxidative stability in complex food systems.

This research could offer new insights into the mechanisms of antioxidant reactivity in MRPs, potentially contributing to the development of breadcrumbs with improved functional properties. The aim of this project is to examine how varying thermal processing parameters affect the antioxidant activity of breadcrumbs through MR pathways. Furthermore, some Maillard reaction products (MRPs) may have adverse health effects [4,6]. It is crucial to maximizing health benefits while minimizing potential risks. Further work involving the combination of breadcrumbs with oils to assess their effect on lipid oxidation. Future investigations of the complexity of MRPs and their diverse chemical nature requires comprehensive analysis by advanced spectrophotometric and chromatographic methods to fully understand and optimize the protective effects of MRPs in food applications.

2. PhD Thesis Objectives and Milestones

A. Evaluating the impact of thermal treatment (temperature and time) on the formation of antioxidant compounds in breadcrumbs.
B. Identifying the key Maillard reaction products responsible for antioxidant activity in breadcrumbs.
C. Maximizing antioxidant activity while minimizing harmful MRP formation.
D. Investigating the ability of breadcrumb-derived compounds to inhibit lipid oxidation in oil systems, with potential applications in products such as cookies, potatoes, and other bakery or snack items.
E. Evaluating the radical scavenging activity of breadcrumb extracts using the DPPH assay as a measure of antioxidant reactivity.

Table 2 Diagram for PhD thesis project.

		1st year				**2nd year**				**3rd year**			
Activity	Years	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A1)	***Literary review***												
A2)	***Antioxdant in breadcrumbs: effect of Temperature and time***												
A3)	***Antioxdant in breadcrumbs in formulation: Oil/Water emulsions vs bulk oil***												
A4)	***Antioxdant in breadcrumbs: effect of digestion***												
A5)	***Antioxdant in breadcrumbs: review***												

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Use of innovative plant-derived ingredients in the production of functional foods for the enhancement of marginal areas (P-I-39)

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This PhD thesis research project aims to valorize the marginal areas by evaluating underutilized native plant species from these lands as potential innovative food ingredients, with special regard to *Quercus* L. spp., *Fraxinus excelsior* L. and *Astragalus* L. spp. This objective falls within the "MARginal Areas: Enhancement of ecosystem resources for a fair and sustainable livelihood" (MAR.V.E.L.) Project of Excellence of the DISSPA Dept. of the University of Bari (Area 3: "Food sciences and food design" – Objective ii: "Search for new, sustainable, food sources").

Uso di ingredienti innovativi di origine vegetale nella produzione di alimenti funzionali per la valorizzazione delle aree marginale

Il progetto di tesi di dottorato ha come obiettivo principale la valorizzazione delle aree marginali, attraverso la valutazione delle specie vegetali ivi presenti come ingredienti innovativi per uso alimentare, con particolare attenzione a tre specie, Quercia, Frassino e Astragalo. Tale obiettivo rientra nel Progetto di Eccellenza "Aree marginali: Valorizzazione delle risorse dell'ecosistema per un sostenimento equo e sostenibile" (MAR.V.E.L.), promosso dal Dipartimento DISSPA dell'Università degli Studi di Bari (Area 3: "Scienze alimentari e progettazione degli alimenti –obiettivo ii: "Ricerca di risorse alimentari nuove e sostenibili").

1. State-of-the-Art

"Marginal lands" or "marginal areas" are defined as "agriculturally inefficient lands", "land with little or no agricultural or industrial value", or "land with low production levels or unproductive". In these areas, the costs of labor and material costs highly exceed the value of yields (Wójcik-Leń and Sobolewska-Mikulska, 2017), so cost-effective production is not possible due to low soil fertility and ineffective cultivation techniques. In addition, while land in Europe is considered marginal from an economic perspective, the global definition of marginal land is broader and includes social and environmental factors, such as unemployment and abandonment of youth population; soil health and topography, and also extreme weather events, such as floods and drylands. The wider term of "marginality" indicates, therefore, a multidimensional phenomenon driven by several interlinked environmental and socio-economic factors. It must be considered, however, that the classification of a land as "marginal" for one purpose may not necessarily mean that the same land is unproductive for all purposes (Alhajj Ali et al., 2024). In fact, marginal lands can show many underutilized botanical species with great potential in terms of bioactive compounds that can be introduced into the food chain in the form of innovative ingredients. In the Mediterranean environment, including southern Italy (the area where this doctoral project is being developed), three species can be considered as case study, two tree species and one herbaceous species, all of which are wild: *Quercus* L. spp., *Fraxinus excelsior* L., and *Astragalus* L. spp. Over 300 species of *Quercus* have been identified in the literature, with the most common species in Apulia being *Q. ilex* L., *Q. pubescens* W. and *Q. trojana* W.. Acorns are the edible fruits of oak trees and are recognized for their nutritional value, including high content of carbohydrates, mainly starch, along with fiber and fats. They have been an important food source in different cultures worldwide and were used in bread production and in coffee substitutes (Zocchi et al., 2022). However, they become under-appreciated in modern diets, despite their long history in culinary applications (Szabłowska and Tańska, 2021). *Fraxinus excelsior* L. is the source of "*manna*", that is a particularly unique vegetable product rich in mannitol, obtained by the spontaneous solidification of phloem sap gathered from selected *Fraxinus* species (*Oleaceae*) or "ash tree". More than 80% of the dry weight of *Fraxinus manna* is composed of sugars, mono- and oligosaccharides. The primary component among them is mannitol, a polyol, used in dermatological treatments for its soothing and anti-aging properties, as well as in syrups as a fluidizer, expectorant, and cough suppressant (Attanzio et al., 2019). Due to mannitol, *manna* has sweetening properties and was used in the past but then gradually declined, although it is also suitable for diabetics (Spadaro et al., 2022). *Astragalus* L. spp., including about 3000 species widely distributed throughout the temperate and arid regions of the word, is one of the largest genera of *Fabaceae* (Shahrivari-Baviloliaei et al., 2025). Plants belonging to *Astragalus* genera are commonly used as fodder crops for wild animals and livestock. Additionally, several *Astragalus* species have been utilized in pharmaceuticals and cosmetics. The use as coffee substitute of *Astragalus* seeds, after toasting and milling, was traditional in the Apulia region of southern Italy during the second world war. Due to their high level of bioactive compounds, these plants exhibit strong antioxidants, anti-inflammatory and antimicrobial properties (Shahrivari-Baviloliaei et al., 2024) and could be effectively used in the production of functional foods.

2. PhD Thesis Objectives and Milestones

Based on the above reported state of the art, this PhD project has the main aim of valorizing three underutilized plant species from marginal lands, *Quercus* L. spp., *Fraxinus excelsior* L., and *Astragalus* L. spp., by using them

as a source of innovative food ingredients. The activities planned for the PhD project, the duration of which is illustrated in the Gantt chart shown in Figure 1, are subdivided according to the species studied, as follows:

A) **Bibliographic research** on marginal areas in the Mediterranean environment to identify the main botanical species from these areas potentially suitable for food use, then for deepening the knowledge on analytical methods to apply for their characterization, food preparation methods, and all the needed for the PhD project aims.

B) Focus on ***Quercus* L. spp.**: Chemical characterization of acorns (B1) from at least three species, shelf-life study of a corn flour under different conditions (B2), formulation of innovative foods with acorn flour (B3), physicochemical characterization of acorn-based foods ("*focaccia*" flatbread) (B4).

C) Focus on ***Astragalus* L. spp.**: Chemical characterization of *Astragalus* L. spp. roots, leaves and seeds from at least three species (C1), formulation of innovative foods (biscuits) with *Astragalus* L. spp. powdered roots, leaves and seeds (C2), physicochemical characterization of foods enriched with *Astragalus* L. spp. (C3)

D) Focus on ***Fraxinus excelsior* L.**: chemical characterization of *manna* (D1), formulation of innovative foods with *manna* (ice-cream) (D2), physicochemical characterization of *manna*-based foods (D3).

E) **Data elaboration, thesis and paper preparation**: Statistical treatment of data, writing and editing of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	***Bibliographic search***																								
B	***Quercus* L. spp.**																								
B1	Chemical characterization of acorns																								
B2	Shelf-life study under different conditions																								
B3	Formulation of innovative foods with acorn flour																								
B4	Characterization of acorn-based foods																								
C	***Astragalus* L. spp.**																								
C1	Chemical characterization of *Astragalus* L. spp.																								
C2	Formulation of innovative foods with *Astragalus* L. spp.																								
C3	Characterization of foods enriched with *Astragalus* L. spp.																								
D	***Fraxinus excelsior* L.**																								
D1	Chemical characterization of *manna*																								
D2	Formulation of innovative foods with *manna*																								
D3	Characterization of *manna*-based foods																								
E	***Data elaboration, thesis and paper preparation***																								

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Sustainability evaluation of plant-based protein sources by integrating nutritional, environmental and food safety dimensions (P-I-40)

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Tutor: Prof. Emma Chiavaro and Prof. Francesca Scazzina

This PhD thesis research project aims to evaluate the sustainability of the Italian supply chain for alternative protein sources, specifically legumes, by integrating nutritional value, food safety and environmental impact. The analysis will combine food consumption data with the assessment of emerging contaminant risks and environmental sustainability metrics derived from Life Cycle Assessment (LCA). Moreover, food consumption data will be used to model scenarios of increased legumes consumption in Italy.

Valutazione della sostenibilità delle fonti proteiche vegetali integrando le dimensioni nutrizionali, ambientali e di sicurezza alimentare

Questo progetto di tesi di dottorato ha l'obiettivo di valutare la sostenibilità della filiera italiana fonti vegetali proteiche, con particolare riferimento ai legumi, integrando aspetti nutrizionali, di sicurezza alimentare e di impatto ambientale. L'analisi combinerà i dati relativi ai consumi alimentari con la valutazione del rischio da contaminanti emergenti e con metriche di sostenibilità ambientale derivate dall'Analisi del Ciclo di Vita (LCA). Inoltre, i dati ottenuti saranno utilizzati per modellare scenari di aumento del consumo di legumi in Italia.

1. State-of-the-Art

In recent decades, the impact of human activities on terrestrial ecosystems has increasingly attracted the attention of the scientific community and international institutions. The Sixth IPCC Report highlights the urgency of taking action to tackle climate change by combining mitigation and adaptation measures. According to WWF (2024), the average Italian lifestyle would require 1.7 Planets to be sustained, a clear indication that we are consuming more resources than the Earth can regenerate. The main environmental challenges (from the overuse of non-renewable resources to biodiversity loss, greenhouse gas emissions and soil degradation) demand a profound reshape of the agri-food system as well.

The link between nutrition, health and sustainability is now at the center of scientific and institutional debate. The United Nations 2030 Agenda established 17 Sustainable Development Goals (SDGs), including Goal 2: "End hunger, achieve food security and promote sustainable agriculture," which directly involves food production and consumption. The FAO, EFSA and WHO emphasize the need for a systemic approach that integrates environmental protection, social equity, and nutritional well-being.

In this context, legumes represent a strategic food source. They are rich in proteins, fibers, and micronutrients and have a low environmental impact due to their ability to fix atmospheric nitrogen and improve soil fertility, thereby reducing the use of synthetic fertilizers. The latest Italian Dietary Guidelines (CREA, 2019) explicitly recognize the value of plant-based protein sources to promote healthier and more sustainable dietary patterns, in line with the principles of the Mediterranean Diet.

However, despite their many benefits, legume consumption in Italy remains well below recommended levels. Data from the CREA IV SCAI survey (2023) show an average daily intake of only 9 grams per person, with 31% of the population reporting consumption. This trend highlights the need for more effective strategies to encourage legume inclusion in dietary habits, including nutrition education, product innovation and supply chain studies.

Production also faces significant challenges. In regions such as Emilia-Romagna, which holds a key share in the cultivation of beans, the effects of climate change are already evident: high summer temperatures hinder fruit set, while autumn humidity favors fungal diseases. Improving the resilience of legume crops thus becomes a central objective, to be addressed through innovative agronomic approaches and climate risk management strategies.

Alongside nutritional and production aspects, emerging issues related to food safety are also gaining attention. Beyond traditional contaminants such as heavy metals and pesticides, climate change and environmental pollution are promoting the emergence of new substances potentially harmful to human health (Awino et al., 2025). Moreover, there has been a global increase in legume-related allergies, a complex phenomenon that may be linked to epigenetic factors influenced by lifestyle and environmental conditions (Abu Risha et al., 2024).

In light of this context, it is increasingly urgent to rethink the role of legumes within food systems, not only for their nutritional value but also for the contribution they can make to environmental sustainability and food safety. This doctoral project fits into this perspective by proposing an integrated analysis of the Italian legume supply chain, with particular attention to environmental, nutritional and production aspects, as well as potential emerging risks.

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2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Database (DB) completion** with nutritional and environmental impact information from specific database (A1.1) and then modelling new dietary scenarios (A1.2).

A2) **Food safety risk assessment** information integration through literature research (A2.1) and effective assessment (A2.2).

A3) **Study of a prototype meat alternative** first selecting the raw material (legume) that has been shown to have the best nutritional characteristics and the lowest environmental impact (A3.1) which will be used to produce the prototype of the alternative to animal sources (A3.2).

A4) **Study of the final product** from environmental sustainability point of view (A4.1) and the perception of the end consumer will be investigated (A4.2).

A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity \ Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Database (DB) completion***																								
	1) Nutritional and Environmental data																								
	2) Modelling new dietary scenarios																								
A2)	***Food safety risk assessment***																								
	1) DB information integration																								
	2) Assessment of emerging risks																								
A3)	***Study of a prototype meat alternative***																								
	1) Selection of the best raw material																								
	2) Definition of a prototype																								
A4)	***Study of the final product***																								
	1) LCA analysis of the supply chain																								
	2) Consumer perception study																								
A5)	***Thesis and Paper Preparation***																								

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Development of Innovative Processes to produce micro- and nano-sized Extracellular Vesicles from Plant matrices intended for Nutraceuticals and Food applications (P-I-41)

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This PhD thesis project aims at the development of an efficient and scalable process to produce micro- and nano-sized extracellular vesicles from plant matrices (PDEVs) with high healthy and technological functionalities and assess the feasibility of their application for nutraceuticals and food applications (e.g. antioxidant, antimicrobial, other heath properties). The application of conventional (e.g. blanching) and innovative technologies such as Pulsed Electric Field (PEF), ultrasound for the pretreatment of the raw materials to enhance process efficiency and product stability and safety will be explored. Moreover, alternative technologies (e.g. Tangential Flow Filtration, TFF) to the currently applied at the extraction step will be tested to enhance the yield and purity of PDEVs. Finally, the obtained PDEVs will be used to produce PDEVs-enriched beverages or nutraceuticals.

Sviluppo di un processo innovativo per la produzione di micro e nano vescicole extracellulari da matrici vegetali allo scopo di usarle nella formulazione di nutraceutici o alimenti funzionali

Questo Progetto di tesi ha l'obiettivo di sviluppare un processo efficiente e scalabile per l'estrazione di micro e nano vescicole extracellulari da matrici vegetali con proprietà salutari e tecnologiche e appurare la fattibilità del loro utilizzo nella produzione di nutraceutici o alimenti funzionali (proprietà antiossidanti, antimicrobiche etc..). L'uso di tecnologie convenzionali (blanching) e innovative come i campi elettrici pulsati e ultrasuoni per il pre-trattamento della materia prima saranno indagati allo scopo di aumentare l'efficienza di estrazione, stabilità e sicurezza del prodotto finito. Inoltre, l'uso di tecnologie alternative (membrane a filtrazione tangenziale) a quelle attualmente utilizzate per il processo di estrazione (ultracentrifuga) saranno studiate allo scopo di aumentare la resa e la purezza delle vescicole. Infine, le vescicole ottenute saranno utilizzate per produrre bevande arricchite dalle suddette oppure nutraceutici.

1. Scientific State of Art

Extracellular vesicles (EV) are defined as "particles naturally released from the cell that are delimited by a lipid bilayer and cannot replicate, i.e. do not contain a functional nucleus "(April et al., 2012). Exosomes are a specific size-range of EVs (40-160 nm) abundant in proteins, lipids, RNA, metabolites, and other components which are released by them in the extracellular environment to carry out their functions. In recent years there have been an increasing interest in the plant derived extracellular vesicles (PDEVs), which were found to exert natural anticancer, antitumor, antioxidant, anti-inflammatory, and wound-healing effects. In addition, they do not have adverse effects on intestinal immune homeostasis, can strengthen host anti-inflammatory capacity and can be used for drug delivery also for pregnant women (Wang, Zhuang et al. 2014). Furthermore, their ability to carry bioactive compounds targeting specific area of the body make them a suitable resource for the nutraceuticals and food industry (Liu, Ren et al. 2024). Thus, growing is the use of PDEVs in the medical, pharma and cosmetics sectors and in recent times also the food sector showed an increased interest for the natural origin of PDEVs and health potentialities.

So far, various are the methods for their extraction and purification starting from plant-based raw materials such as ultracentrifugation or ultrafiltration, but there is still a lack of optimised methodologies able to isolate high-yield, high-purity EVs. Specifically, the largely used ultracentrifugation method, even if suitable for EVs extraction and purification, is expensive, time-consuming and with a low yield of process. On the other hand, ultrafiltration has been used to successfully isolate EVs from *Citrus lemon* and other plants providing a cost-effective and scalable method that could lead to the industrial production of PDEVs (Paganini et al., 2019, Giancaterino et al., 2025). Thus far, there have been only two applications of PDEVs as novel ingredient to produce functional beverages with positive results that assess the potential of their use in food industry (Tinnirello V. 2025, Zhu et al., 2025). However, the full process of PVES production remains a challenge for food and nutraceuticals application as scarce are the investigations aimed to define their stability and technological functionality in real food systems. Moreover, low yield process and the high quantity of extraction by-products, that will become waste

if not valorised, makes the PDEVs extraction process of limited sustainability, despite the natural origin of final products aligned with the green transition of the food system.
Systematic investigations considering the factors that could improve process yield and technological functionality of PDEVs intended for food purposes are needed, including the understanding of the effects of extraction pre-treatments on the raw materials (e.g. blanching) and the use of innovative technologies (e.g. Pulsed Electric Fields, Vacuum Impregnation).
Being PDEVs a potential valuable "green" new ingredient for high quality and health food formulations and nutraceuticals, this thesis project will, thus, focus on the optimization of a scalable method for PDEVs production, their technological characterisation (e.g. antioxidant properties) and stabilisation; an application in real food formulation will be explored. Carrots will be taken as raw material for the investigations.

2. PhD Thesis Objectives and Milestones

The aim of this project can be in the following activity, according to the Gantt diagram in Table 1:
A1) **Understanding of process conditions affecting PDEVs extraction efficiency and technological functionalities (M1-M12).** Conventional centrifugation-based PDEVs extraction methodology will be used as reference. Innovative technologies such as tangential flow filtration and Vacuum Impregnation and/or green solvents will be tested. Mass balance and yield will be investigated along with the PDEVs characterisation (i.e. number, size, shape, antioxidant capacity, bioactive content, protein/lipid/RNA content, other health properties). Blanching (under various temperature and time conditions) will be tested as pre-treatment to PDEVs extraction.
A2) **Application of innovative technologies to enhance process efficiency and PDEVs technological functionalities (M13-M18).** Pre-treatment such as ultrasound and pulsed electric field (PEF) treatments will be carried out under various process conditions. PDEVs process efficiency and technological functionalities will be evaluated as in A1).
A3) **PDEVs for food application (M19-M24).** Freeze-drying and spray-drying will be used to obtain stable encapsulated PDEVs for food purposes. The encapsulated materials will be used in a formulated real food product (e.g. dairy derivatives, dressings) and evaluations on the quality, stability and health properties will be carried out.
A4) **Writing and editing (M1-M24)** PhD thesis, scientific papers writing, oral/poster presentations at conferences.

Table 1. Thesis project's Gantt diagram.

Activity		Months																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	**Process Development**																								
	1) Extraction techniques comparison																								
	2) Process optimization																								
A2)	**PDEVs characterization**																								
	1) Physical, functional and chemical properties																								
	2) In vitro effect evaluation																								
A3)	**PDEVs Food application**																								
	1) PDEVs encapsulation and food formulation																								
	2) Food stability and properties																								
A4)	**Thesis and paper preparation**																								

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Innovative food formulations with plant protein-based bigels (P-I-42)

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This PhD research focuses on developing plant-based bigels as a sustainable and health-conscious alternative to conventional fats and synthetic additives. The project aligns with growing consumer demand for clean-label and environmentally friendly products. It aims to improve the texture and mouthfeel of low-fat food formulations and verify the possibility to use bigel as carriers of hydrophilic and/or lipophilic bioactive compounds, for creating innovative, nutritious, and appealing food products.

Formulazioni alimentari innovative con bigels a base di proteine vegetali

Questo progetto di dottorato si concentra sullo sviluppo di bigel a base vegetale come alternativa sostenibile e salutare ai grassi convenzionali e agli additivi sintetici. Il progetto risponde alla crescente domanda dei consumatori di prodotti a etichetta pulita e rispettosi dell'ambiente. Inoltre mira a migliorare caratteristiche quali texture e mouthfeeling dei prodotti a basso contenuto in grasso e a investigare il loro utilizzo come carrier di composti idrofilici e/o lipofilici per creare alimenti innovativi, con migliorate caratteristiche nutrizionali e appetibili per i consumatori.

1. State-of-the-Art

There is an increasing interest in creating healthy solid fat alternatives to replace conventional fats in the food industry because the growing concerns on the impact of saturated fatty acid consumption on cardiovascular disease, cerebrovascular illness, obesity, hypertension, and diabetes (Liu *et al*., 2025). Bigel, or hybrid gel, is an innovative solid-like oil product generated by the combination of an oleogel and a hydrogel using high-speed shear at a specific temperature, resulting in an interesting set of properties such as enhanced stability, nutritional values, and texture, which might also be tailored for specific applications (Hu *et al*., 2025). Such bigels represent an interesting biphasic system, possessing the specific advantages of both oleo and hydrogel. However, despite the potential bigels benefits, the food sector still has issues with ingredient safety, legal compliance, and consumer acceptance.

When incorporating bigels into food products, their physicochemical characteristics and interactions with other components must be carefully considered (Farzana *et al*., 2025). Real food systems include several interacting elements that are processed mechanically and thermally, such as dairy substitutes, meat substitutes, or baked foods. These circumstances may affect bigels' stability and structural integrity, resulting in problems like phase separation, oxidative instability, and undesired texture (Li *et al*., 2023). Achieving a stable and uniform interface between the water and the oil phases is a significant difficulty and the intrinsic incompatibility of these phases may lead to undesirable rheological characteristics, such as low mechanical strength, and a limited shelf life (Chao *et al*., 2024). To address these issues, plant proteins represent an ongoing novelty in food science, being an interesting base for producing either oil-based oleogels or water-based hydrogels, which can replicate the texture and functionality of conventional animal-derived fats. The incorporation of plant proteins such as soy protein isolate, pulses (e.g. peas, chickpeas, and lentils) and cereals (e.g. wheat, barley, and zein) and canola proteins can enhance both the nutritional profile and the structural/sensory attributes of bigels.

Plant-based bigels might represent a flexible, clean-label solution as fat replacer in several food products, such as meat and cheese substitutes, and taking into account their nutritional aspects, the affordable and relatively easy of production their application in food bigels design is going to grow in the next future. Furthermore, plant protein based bigels might represent a promising opportunity for creating of a variety of plant-based products, opening the door to more sustainable and healthful dietary options (Lin *et al*., 2024). However, at present much research is still limited to laboratory-scale tests without pilot-scale trials or testing procedures that are valuable to industry.

Thus, this PhD project aims to design plant protein-based bigels investigating how different oleogel and hydrogel might affect their properties and their suitability for being incorporated into food items. By leveraging plant protein-based bigels' dual functionality, the study aims to overcome current limitations in bigel design and move toward clean-label, scalable, and consumer-acceptable food innovations.

2.PhD Thesis Objectives and Milestones

Within the overall objectives mentioned above, this PhD project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Bibliographic research** on the oleogels and hydrogels component and structure, the role of oleogels and hydrogels in bigel formation, natural food emulsifier's structure and emulsifying functionality such as soy protein isolate, chickpea, potato, pea, canola proteins and preparation bigel and food applications of edible bigels.

A2) **Determination of components and structure of a few selected oleogels and hydrogels** (e.g., bees wax, rice bran wax, carrageenan, soy protein isolate, quinoa starch-based emulsions) in edible oils to physicochemical characterization such as structural analysis and thermal stability, rheological properties, and oxidation and chemical properties.
A3) **Assessment of oleogel/hydrogel combinations for optimal bigel formulation** such as rheological and textural properties, microstructure, oil binding capacity, stability and shelf-life.
A4) **Assessment of plant protein -based bigels on different food application** such as baked goods, dairy products, spreads and sauces, confectionary, meat analogues and functional foods.
A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Bibliographic research inherent to the topic***																								
	Review existing studies on oleogels, hydrogels and natural emulsifiers, component and structure as well as on bigels.																								
A2)	***New oleogel and hydrogels formulation***																								
	Oleogel and hydrogels formulation - physicochemical characterization and stability tests.																								
A3)	***Assessment of oleogel/hydrogel combinations for optimal bigel formulation***																								
	Physicochemical bigel characterization (rheological and textural properties, microstructure, oil binding capacity, stability, shelf-life)																								
A4)	***Assessment of plant protein -based bigels in different food application***																								
	Lab and pilot scale trials																								
A5)	***Thesis and paper preparation***																								

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Development of a body of knowledge of selected microbial bioresources for the production of an innovative kombucha (P-I-43)

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Kombucha, a fermented functional beverage rich in bioactive compounds, gained popularity as a healthier alternative to soft drinks. Its microbial ecosystem, comprising acetic and lactic acid bacteria along with diverse yeasts, drives fermentation and shapes its sensory and nutritional profile. However, the use of traditional SCOBYs may cause product variability and safety concerns. This PhD project aims to isolate and characterize key microbial strains from artisanal kombucha produced in Verona, Italy. The goal is to evaluate their technological potential and safety, paving the way for their inclusion in the EFSA QPS list and enabling standardized, scalable, and high-quality kombucha production.

Sviluppo di un *corpus* di conoscenze per l'autorizzazione all'utilizzo di biorisorse microbiche selezionate per lo sviluppo di kombucha innovativo

La kombucha è una bevanda ottenuta dalla fermentazione del tè, sempre più apprezzata come alternativa più salutistica rispetto a bevande zuccherate non alcoliche. La fermentazione è guidata da un consorzio microbico (SCOBY) composto da lieviti e batteri acetici e lattici, che contribuiscono alle caratteristiche sensoriali e nutrizionali. Tuttavia, l'uso dello SCOBY in modo "tradizionale" può introdurre variabilità e problemi di sicurezza. Questo progetto si propone di caratterizzare le principali biorisorse microbiche di una produzione artigianale di kombucha, valutandone sicurezza e proprietà tecnologiche, con l'obiettivo di candidarle per l'inclusione nella lista QPS dell'EFSA, promuovendone un uso intenzionale per una produzione più controllata, sicura e di qualità per la kombucha.

1. State-of-the-Art

In recent years, functional beverages have become one of the fastest growing sectors in the food industry. The widespread availability of artificially sweetened soft drinks and their well-documented link to obesity have intensified the demand for healthier alternatives. Within this context, kombucha has gained considerable attention, as it is characterized by a variety of bioactive compounds associated with antioxidant antimicrobial properties as well as potential health benefits (Kim *et al.,* 2025). The global market size is projected to reach $10.45 billion by 2027, highlighting its significant commercial potential (Kim & Adhikari *et al.,* 2020). From a microbiological point of view, kombucha hosts a complex synergistic microbial community, typically dominated by Acetic Acid Bacteria (AAB, e.g., *Komagataeibacter* spp*., Acetobacter* spp*., Gluconobacter* spp.), Lactic Acid Bacteria (LAB, e.g., *Liquorilactobacillus* spp., and *Oenococcus* spp.) and yeasts (*Brettanomyces* spp*., Dekkera* spp., *Hanseniaspora* spp*., Saccharomyces* spp., *Zygosaccharomyces* spp.) (Wang *et al*., 2022). Microbial composition varies according to geographical origin, substrate, fermentation parameters, and environmental conditions. These microorganisms interact throughout different fermentation stages, releasing several metabolites to shape the chemical and sensorial profile of the final product: for example, yeasts convert sucrose into monosaccharide components through the activity of the invertase enzyme and produce ethanol, which is then oxidized by AAB (*Komagataeibacter xylinus*, *Komagataeibacter saccharivorans*) to produce acetic acid with the concomitant formation of cellulose (essential for SCOBY structure). On the other hand, LAB (i.e. *Liquorilactobacillus nagelii*) enhance short-chain fatty acids production, reduces acetic acid, and improves flavor through alcohol and ester formation (Nguyen & Nguyen, 2024). Moreover, researchers have also investigated the potential of probiotic strains, such as *Kluyveromyces marxianus* B0399, to create probiotic-rich kombucha with improved health benefits (Fensterseifer *et al*., 2023).
Even though kombucha is a promising product, the use of traditional SCOBY as a starter culture may lead to uncontrolled microbial diversity. To address this issue, current research is focusing on the selection of dominant, safe, and well-characterized microbial strains to ensure consistent fermentation. The identification of specific microbial bioresources not only refines and optimizes the fermentation process but also improves the reproducibility, as in other fermented products. This approach also mitigates the risk of contamination, making kombucha more suitable for industrial-scale production.
Remarkably, from a regulatory perspective, the use of pitched communities to be deliberately added for kombucha production can be limited as very few species associated with it are currently included in the Qualified Presumption of Safety (QPS) list of the European Food Safety Authority (EFSA). To overcome this issue, the aim of the present project is setting up a collection of the main microbial players of an artisanal kombucha manufactured at the facility of the partner company in Verona, Italy and their comprehensive characterization in terms of pro-technological

features and safety, a prerequisite for their consideration under the EFSA QPS framework. This body of knowledge is crucial to support the deliberate inclusion of these microorganisms to produce safer, higher-quality kombucha beverages.

2. Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1.

A1) **Bioresource collection** step is related to isolation, dereplication and unequivocal identification of main microbial players associated to kombucha manufactured in facility in Verona, Italy. The goal is to set up curated collection of bioresources as a platform for the assessment of their applicability.

A2) **Safety Assessment** includes i) extensive literature search to assess history of use, ecology and degree of exposure to humans and animals in food and feed of isolated species; ii) a combination of genome sequencing as well as *in vitro* and *in vivo* analyses of the candidate strains, to exclude strains with deleterious traits. The goal is the selection of a subset of safe strains to be used for kombucha production.

A3) **Characterization of protechnological features:** once safety is ensured, selected strains are characterized based on their destination of use in kombucha, such as starter cultures. This step includes microfermentation trials, including also assessment of fermentation stability (in collaboration with UNICAEN) and analysis of biotechnological activities, i.e., production of antimicrobial compounds.

A4) **Writing and Editing** includes drafting of the PhD thesis, scientific papers and oral and/or poster communications, further a notification study of the applicability of the most promising and safe bioresources to EFSA is also expected.

Table 1: Timeline for this PhD project

	Activity / Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Bioresource Collection***																								
	1) Literature review																								
	3) Sampling																								
	4) Microbial Isolation and Identification																								
A2)	***Safety Assessment***																								
	1) Extensive Literature Research																								
	2) *In vitro* analysis																								
	3) Genome sequencing and analysis																								
A3)	***Characterization***																								
	1) Protechnological characterization																								
	2) Micro-fermentation trials																								
A4)	***Common Activities***																								
	1) Thesis and Paper writing																								
	2) EFSA dossier drafting																								

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Parthenocarpy for enhanced food quality in eggplant: An approach to breeding seedless fruits (P-I-44)

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Giugno 2025

Parthenocarpy, the production of seedless fruits, enhances fruit quality, texture, flavor, and shelf life. This project aims to identify genes associated with parthenocarpy in eggplant (*Solanum melongena* L.) using existing progenies. We will employ BSA-SEQ and RNAseq for gene identification and validation. Understanding the genetic basis of parthenocarpy will enable Marker-Assisted Selection (MAS) for breeding new seedless eggplant varieties, improving food quality.

Partenocarpia per migliorare la qualità alimentare della melanzana: Un approccio alla riproduzione di frutti senza semi

La partenocarpia, ovvero la produzione di frutti senza semi, migliora la qualità, la consistenza, il sapore e la conservabilità dei frutti. Questo progetto mira a identificare i geni associati alla partenocarpia nella melanzana (*Solanum melongena* L.) utilizzando le progenie esistenti. Saranno adottate analisi BSA-SEQ e RNAseq per l'identificazione e la validazione dei geni. La comprensione delle basi genetiche della partenocarpia consentirà la selezione assistita da marcatori (MAS) per la selezione di nuove varietà di melanzane senza semi, migliorando la qualità degli alimenti.

1. State-of-the-Art

Recent studies have shown that parthenocarpy is not a character sought solely for consumer liking (Rahat et al.; 2022); rather, it is capable of improving fruit yields, as seedlessness allows for fewer seed cavities in the mesocarp (Liu et al.; 2018). Surprisingly, fruit quality also showed benefits. For example, browning and bitterness caused by seeds in some crops, including eggplant, are avoided altogether. In tomato, the hfs (*high fruit set under stress*) mutants showed better organoleptic properties, with corrections on acidity and sugar content in the berry compared with the corresponding wild type (Meco et al., 2019), while in sweet chili pepper showed a greater pungency than pollinated fruits (Kondo et al., 2021). Finally, parthenocarpic cucumber fruit showed better shelf life, due to increased fruit hardness, an important characteristic for storage (Qian et al., 2018). Although biotechnology has made it possible to identify some key transcription factors associated with parthenocarpy (Subbaraya et al., 2020), parthenocarpy still appears to be a complex trait to elucidate, being polygenic and often associated with abiotic stresses.

2. PhD Thesis Objectives and Milestones

The experimental design consists of five **Working Packages (WP)**, organized in **Tasks (T)**, described in detail below (**Figure 1**).

***WP 1* - Plant material & sampling:** Starting from a parthenocarpic (P) and a normal (NP) line, F1 and F2 populations will be generated (T1.1). F2's phenotypic variability enables QTL mapping to detect markers and mutations linked to parthenocarpy. For BSA-seq (T1.2), two DNA bulks (~15–20 individuals each) will be formed from parthenocarpic and normal plants.

***WP 2* - DNA sequencing of two contrasting bulks:** DNA will be extracted from parents and bulk individuals using plant-specific kits, then quantified with a Qubit 2.0 fluorometer to prepare libraries with equal pooled DNA per bulk (T2.1). Whole-genome sequencing of bulks and parents will be performed via Illumina (2×150 PE) at ~100× depth (~5–10× per individual). Though low for rare variants, this depth suffices to detect key group differences and is supported by previous studies balancing diversity detection and cost (T2.2; Clevenger et al., 2018, Huang et al., 2018).

***WP 3* - BSA-seq analysis and candidate gene analysis:** Raw reads from bulks will be cleaned with *fastp*, mapped to the reference genome using *BWA-mem2*, and variants (SNPs/INDELs) called with *GATK* (T3.1). *QTLseqR* (T3.2; Mansfeld et al., 2018) will calculate SNP-indexes, with deltaSNP and G' statistics highlighting significant QTLs, visualized genome-wide. Candidate regions will be analyzed with *SnpEff* (T3.3; Cingolani et al., 2012) to predict variant impacts, ranked by effect on gene function. Functional annotation and literature comparison of candidate genes will be performed; if no matches exist, tools like *InterProScan*, *Pfam*, or *BLAST* will assist functional prediction and conservation analysis (T3.4).

***WP 4* - RNA-seq and validation:** RNA-seq will validate if SNPs/indels affect gene expression (T4.1). RNA from leaves (3 replicates per bulk) will be extracted, sequenced (*Illumina*), cleaned (*fastp*), mapped (*bowtie2*), and

analyzed with DESeq2 to identify differentially expressed genes (DEGs). Enrichment analysis will determine if DEG expression matches phenotypes and if variants affect splicing, producing alternative isoforms. Key RNA-seq results will be confirmed by RT-qPCR to ensure robust expression changes.

***WP 5* - Data dissemination:** As results emerge, findings on parthenocarpy in eggplant will be shared to promote collaboration and scientific engagement. Results will be presented at scientific conferences (T5.1) and findings will also be published in peer-reviewed journals (T5.2).

Figure 1 - *Gantt chart of the projec*t.

WP	Task	Activity name	TIMESCALE (QUARTERS)												
			0	3	6	9	12	15	18	21	24	27	30	33	36
0		**Experimental scheme**													
1		**Plant material & sampling**													
	1.1	F1/F2 Progeny													
	1.2	Phenotypic analysis													
2		**DNA sequencing of two contrasting bulks**													
	2.1	DNA extraction, quantification, library preparation													
	2.2	Illumina sequencing													
3		**BSA-seq analysis & Candidate gene analysis**													
	3.1	Reads cleaning, mapping & variant calling													
	3.3	BSA analysis & deltaSNP/G'													
	3.5	Genetic variants effects													
	3.6	Candidate gene lists annotation													
4		**RNA-seq and validation**													
	4.1	RNA extraction & data preparation													
	4.3	DEGs identification & Enrichment analysis													
	4.4	RT-qPCR validation													
5		**Data Dissemination**													
	5.1	Congresses & conferences													
	5.2	Writing for pubblication													

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Tackling Sustainability and Quality in Beef Production: a Consumer-Driven Approach (P-I-45)

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This PhD project aims to develop an integrated approach to characterize and promote intrinsic and extrinsic quality attributes of beef throughout the entire supply chain. The research emphasizes aspects of product quality and sustainability, alongside consumer behavior, marketing strategies, and innovation. A key objective is to design a targeted marketing mix that supports effective product differentiation, systems sustainability and consumers' segmentation.

Affrontare la sostenibilità e la qualità nella produzione di carne bovina: un approccio orientato dal consumatore

Questo progetto di dottorato mira a sviluppare un approccio integrato per caratterizzare e valorizzare gli attributi di qualità intrinseca ed estrinseca della carne bovina, lungo l'intera filiera. La ricerca si focalizza sugli aspetti legati alla qualità del prodotto e alla sostenibilità, insieme allo studio del comportamento dei consumatori, delle strategie di marketing e dell'innovazione. Un obiettivo centrale è la progettazione di un marketing-mix mirato, capace di supportare una differenziazione efficace del prodotto, la sostenibilità dei sistemi produttivi e la segmentazione dei consumatori.

1. State-of-the-Art

Beef production plays a critical role in global food systems, contributing to human nutrition, rural economies, and cultural heritage. Nutritionally, beef is a rich source of high-quality proteins, essential amino acids, vitamin B12, and key minerals such as iron and zinc. However, in recent decades, beef consumption, especially in high-income countries, has faced increasing criticism due to concerns about environmental sustainability, animal welfare, and health implications (Wood, 2017; Henchion et al., 2014).

In response to these pressures, the beef industry and researchers have made notable efforts to align production with sustainability goals. Advances in livestock management, circular economy practices and environmentally conscious processing techniques have helped reduce the sector's footprint (Vitali et al., 2018). Simultaneously, improvements in packaging and cold chain logistics have enhanced product quality and shelf life.

Despite these advances, the added value of sustainably and ethically produced beef is often poorly recognized by consumers. As a result, high-quality products are frequently forced to compete on price with undifferentiated or lower-standard alternatives, undermining producers' profitability and disincentivizing further innovation (Henchion et al., 2017). Disconnection between production efforts and market reward highlights a fundamental challenge for the sector.

Moreover, beef quality itself is multifaceted, encompassing both intrinsic (e.g., tenderness, color, nutritional profile) and extrinsic attributes (e.g., origin, animal welfare, environmental impact). While consumers increasingly value these dimensions, they do so in highly variable ways. Evidence suggests that purchasing decisions are not only influenced by product characteristics but also by personal values, lifestyle, and trust in labelling and certification systems.

In Italy, these dynamics are particularly relevant. Although consumer awareness about sustainability and food quality is growing (ISMEA, 2024), product differentiation in the marketplace remains weak. Certified, locally sourced, or animal welfare-oriented products often fail to command adequate recognition or premium pricing, due in part to fragmented communication strategies and limited consumer segmentation.

In this context, there is a clear need for integrative research that bridges production practices, consumer expectations, and market strategies. A deeper understanding of how different consumer groups perceive and prioritize beef quality can provide more effective product positioning and value communication. This calls for a multidisciplinary approach - one that combines food science, marketing, and behavioral research - to support the transition toward a more sustainable and consumer-driven beef sector.

In summary, this PhD project aims to support the beef sector in enhancing product value through the characterization of quality attributes and the development of consumer-driven marketing strategies.

2. PhD Thesis Objectives and Milestones

This PhD project is structured into six main activities aimed at supporting the sustainable differentiation of beef products through quality characterization, consumer insight, and strategic marketing.

A1) Analysis of Commercial Products and Labelling Strategies

This activity examines beef products currently available on the Italian market, focusing on how quality, origin, and sustainability attributes are communicated through labels and claims. The goal is to assess existing valorization strategies and identify opportunities for improvement.

A2) Consumer Segmentation Based on Preferences

This phase explores how consumer preferences, attitudes, and personal values influence the perception of beef quality. A value-based segmentation approach will be applied to identify distinct consumer profiles and inform targeted communication strategies.

A3) Characterization of Intrinsic Quality Attributes in Relation to Farming and Processing Systems

This activity evaluates safety, nutritional and sensorial traits of beef - such as biogenic amines, vitamin B12 and zinc content, tenderness, color, pH, drip loss, and cooking loss - across different livestock and processing systems, to understand how production choices affect intrinsic quality.

A4) Characterization of Extrinsic Quality Attributes and Sustainability Indicators

Focusing on environmental and socio-economic aspects, this activity applies Life Cycle Assessment and other methodologies to compare farming and processing systems in terms of sustainability, animal welfare, and territorial value.

A5) Co-Design of Targeted Marketing Strategies

Based on previous findings, this activity involves the development of consumer-oriented marketing strategies to enhance the visibility and perceived value of sustainable beef. Strategies will be co-designed with the industrial partner to ensure practical relevance.

A6) PhD Thesis Writing and Dissemination

The final phase includes the writing of the doctoral thesis and the dissemination of results through publications and presentations, ensuring the transfer of knowledge to both academic and applied contexts.

Table 1 - Gantt diagram for this PhD thesis project.

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Beef products labels analysis***																								
	1) Data collection																								
	2) Labels content analysis																								
A2)	***Consumers segmentation***																								
	1) Beef quality claims selection																								
	2) Cluster identification and profiling																								
A3)	***Intrinsic quality characterization***																								
	1) Analytical methods optimization																								
	2) Beef cuts analysis																								
A4)	***Extrinsic quality characterization***																								
	1) System and inventory definition																								
	2) Data gathering and elaboration																								
A5)	***Marketing mix co-design***																								
A6)	***Thesis and Paper Preparation***																								

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Application of pre-labelled ACF filters for micropollutants extraction in foods and beverages (P-I-46)

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This PhD research project aims to investigate the application of activated carbon fibers (ACFs) as a valuable analytical tool for the extraction and detection of chemical contaminants in the food chain. The adsorption properties of ACFs will be evaluated on a variety of food and beverage samples, with the goal of controlling, monitoring, and removing micropollutants. This could contribute to ensuring greater safety and quality food products.

Applicazione di filtri ACF premarcati per l'estrazione di microinquinanti in alimenti e bevande

Questo progetto di ricerca mira a studiare l'applicazione delle fibre di carbone attivo (ACF) come valido strumento analitico per l'estrazione e il rilevamento di contaminanti chimici nella catena alimentare.
Le proprietà di adsorbimento delle ACFs saranno valutate su una varietà di campioni di alimenti e bevande, con l'obiettivo di controllare, monitorare e rimuovere i microinquinanti. Ciò contribuirebbe a garantire una maggiore sicurezza e qualità dei prodotti alimentari.

1. State-of-the-Art

Adsorption has emerged as a promising and versatile approach for the removal and mitigation of micropollutants from the environment, and consequently, from food sources. The selection of adsorbents is based on factors such as chemical composition, multifunctional groups, surface area, porosity, structural stability, and surface morphology (Bensalah *et al.*, 2024). Activated carbon fibers (ACFs) are particularly effective in adsorption applications due to their excellent volumetric capacity, high packing density, large specific surface area with uniform micropore distribution, significant adsorption capacity, rapid sorption/desorption rates, enhanced design flexibility, and ease of regeneration. They are typically produced from carbon-rich synthetic polymers, including rayon, polyacrylonitrile (PAN), pitch, or phenolic resins through carbonization followed by activation (Beltrame *et al.*, 2018). However, to promote the use of renewable resources and improve environmental sustainability, various alternative precursors such as hemp, bamboo, wood, seaweed, cellulose, or biomass sources have also been employed. These materials contribute to reducing greenhouse gas emissions associated with conventional disposal methods. The surface properties of ACFs can be further enhanced by introducing functional groups, which act as reactive sites and improve adsorption efficiency, particularly for organic and hydrophobic compounds (Joo *et al.*, 2024). The adsorption process generally involves four main steps: *i)* transport through the bulk solution, *ii)* diffusion across the boundary layer, *iii)* diffusion within the pores, and *iv)* diffusion into the adsorbent particles. The interaction between the adsorbate and ACFs depends on several factors, including the characteristics of both the adsorbate and the adsorbent, as well as the environmental conditions. Adsorption isotherms are used to evaluate the mechanism of adsorption by which adsorbate molecules interact with the adsorbent (Jaber *et al.*, 2024). These models provide the maximum adsorption capacity of adsorbent. For example, Langmuir and Freundlich isotherm models describe monolayer or multilayer adsorption, respectively. Other commonly applied models include the Temkin, Redlich–Peterson, and Dubinin–Radushkevich isotherms. Conversely, adsorption kinetics describe the rate at which a solute is adsorbed and provide insight into the mechanisms that influence how long the adsorbate remains at the solution–adsorbent interface. Several models are commonly used to analyse such data, include the pseudo-first order and pseudo-second-order models, the intraparticle diffusion model, and the Elovich model (Sharma *et al.*, 2022). The validity of using the ACFs as SPE medium for polychlorinated dibenzo-p-dioxins (PCDDs), di- benzofurans (PCDFs), and polychlorinated biphenyls (PCBs) from water and air samples has already been demonstrated (Cerasa *et al.*, 2020). Considering the adsorption capacity of ACFs as sampling and analytical medium for the determination of chemical contaminants from environmental matrices, the same material may also prove to be a valid alternative for the analysis of chemical contaminants in food matrices. Therefore, this PhD research project will focus on the study of adsorptive properties of ACFs for selected classes of compounds (*i.e.*, PAEs, PAHs, and PFASs) which will be tested on food and beverage matrices. Several extraction and analytical procedures will be evaluated to identify the most suitable protocol for the determination of the compounds of interest. Furthermore, all the processes governing the spontaneity and nature of the adsorption reaction will be investigated through the development of the specific models above cited.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Bibliography research on ACFs as adsorbents** focusing on manufacturing processes and raw materials (A1.1), characterization of ACFs (A1.2), interaction with micropollutants (A1.3), adsorption efficiency (A1.4).
A2) **Chemical and physical characterization of ACFs** to measure specific surface area (SSA) and pore size distribution (A2.1; A2.2).
A3) **Study and comparison of the most common extraction and analytical methods** using ACFs as adsorbent media for the separation and quantification of selected classes of compounds.
A4) **Study of adsorption capacity, kinetics, and thermodynamic processes** by means of isotherm models (*e.g.*, Langmuir, Freundlich, Temkin, Redlich–Peterson, and Dubinin–Radushkevich) (A4.1), kinetic models (*e.g.*, pseudo-first order and pseudo-second-order models, the intraparticle diffusion model, and the Elovich model) (A4.2), and thermodynamic parameters (*e.g.*, ΔS^0, ΔH^0, ΔG^0) (A4.3).
A5) **Application of optimized protocols to real samples**, with a particular focus on adsorption tests using ACFs for the assessment of contaminants in food and beverages matrices.
A6) **Writing and Editing** of scientific papers, abstracts, oral and/or poster communications.

Table 2 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Bibliography research on ACFs adsorbent***																								
	1) Manufacturing processes and raw materials																								
	2) Characterization of ACFs																								
	3) Interaction ACFs – micropollutants																								
	4) Adsorption efficiency of ACFs																								
A2)	***Chemical and physical characterization of ACFs***																								
	1) Specific surface area																								
	2) Pore size distribution																								
A3)	***Study and comparison of the most common extraction and analytical methods***																								
A4)	***Study of adsorption capacity, kinetics, and thermodynamic processes***																								
	1) Isotherm models																								
	2) Kinetic models																								
	3) Thermodynamic parameters																								
A5)	***Application of optimized protocols to real samples***																								
A6)	***Paper, abstract and poster preparation***																								

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Roasting Effects on the Nutritional and Flavor-Related Biochemical Composition of *Pistacia vera* L. (P-I-47)

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I'm Giacomo Pedron, a first-year PhD student in Drug Innovation at the Università del Piemonte Orientale, specializing in Food Chemistry.
I was awarded the Best Poster Presentation at the recent SISTAL 2025 Congress in Bologna for my poster titled:" Examining the Alterations in Lipid, Protein, and Flavour Profiles During Pistachio Paste Processing"
My current research topic explores the effects of roasting on nut-based products, with a particular focus on quantifying changes in volatile compounds, natural pigments like chlorophylls and anthocyanins, and other significant phytochemicals.

1. State-of-the-Art

The consumption of pistachios (Pistacia vera L.) has increased globally in recent years due to their nutritional and health benefits, which are attributed to beneficial phytochemical compounds, high levels of polyunsaturated fatty acids, and low carbohydrate content. Pistachios are consumed in various forms, including raw, roasted, and as a cream (Mateos et al., 2022).
The roasting of nuts is a thermal process that aims to improve the sensory attributes of nuts through a series of physical and chemical reactions, including the Maillard reaction. This process can lead to alterations in various compounds, such as fatty acids, peptides, and vitamins. Significant changes that occur during nut roasting include starch gelatinization and protein denaturation. Moreover, the roasting process can extend the shelf life of nuts by inactivating the oxidative enzyme system and reducing the water activity.
However, thermal processing can trigger the formation of thermal pollutants, such as acrylamide, and the degradation of certain phytochemical compounds. During the roasting process, Maillard reactions are responsible for the formation of substances that impart desirable roasted aromas and flavours, such as pyrazines, furans, and pyrroles. These reactions occur between the carbonyl groups of reducing sugars and the amino groups of proteins. Additionally, the loss of moisture during roasting contributes to the development of a crunchy mouth feel, characterized by hardness, fracture force, and firmness. For these reasons, the evaluation of several industrial process parameters with innovative assays has become crucial to guarantee the preservation of nutritional properties, taste, color, flavor, and several other characteristics (Pedron et al., 2025).

2. PhD Thesis Objectives and Milestones

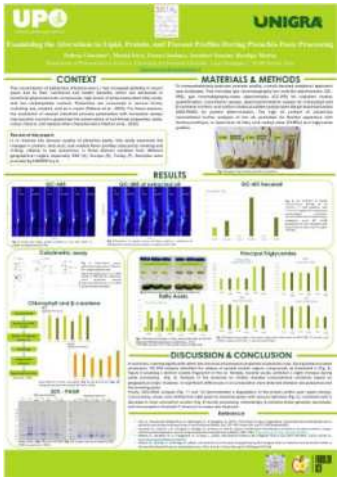
1° years:
Investigation of different aroma and flavours compounds in different raw and roasted *Pistacia vera* L. nuts whit GC. Explore different conditions of roasting process: time, temperature and recipe.

In this phase of the project, I will employ the following analytic methodology: gas chromatography -mass spectrometry or ion mobility spectrometry (GC-MS and GC-IMS). Also combined whit other analytical techniques such as SDS-PAGE, spectrophotometer assay, colorimetric evaluation, HPLC Uv-Vis for quantification of (poly)phenol and carbohydrates, FT-IR spectroscopy or other biochemical methods.

For the roasting process, I will conduct my experiments at UNIGRA' s.p.a. company, while data collection will be performed at the University of Piemonte Orientale.

During first period I published my first scientific review:

- Pedron, G., Jaouhari, Y., & Bordiga, M. (2025). Conventional and Innovative Drying/Roasting Technologies: Effect on Bioactive and Sensorial Profiles in Nuts and Nut-Based Products. Applied Sciences, 15(3), Article 3. https://doi.org/10.3390/app15031258.



Additionally, another study focusing on walnut and flaxseed oil extraction is currently awaiting publication.

Award:
During the recent SISTAL 2025 Congress in Bologna, I was recognized with the 'Best Poster Award' for my presentation: 'Examining the Alterations in Lipid, Protein, and Flavor Profiles During Pistachio Processing".

2° years:
Monitoring the formation of different thermal contaminants during the roasting process, while simultaneously monitoring the formation of VOCs and studying their correlation.
Focus on the identification of specific VOCs as biomarkers for thermal contaminants formation such as pyrazines or another oxidation marker such as hexanal. In this part of my study, I will explore deeply science of sensory science.

My forthcoming research abroad will encompass a multi-faceted investigation into key chemical changes influencing product quality. Specifically, I will:

- Elucidate the role of free amino acids and small peptides and their interaction with sugars in the development of desirable flavor and aroma compounds during the roasting process.
- Conduct an in-depth analysis of lipid oxidation and its contribution to the formation of off flavors.
- Investigate the degradation kinetics of chlorophyll during both the roasting and subsequent paste-making stages, recognizing its critical impact on the final product's characteristic green color.

In order to complete my studies, I am willing to visit several foreign universities, including those outside of Europe.

3° years:
The concluding year of my doctoral research will involve completing my international placement and finalizing data acquisition at UNIGRA S.p.A. My focus will then shift to thesis completion and the dissemination of research findings through scientific publications

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Strategies for Reducing Emissions and Enhancing Sustainability in Livestock Systems (P-I-48)

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Tutors: Prof. Paolo Cornale, Prof. Davide Biagini

This PhD thesis project aims to assess the environmental impact of livestock systems and explore strategies to mitigate it. Data from Piemontese cattle farms will be collected and analysed to develop a software tool enabling farmers to self-assess their farm's sustainability. Subsequently, *in vitro* and *in vivo* trials on dairy cows will be carried out to evaluate the inclusion of agro-industrial by-products (i.e. hazelnut skin and cocoa bean shell) in their diet to increase supply chain circularity and reduce enteric methane emissions.

Strategie per la riduzione delle emissioni e il miglioramento della sostenibilità dei sistemi zootecnici

Questo progetto di tesi di dottorato mira a valutare l'impatto ambientale del settore zootecnico, esplorando metodologie per ridurne l'entità. Verranno raccolti e analizzati dati da allevamenti di bovini di razza Piemontese, con l'obiettivo di sviluppare un software che consenta agli allevatori un'autovalutazione della sostenibilità aziendale. Successivamente, saranno condotti test *in vitro* e *in vivo* su bovine da latte per valutare l'utilizzo di sottoprodotti nella loro alimentazione (ad esempio la cuticola di nocciola e il guscio della fava di cacao) con l'obiettivo di aumentare la circolarità della filiera e ridurre le emissioni di metano enterico.

1. State-of-the-Art

Animal-derived products play a fundamental role in human nutrition. Livestock production accounts for 40% of global agricultural gross domestic product (GDP) and provides approximately 33% of the world's protein supply and 17% of total calorie intake. It also represents a crucial source of livelihood for rural communities, especially in developing countries, where livestock is a cornerstone of food security.

The global population growth and rising average income are expected to increase the demand for animal-based products. However, livestock production is responsible for approximately 14.5% of anthropogenic greenhouse gas (GHG) emissions (Cheng et al., 2022). Given the growing public concern about environmental sustainability, increasing productivity within the livestock sector must be coupled with the implementation of more sustainable practices. A fundamental step in this process is the assessment of the sustainability of current livestock systems, acknowledging their complexity and the necessity for interdisciplinary approaches.

Several methodologies can be applied to evaluate environmental impacts, such as mass balance models and Life Cycle Assessment (LCA). Carbon and water balance models, for example, are relatively simple to implement and allow farmers to assess environmental performance using easily accessible data, while supporting informed management decisions to reduce environmental impact (Leback et al., 2013).

From a nutritional standpoint, agro-industrial by-products represent a valuable resource within the circular economy framework, helping reduce feed-food competition and environmental impact. Their use may also lower enteric methane production, a gas with a global warming potential far higher than CO_2 (Salami et al., 2019). Hazelnut processing (1.1 million tons in 2023; FAOSTAT, 2025) and cocoa bean processing (5.6 million tons in 2023; FAOSTAT, 2025) generate residues, such as hazelnut internal skin and cocoa bean shell, which have been studied in terms of meat and milk quality (Afedzi et al., 2023; Ollani et al., 2024). However, few studies have investigated their individual effects on ruminal methanogenesis, and no data exist on the potential synergistic impact of their combined use.

This thesis project aims to assess the environmental impact of different farming systems involving Piemontese cattle, Italy's most widespread beef breed. The goal is to develop user-friendly software for farmers to evaluate the environmental sustainability of their management practices. Furthermore, the project will investigate the use of hazelnut fruit internal skin and cocoa bean shell as dietary components to reduce enteric methane emissions—through *in vitro* trials and *in vivo* measurements on dairy cows using the GreenFeed system. The research adopts an interdisciplinary approach, examining animal metabolism, digestion, health, and welfare. Additionally, it will assess the effects of these diets on dairy products (spray-dried milk, butter), considering nutritional, technological, organoleptic, and shelf-life characteristics.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Data collection and processing** of Piemontese cattle farms by direct visit (A1.1). Processing involves the development of algorithms for the determination of secondary data (A1.2).

A2) **Preliminary *in vitro* tests** to establish the optimum doses of by-product inclusion in the diet of cows for conducting the *in vivo* trial. The proximate composition of all feeds used in the ration will be defined (A2.1), together with fermentation tests to define digestibility and gas production, including methane and ammonia (A2.2).

A3) **Software development** for calculating the environmental impact through carbon balance, which can be used directly by farmers and other stakeholders (A3.1). Subsequently, the software will be implemented to calculate the water balance (A3.2).

A4) ***In vivo* tests** in which blood, faeces, gaseous emissions, milk, and derived products will be sampled and analyzed (A4.1). This will be followed by a detailed exploitation of the data obtained (A4.2).

A5) **Writing and editing** the PhD thesis, scientific papers, and oral and/or poster communications.

Table 1 Gantt diagram is divided into 36 months (3-year PhD) for this PhD thesis project. The yellow color indicates the current month at the time of the workshop, where the overlapping areas between yellow and the two shades of grey are colored green.

	Activity / Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
A1)	***On-farm data collection and processing***																																				
	1) On-farm visits																																				
	2) Data processing																																				
A2)	***In vitro analysis***																																				
	1) Proximate composition																																				
	2) Gas production and digestibility																																				
A3)	***Software developing***																																				
	1) Carbonic balance																																				
	2) Water balance																																				
A4)	***In vivo trial***																																				
	1) Sampling and analysis																																				
	2) Data processing																																				
A5)	***Writing and sharing***																																				

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Extraction and characterisation of biomolecules from insects reared on food by-products for food and feed applications (P-I-49)

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Tutor: Prof. Augusta Caligiani

This PhD project aims to extract and characterize biomolecules from insects reared on substrates derived from agri-food by-products, with the goal of recovering macronutrients and functional compounds of interest for the food, feed, chemical, and pharmaceutical sectors. Expected outcomes include the broadening of the range of organic waste streams suitable for insect bioconversion, enhanced substrate digestibility, and a deeper understanding of the role of both endogenous and exogenous enzymes in insect-based applications.

Estrazione e caratterizzazione di biomolecole da insetti allevati su scarti alimentari per applicazioni alimentari e mangimistiche

Questo progetto di dottorato mira a estrarre e caratterizzare biomolecole da insetti allevati su substrati derivati da sottoprodotti agroalimentari, con l'obiettivo di recuperare macronutrienti e composti funzionali di interesse per i settori alimentare, zootecnico, chimico e farmaceutico. I risultati attesi includono l'ampliamento della gamma di scarti organici idonei alla bioconversione tramite insetti, il miglioramento della digeribilità dei substrati e una comprensione più approfondita del ruolo degli enzimi endogeni ed esogeni nelle applicazioni basate sugli insetti.

1. State-of-the-Art

The reduction of food waste and the valorization of food by-products represent two crucial challenges in the current context of a growing global population and increasing demand for animal-based protein (Oonincx *et al*., 2012). In this scenario, insects emerge as a sustainable and efficient solution, since several insect species have the natural ability to grow on a wide range of organic substrates. However, optimal substrate processing methods and the potential of insects to grow on unconventional substrates, are still poorly explored. One of the main barriers to efficient insect-based bioconversion is the presence of structural components in agro-food by-product, particularly lignocellulosic compounds. Controlled fermentation with selected microorganisms can enhance substrate digestibility through the action of microbial enzymes that hydrolyze fiber into simple sugar, organic acid, and other compounds, which are subsequently metabolized into lipids and proteins by larvae (Raksasat et al., 2020). However, the potential of spontaneous fermentation driven by native microorganisms remains largely underexplored. In parallel, a large fraction of plastic packaging waste remains unrecycled due to heterogeneous material composition and food residue contamination. Expanded plastic packaging materials like expanded polyethylene (EPE) and expanded biopolymers (e.g. PLA and starch) theoretically promotes microbial and enzymatic activity, potentially accelerating biodegradation times, making them compatible with those of household organic waste (Wang et al., 2022). However, further investigation is required to assess whether the presence of plastic hinders substrate utilization by the larvae, as well as to evaluate the potential risk of bioaccumulation of harmful chemical compounds in the insect body. In addition, further research with a strong innovative character on the role of enzymes in insect-based biotechnological applications is expanding. A recent study demonstrated significant lipase activity in insects (Caligiani et al., 2019). However, comprehensive analyses of lipase activity across different insect species and under various killing methods, as well as insect lipase applications, remain largely unexplored. Enzymes can also be proposed as biotechnological pre-treatments to reduce the allergic potential of insect protein, improving the digestibility and the biofunctional properties of insect meal. Chitinase can produce chitooligosaccharides (COSs), which are environmentally friendly and safe for human use, while protease enhance protein extraction, lower allergenic potential, and generate bioactive peptides. In this new emerging research topic, further efforts are needed to understand the possibility of using enzymes to improve the nutritional quality of insect-based products and reduce allergenic potential. This PhD project addresses these new emerging challenges and is conducted within the framework of the BIO R&R PR FESR project, which aims to explore sustainable strategies for valorizing food waste by-products into high-quality and safe insect-derived products.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above, this PhD thesis project will be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Evaluation of the effect of spontaneous and controlled fermentation of substrates on the nutrient composition of insect larvae.** The chemical characterization of fermented rearing substrates, focusing on lipids, proteins and fiber, will provide a comprehensive framework for understanding the effects of fermentation on substrate composition (A1.1). A reduced fiber content in the substrates is expected to enhance larval bioconversion, contributing to an improvement in their nutritional composition (A1.2). Milestone: Scientific paper on the effect of substrate fermentation on larval development and composition.

A2) **Evaluation of the effect of plastic residues in the substrate on the chemical composition of the larvae and the potential bioaccumulation of harmful plastic-derived compounds.** Analysis of the chemical composition of larvae will clearly define whether the presence of plastic in the substrate hinders substrate utilization, altering the larval nutrient profile (A2.1). Identification of harmful plastic-derived compounds in the larval tissues, in order to clarify the presence of potential bioaccumulation risks (A2.2). Milestone: data supporting the potentiality of insects to bio-convert wastes containing packaging residues.

A3) **Evaluation of the biotechnological potential of insect-derived enzymes.** Characterization of lipase activity in selected insect species under different killing methods, in order to compare it with commercial lipases used in the industrial sector (A3.1). Milestone: production of an insect protein extraction with high lipase activity.

A4) **Improvement of insect meals through biotechnological approaches.** Application of commercial enzymes (chitinase and protease) to insect meals, in order to obtain more digestible products with reduced allergenic potential (A4.1). Milestone: production of an insect meal, fully characterized, with improved nutritional properties.

A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table1 Gantt diagram for this PhD thesis project.

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Effect of fermentation on the nutritional composition of larvae***																								
	1) Chemical characterization of fermented substrates																								
	2) Analysis of larval chemical composition in fermented substrates																								
A2)	***Effects of plastic residues***																								
	1) Analysis of the chemical composition of larvae																								
	2) Identification and assessment of harmful plastic-derived compounds																								
A3)	***Insect enzymes biotech potential***																								
	1) Characterization of lipase activity																								
A4)	***Improvement of insect meals***																								
	1) Enzymatic treatment of insect meals																								
A5)	***Thesis and Paper Preparation***																								

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Quality and sustainability of Pasta as social integration tool (P-I-50)

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Tutor: Prof.ssa Maria Martuscelli

This PhD thesis project aims to integrate quality and environmental sustainability aspects of dried pasta production with the social reintegration process of young prisoners. The characteristics of pasta produced with conventional and ancient grains will be analysed and the environmental impact of the production cycle will be assessed by LCA analysis. A qualitative analysis will be carried out through interviews and questionnaires in order to measure and understand the learning and change process of the young inmates.

Qualità e sostenibilità della Pasta come strumento di integrazione sociale

Questo progetto di tesi di dottorato mira ad integrare aspetti qualitativi e di sostenibilità ambientale legati alla produzione di pasta secca con il processo di reinserimento sociale dei giovani detenuti. Verranno valutate le caratteristiche della pasta prodotta con grani convenzionali e grani antichi e l'impatto ambientale del ciclo produttivo mediante analisi LCA. Verrà eseguita un'analisi qualitativa attraverso interviste e questionari al fine di misurare e comprendere il processo di apprendimento e di cambiamento dei giovani detenuti.

1. State-of-the-Art

According to the Ministry of Justice, at the end of April 2025, approximately 600 youths are detained in Juvenile Penal Institutions (IPMs) across Italy, and the number of entrances is increasing. The social reintegration of young prisoners held in these facilities is an issue of vital importance for our society and work could provide them with an increase of dignity and personal fulfilment, contributing significantly to lowering recidivism (1). At the end of their sentence, prisoners struggle to find a job that allows them to be economically independent and without the ability to meet their material needs, they are unable to adopt conventional lifestyles. Confirming this, a recent study (2) states that positive experiences in the field of activity during imprisonment are associated with lower re-offending rates two years after release from prison.
Socio-economic changes in recent years have made consumers more aware and responsible, leading agri-food companies to adopt strategies inspired by sustainability and social responsibility (3). Consumers attribute great importance to the origin and type of raw material used in the production of pasta, recognizing a high economic value to pasta made from ancient varieties of wheat (4). Wheat is the most cultivated plant species in the world and the main basis of most foods. The scientific community considers ancient varieties a valid solution to the climate change problem, while consumers appreciate pasta made with this type of grain because it has cooking qualities and properties comparable to classic semolina pasta but is perceived as "better" (5). Looking at the environmental impact of pasta production, wheat cultivation remains the most impactful phase in the chain, although the use of organically grown ancient varieties has good potential in reduction. For this type of analysis, many researchers have used the LCA method with reference to water consumption, nitrogen input and the comparison of different agronomic practices.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 2:

A1) **Study of the characteristics of ancient grain varieties,** the rheological behavior (1) and sensory analysis of pasta produced (2).
A2) **Evaluation of Environmental impact of pasta process** by LCA analysis.
A3) **Study of young inmates' behavior** by questionaries and interviews.
A4) **Market research** on consumers and their willingness to buy a sustainable product using questionaries.
A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 2 Gantt diagram for this PhD thesis project.

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Ancient grains analysis***																								
	1) Rheological Behaviour																								
	2) Pasta sensory analysis																								
A2)	***LCA analysis***																								
A3)	***Study of young inmates' behaviour***																								
A4)	***Market Research***																								
A5)	***Thesis and Paper Preparation***																								

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Processing and valorisation of agri-food by-products and their use as elicitors in agriculture (P-I-51)

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This project focuses on the valorization of agri-food by-products such as peels, seeds, and pomace, which contain bioactive compounds with antioxidant and elicitor potential. Conventional extraction methods are often inefficient and unsustainable, whereas emerging technologies, like ultrasound-assisted extraction (UAE), pulsed electric fields (PEF), ohmic heating (OH), and high-pressure processing (HPP), offer greener and more effective alternatives. In addition, fermentation further enhances bioactivity and enables the production of natural plant elicitors that support crop defense and reduce the agro-chemical use. However, integrated and scalable strategies remain limited, highlighting the need for innovative approaches.

Trasformazione e valorizzazione dei sottoprodotti agroalimentari e il loro utilizzo come elicitori in agricoltura.

Questo progetto mira a valorizzare i sottoprodotti agroalimentari (bucce, semi, sottoprodotti), ricchi di composti bioattivi, come potenziali elicitori. I metodi convenzionali di estrazione di questi composti bioattivi sono a volte inefficienti e poco sostenibili; per questa ragione si privilegiano tecnologie non termiche e più sostenibili come: ultrasuoni (UAE - ultrasound-assisted extraction, PEF - pulsed electric fields, OH - ohmic heating, e HPP - high-pressure processing). Inoltre, altri processi, come la fermentazione aumenta la bioattività, creando elicitori naturali che rafforzano le difese delle piante, riducendo in questo modo l'utilizzo di agrofarmaci. Mancano però strategie integrate e scalabili e per questo motive è necessario un approccio innovativo.

1. State-of-the-Art

Agri-food by-products like peels, pomace, stems, and leaves are rich in bioactive compounds with antioxidants, antimicrobial, and elicitor properties. Conventional extraction methods are often inefficient and environmentally unfriendly. Green technologies such as UAE, PEF, OH, and HPP could offer yield improvement, reduce the solvent use, and shorten processing times. These have been effectively applied to waste like tomato peels, grape pomace, and olive leaves (Teles et al., 2021).
Microbial fermentation is increasingly recognized for enhancing bioactivity and converting agri-food waste into plant elicitor compounds that trigger plant defense responses. These offer eco-friendly solutions for crop protection and growth, supporting the circular economy and zero-waste goals. However, integrated approaches combining advanced extraction, fermentation, and formulation remain limited. This project addresses that gap by developing a unified method integrating green extraction, fermentation, and delivery systems to produce effective elicitors from various agri-food by-products. The outcome will support sustainable agriculture and advance bioeconomy-driven food system innovations.

Table 1 Potential applications of agri-food waste.

Application Area	*Description*
Biostimulants	Enhanced Plant growth and defence.
Animal Feed	Nutritious supplements for livestock.
Plant Elicitors	Stimulate plant immune responses for better yield.
Cosmetic Industry	Source of natural antioxidants and bioactives.
Pharmaceutical Industries	Used in drug development and nutraceuticals.

PEF is an effective eco-extraction technique for bioactive compounds as it enhances cell disruption and membrane pore formation in agri-food by-products, for example, key components (peonidin 3-O-glucoside and epicatechin) were extracted from avocado seed waste with no degradation (Carpentieri et al., 2023; Faizah et al., 2024), additionally, it improved the recovery of polyphenols and methylxanthines (such as caffeine and theobromine) from coffee silver skin (Barbosa et al., 2018). OH can induce electroporation and disrupt plant cell membranes, which facilitates the release of intracellular compounds. Similarly, HPP extraction techniques also showed promise in recovering valuable compounds from food industry waste like grape pomace, olive pomace, cherry pomace, and spent coffee grounds (Okur et al., 2019; Andreou et al., 2020). Conventional fermentation can harness microbial activity to transform bioactive-rich by-products into powerful elicitors for enhanced plant and crop production. Based on scientific literature and accessibility, promising techniques like UAE, PEF, OH, HPP

and or their combination, along with comparative fermentation processes, will be explored in this Ph.D. work.

2. Ph.D. Thesis Objectives

Table 2 presents the Gantt chart outlining the projected timeline and key milestones of this PhD project, illustrating the planned progression of research activities.

A1) Utilize and analyse agri-food waste, by-products (BP), and plant growth promoters (PGPs): Selection and classification of BP and PGPs (A1.1), assessment of synergistic potential between BP and PGPs (A1.2)

A2) To apply innovative technologies combined with fermentation for efficient elicitor extraction and to characterize their techno-functional and chemical properties through in vitro and vivo tests: Novel technology application (A2.1), scrutinizing of the suitable microorganism for the fermentation, and exploring the synergetic effect of fermentation and novel technologies (A2.3)

A3) Develop a technical protocol for valorization using new processes and fermentation to help companies produce sustainable materials from waste, supporting the circular economy: Assessment of novel technologies applied for optimization (A3.1), standardization of combined technological application (A3.2)

A4) To transform elicitor compounds into value-added applications: Optimized potential of elicitors by assessing in vitro/in vivo study (A4.1), and bioactivity assessment along with delivery system optimized for agricultural elicitors by in vitro/in vivo study (A4.2)

Table 2 Gantt diagram of Ph.D. thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Selection of BP and PGPs***																								
	1) Selection of BP and PGPs																								
	2) Synergetic potential																								
A2)	***Valorization stage***																								
	1) Novel technologies : UAE PEF, OH, HPP																								
	2) Selection of microbes for fermentation																								
	3) Innovative extraction + Fermentation																								
A3)	***Process parameter setup***																								
	Novel technique assessment																								
	Integration with the fermentation technique																								
A4)	***Elicitor analysis from agri-food waste***																								
	1) Elicitor analysis (in vitro/in vivo)																								
	2) Bioactivity assessment																								
A5)	***Ph.D. Thesis and Paper Preparation***																								

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Valorisation of agri-food by-products through innovative technologies: circular economy in the brewing supply chain (P-I-52)

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The main objective of the Ph.D. project is to design and evaluate innovative solutions for the recovery and valorisation of by-products from the brewing supply chain, through a dual approach: to utilize brewing by-products for the development of new food and beverages intended for human consumption, using emerging processing technologies and to recover by-products from other agri-food sectors and functionally reintegrate them into the brewing supply chain.

Valorizzazione di sottoprodotti agroalimentari attraverso l'uso di tecnologie innovative: economia circolare nella filiera brassicola

L'obiettivo principale del progetto di dottorato è progettare e valutare soluzioni innovative per il recupero e la valorizzazione dei sottoprodotti della filiera brassicola, attraverso un duplice approccio: utilizzare i sottoprodotti derivanti dalla filiera brassicola per lo sviluppo di nuovi alimenti e bevande destinati al consumo umano, impiegando tecnologie innovative, e recuperare sottoprodotti provenienti da altri settori agroalimentari per reintegrarli in modo funzionale nella filiera brassicola.

1. State-of-the-Art

The impact of climate change, the inefficient use of natural resources, and the exponential growth of the global population, projected by the United Nations to reach 9.8 billion by 2050, represent some of the most pressing challenges we are expected to face in the near future. Addressing these issues requires the optimized management of natural resources, including the valorization of high-value by-products/waste and a significant reduction in food loss and waste (Berenguer et al., 2022). In the agrifood sector, given the 1.86 billion hectoliters of beer produced globally in 2021, a figure that continues to grow, optimizing waste from the production processes of the world's most consumed alcoholic beverage is important. The brewing industry is a global business involving major multinational corporations, primarily based in China, the United States, and Brazil, alongside thousands of smaller breweries, craft brewers, and brewpubs worldwide (Carvalho et al., 2023). The brewing supply chain encompasses the entire beer production process, which includes several processing steps, each generating different types of by-products such as rootlets, spent grain, spent yeast and trub. Today, to reduce waste from beer production and enhance environmental sustainability, these by-products are being increasingly valued in various ways. Most of the current valorisation strategies do not involve the production of food or beverages intended for human consumption but rather focus on the generation of animal feed and biofuels. This approach, driven by economic considerations and the lack of industrial developments enabling large-scale use of these by-products in human nutrition, raises ethical concerns about the diversion of food-grade resources for energy production, potentially subtracting food from the human supply chain. It is therefore essential to explore how these by-products can be more sustainably utilized. Although the greatest environmental impact in the beer life cycle is associated with the production and manufacturing of packaging materials, as well as the cultivation and transportation of cereals, the brewing process itself still contributes to a non-negligible environmental burden (Hospido et al., 2005). While considerable scientific, technological, and industrial efforts have been dedicated to mitigating the most impactful environmental aspects of the brewing supply chain, relatively little research has focused on the study and potential food applications of brewing by-products, contributing to a notable scientific gap in this area. In parallel with the valorization of brewing-derived by-products, an equally promising approach involves the recovery of nutrient-rich residues from other food processing chains and their functional reintegration into the brewing supply chain. Redirecting these by-products into the brewing process, for instance, to produce enriched beers, fits perfectly within the framework of the circular economy. In recent years, emerging processing technologies, such as pulsed electric fields (PEF), ohmic heating (OH), and high hydrostatic pressure (HPP), have shown great potential in extracting functional compounds from complex food matrices, enhancing microbiological stability, and enabling sustainable food transformation. The targeted application of these technologies can serve as a bridge between environmental sustainability and technological innovation.

2. PhD Thesis Objectives and Milestones

In order to achieve the main objective with the dual approach mentioned above, the project is structured into the following activities according to the Gantt diagram given in Table 1:

A1) **Identification and Physico-chemical Characterization of By-products.** Selection of at least 3 by-products

from the brewing supply chain and at least 3 by-products from other food production chains based on their functional properties and characterization through the following analyses: moisture content, ash content, total lipids, crude proteins, , soluble and insoluble dietary fiber, (A1.1) (Olivares-Galván et al., 2022), water-holding capacity, swelling capacity, water solubility, emulsifying capacity and stability, foam formation capacity, viscosity, texture, color and particle size distribution (A1.2)

A2) **Application of Innovative Technologies.** Application of PEF to enhance the extraction of bioactive compounds from brewing by-products such as spent grains or barley rootlets, as well as from fruit and vegetable residues, for the formulation of functional beers and beverages. Additionally, PEF can be applied to barley or hop seeds to stimulate germination and improve enzymatic activity, potentially enhancing the quality of malt and fermentable substrates (A2.1). Application of HPP to improve extraction and stabilize complex matrices without thermal degradation, preserving nutritional and sensory qualities. Useful for the preservation of fermented infusions obtained from brewing residues or for the treatment of fruit-based ingredients intended for beer production, such as residual fruit juice or pulp (A2.2). Application of OH to achieve rapid and uniform heating, enabling controlled disintegration of plant cell walls. This improves the release of nutrients and enhances the technological functionality of ingredients derived from brewing by-products or external agri-food residues; an example is the application of ohmic heating to grape must, which improves polyphenol extraction while preserving aroma and microbial stability for use in innovative beer formulations (A2.3). Additionally, an evaluation of the effects arising from the combination of these technologies. (A2.4).

A3) **New Product Development** formulation and optimization of new products (food and beverage) for human consumption (A3.1), shelf-life evaluation through the following analyses: microbial stability, chemical and physico-chemical stability (pH, water activity, lipid oxidation, color, aroma profile, antioxidant activity, residual sugars and alcohol for fermented beverages), structural stability (texture profile, viscosity, phase separation or sedimentation), sensory analyses, (A3.2). A progress check is scheduled at the end of activity A3 and is indicated in the Gantt chart by a diamond symbol.

A4) **Thesis and Paper preparation.** During the Ph.D. at least 3 papers will be published in international peer-reviewed journals. Participation in at least 2 conferences per year is expected.

Table 1 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Identification and Physicochemical Characterization of By-products***																								
	1) Chemical composition		M1																						
	2) Functional properties								M4																
A2)	***Application of Innovative Technologies***																								
	1) PEF treatment			M2																					
	2) HPP treatment																								
	3) Ohmic Heating																								
	4) Combined use of treatments																		M5						
A3)	***New Product Development***																								
	1) Formulation and optimization							M3																	
	2) Shelf-life evaluation																							M6	
A4)	***Thesis and Paper Preparation***																								M7

Milestones: completion of basic composition analyses (M1), optimization of process parameters for PEF treatment (M2), prototype products developed (M3), completion of functional property analyses (M4), combined technology evaluation completed (M5), shelf-life study completed (M6), total project completion (M7).

3. Selected References

Berenguer et al., (2022) *Current Challenges in the Sustainable Valorisation of Agri-Food Wastes: A Review*, Processes.

Carvalho et al., (2023) *The Role of Emergent Processing Technologies in Beer Production*, Beverages.

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Characterization and smart par-drying of potatoes belonging to PGI "Patata dell'Alto Viterbese" (P-I-53)

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The research project aims at spreading innovation into Protected Geographical Indication (PGI) "Patata dell'Alto Viterbese" sector. This would be done by studying and proposing a par-dried, sliced product, starting from: cultivars' selection to be implemented, processing tries till optimization to end with a characterization of the output. For this purpose, the final product would be subjected to cooking tests to assess its quality through physico-chemical and sensorial analyses.

Caratterizzazione e semi-disidratazione smart di cultivar di patata afferenti al marchio IGP "Patata dell'Alto Viterbese"

Il progetto di tesi di dottorato ha come scopo lo studio per l'innovazione di processo e prodotto nell'area della Patata dell'Alto Viterbese IGP. La proposta in questo senso è la realizzazione di un prodotto semi-disidratato a fette, a partire dalla selezione varietale, passando per prove di processo pilota fino ad una sua ottimizzazione. Lo scopo ultimo è ottenere un output standardizzabile che, una volta individuato, verrà successivamente utilizzato in test di cottura (bollitura e frittura) e di valutazione sensoriale; ciò per valutare gli effetti di processo sulle proprietà dell'alimento pronto per il consumo e sull'opinione dei consumatori.

1. State-of-the-Art

Solanum tuberosum is a globally relevant crop: in fact, it is a nutritional and cheap source of energy (mainly from carbohydrates) available for almost all the year. Plus, tubers are offered to consumers with various levels of transformation, making the species a valuable commodity from raw (no service content) to ready-to-eat or ready-to-cook usage (highest service content). To assess a proper intended use among the possible ones, it is therefore useful to study potatoes' composition. In particular, dry matter content, starch content and its granules characteristics, Maillard's reaction precursors (i.e., free sugars and amino acids), polyphenols' content, enzymatic activities rate are all features that help to select the best-fitting cultivar to a specific application. Considering this context, this Ph.D. project focuses on studying drying and par-drying applications on potatoes from Tuscia region, a relevant crop for this area since its predisposition for farming. Of the possible processing outcomes, dried ones like flour or fflakeshave a little market share compared to the rest of processed goods offered to consumers. Nevertheless, they are relevant as ingredients or as pre-processed goods for further steps (e.g., frying for industry). As an ingredient, dried output is useful in providing a better texture in bakeries, snacks and any other food to get thickened; in addition, the choice of a drying step, before frying, is supported by oil uptake reduction plus enhanced texture as well (Pedreschi et al., 2007).

About potato par-drying, instead, there is not much information on its use; it could be a new insight to develop as it could produce items belonging to the IMF (Intermediate Moisture Food) category. Consistent with a water activity (a_w) and moisture content (MC) food classification, IMF has a_w between 0.6 – 0.9 and MC < 50 % so that they do not support generally pathogenic bacteria's growth. However, other spoilage microorganisms could, so proper packaging and thermal treatment like pasteurization or low temperature storage should be considered. According to Qiu et al. (2019), the traditional full drying procedure gives out a safe product but with nutritional and sensorial side effects; changing the target could open also a new market share.

Independently from the MC and a_w levels, to dehydrate in an efficient way, it is suggested to run pre-treatments; this step is useful to improve drying kinetics, enhancing both environmental and economic sustainability while reducing enzyme-related side effects, during processing and storing. Thermal and non-thermal solutions with their pros and cons are two approaches to be classified. According to Calder et al. (2012), in particular, blanching solutions are a processing step in which it is possible to act against ACD (after-cooking darkening). This phenomenon is due to an oxidized complex, between ferric ions and chlorogenic acid (a phenolic acid); it causes mainly the cortex region to get an undesirable from grey to violet, so it impacts negatively on drying output quality, beside the enzymatic-induced browning. To decrease the ACD occurrence, it is worth processing cultivars less susceptible to it i.e. to have a lower content of precursors; then, in processing, the most common solution so far has been the addition of SAPP (Sodium Acid Pyrophosphate), an iron-chelating agent to prevent the reaction to occur. Because of environment and health concerns to SAPP, companies are looking for valid substitutes; SAS (Sodium Acid Sulphate), CA (Citric Acid), $CaCl_2$ (Calcium Chloride) and edible coatings could be possible solutions. Moreover, as blanching conditions to reduce browning, other authors have experienced alternatives to plain hot water like combined solutions of organic acids and salts (Severini et al., 2003; Zhao et al., 2024). In the end, when it comes to potato dehydration, in the literature it is reported that different conditions could be applied in terms of thermal and not thermal application, pressure inside the system whatever it is batch or continuous. The

interest in more efficient processing and better output quality has led to explore hybrid processing, smart and intermittent drying; the latter would be implemented through sensors in line to get a real time monitoring: this in order to change running conditions for processing in a more sustainable way (Calín-Sánchez et al., 2020).

2. PhD Thesis Objectives and Milestones

Given the previous description, this Ph.D.'s thesis focuses on the development of par-dried potato products categorized as IMF and compares it to a full-dried one; once obtained and characterized, it will be tested then on cooking quality, namely boiling and frying operations. Within this goal in mind, the project can be subdivided into the following activities, according to the GANTT diagram given in Table 1.

A1) **Development of the research strategy**. (A1.1) Acquisition of the knowledge needed for properly conducting the research activity, regarding the study of the state-of-the-art of drying, with application in the potato sector. This is needed to figure out what are the output's desired characteristics for par-/drying processes, identify analytes and procedures to quantify them. (A1.2) Cultivars will be selected on their potential use for processing; after that, with the information acquired so far, some preliminary blanching tests, combining acid and salt, will be applied on them followed by image analysis: this is in order to look at discoloration is present and how it develops.

A2) **Par-drying and drying process optimization**. After assessing results from different blanching tests, the core part will be i) setting the process space of the air cabinet (i.e., temperature, relative humidity, air velocity); ii) defining the product space in terms of the desired final MC, color, and a_w values, as well as changes in size and shape (e.g., bending, shrinkage, etc.). This is meant to standardize the process and then study how pre-treatments influence the output in terms of water activity and content, color development and shape alteration.

A3) **Output culinary application and characterization**. To evaluate the output cooking quality, the path would include standardizing the cooking processes, namely boiling and frying conditions and when to stop the process; then a list of determination will be performed, like MC, a_w, color, rehydration curve kinetics, oil uptake (only on fried output).

A4) **Mobility period abroad**. Once either a research center or a university has been defined to satisfy PNRR's requirement of a six-month-working period abroad, activities to be developed will be dealt with; they could be related to process or product spaces.

A5) **Project and result dissemination**. Outputs from the previous activities allow the PhD student to: (A5.1) deal with workshops, conferences, fairs, etc.; (A5.2) submit conference proceedings and scientific papers; (A5.3) conclude PhD thesis.

Table 1 GANTT diagram for this Ph.D. thesis project

	Activities	1st year												2nd year												3rd year											
		Q1			Q2			Q3			Q4			Q5			Q6			Q7			Q8			Q9			Q10			Q11			Q12		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
A1)	**Development of the research strategy**																																				
	1. State of the art of potato drying and product characterization										M1																										
	2. Cultivar selection and preliminary processing and quality tests							M2			D1																										
A2)	**Par-drying and drying process optimization**																M3												D2								
A3)	**Output culinary application and characterization**																M4												D3								
A4)	**Mobility period abroad (six months)**																						M5														
A5)	**Project and results dissemination**																																				
	1. Conference, workshops, fairs, etc.																																				D4
	2. Conference proceedings, scientific articles																																				D5
	3. PhD thesis development																												M6								D6

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Vegetable Coagulants and New Dairy Technologies for the Innovation and Diversification of Goat's Milk Dairy Production (P-I-54)

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The rise in global dairy production, coupled with the limited availability and high cost of animal rennet, underscores the need for alternative coagulants. Religious, ethical, and dietary preferences further drive demand for vegetable coagulants. In light of this, the present research project aims to develop standardised vegetable rennet formulations that ensure high cheese yields and optimal sensory quality, supporting their future adoption in the dairy industry.

Coagulanti Vegetali e Nuove Tecnologie Casearie per l'Innovazione e la Diversificazione delle Produzioni Casearie a base di Latte Caprino

L'aumento della produzione casearia globale, unito alla scarsa disponibilità e al costo elevato del caglio animale, richiede coagulanti alternativi. Fattori religiosi, scelte vegetariane e la preferenza per prodotti naturali rafforzano questa necessità. In questo contesto, il presente progetto di ricerca mira allo sviluppo di formulazioni standardizzate di caglio vegetale capaci di garantire elevate rese nel processo di caseificazione e una elevata qualità sensoriale dei prodotti, fattori determinanti per la loro futura implementazione nell'industria lattiero-casearia.

1. State-of-the-Art

The Italian dairy sector is continually evolving, driven by the growing consumer demand for products that meet ethical, health, and environmental standards. One significant aspect of this evolution is the enzymatic coagulation of milk, which is traditionally reliant on animal rennet. The increase in global cheese production, combined with the decreased availability and rising costs of animal rennet, alongside religious considerations in Islam and Judaism, as well as vegetarian preferences, has spurred the search for alternative coagulation methods. Additionally, consumers increasingly favour natural and pleasant products sourced from ethical supply chains that respect production processes and avoid additives (Pacifico *et al.,* 2024). In this context, the use of vegetable-derived curds emerges as an innovative strategy. For many years, vegetable extracts have been utilised in the preparation of artisanal cheeses. Among these, extracts from thistle inflorescences (*Cynara cardunculus* L.) are well-known for their coagulating properties and are primarily used for making sheep and goat cheeses. Other plant-based coagulants include fig latex (*Ficus carica* L.) and sulphur curd (*Galium verum* L.). In Italy, vegetable coagulants are still used in several cheese-making traditions, particularly in regions like Tuscany, Marche, Lazio, Abruzzo, Apulia, and Sardinia. Some notable varieties include "Caciofiore" (Lazio, Pat-Lazio and Slow Food Presidium), "Pecorino delle Balze Volterrane" (Tuscany, DOP), "Fiore Sardo" (Sardinia, DOP), "Caciofiore dell'Abruzzo", "Caciotta vaccina" with vegetable rennet (Marche), "Caprino di latte di fico" (Marche), "Raviggiolo di pecora pistoiese", "Pampanella" from Puglia and Abruzzo, and the Sicilian "Ficu". The enzymatic activity of plant rennet is mainly attributed to plant proteases (aspartic, serine, and cysteine), which vary in number and type depending on the species and part of the plant used. These proteases hydrolyse casein κ, destabilising milk micelles and promoting gel formation. In addition to coagulation, plant proteases affect cheese ripening by contributing to flavour development through the hydrolysis of caseins. The extent of these effects depends on the type, dosage, and enzymatic activity of the coagulant. However, one major limitation of many vegetable curds is the development of a pronounced bitter taste and defects in cheese texture during storage or ripening. This issue arises from excessive proteolytic activity and, specifically, from a low coagulating-to-proteolytic activity (CA/PA) ratio. Maintaining a high AC/AP ratio is crucial for producing quality products. Therefore, evaluating enzyme activities and comparing them with commercial rennet are essential steps in selecting a suitable plant coagulant, particularly for industrial production where maximising yield and ensuring desirable sensory properties are key factors (Mohsin *et al*., 2024). In this context, the present research project aims at 1) investigating the milk-clotting activity of various plants and plant parts from wild Mediterranean flora to identify new coagulants with a high CA/PA ratio, 2) developing and optimising extraction processes to standardise coagulant preparation, 3) designing, producing, and characterising innovative dairy products using the developed coagulants. These processes and product innovations will expand market opportunities for dairy companies, particularly by enabling Halal and Kosher certification. Additionally, the standardisation of coagulants and cheese-making processes could support the attainment of certifications and protection designations, further strengthening the connection between production and its regional heritage.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Production of New Vegetable Curds. (**A1.1) Plant selection - A screening will be conducted to assess the coagulant activity of new plant species, with a particular focus on wild plants from the Mediterranean flora. Different parts of the plants will be tested to select the most suitable vegetable matrices (A1.2) Standardisation of protease extraction processes from plant matrices - The extraction process will be optimised and standardised based on variables such as temperature, pH, ionic strength, and matrix-to-solvent ratio. (A.1.3) Characterisation of New Plant Coagulants - The coagulants will be characterised in terms of protein content and profile, coagulant and proteolytic activity, and collateral enzymatic (e.g., lipolytic and esterase) activities. Technological properties will be defined in relation to pH, temperature, and Ca^{2+} ion concentration to identify optimal utilisation conditions. (A1.4) Definition of coagulant stability under optimised storage conditions- This will be assessed by monitoring the CA over time

A2) **Development and Optimisation of Cheese-making Processes (CMP) Using New Vegetable Rennet.** (A2.1) Selection of CMP - The CMP and type of cheese will be chosen based on the technological characteristics of the vegetable curds. Typical cheese processes and products will be preferred. (A2.2) Optimisation of CMP processes -The production processes will be standardised by defining the optimal parameters for each unit operation.

A3) **Characterisation of Developed Dairy Products.** (A3.1) Microbiological characterisation - Count of lactic acid bacteria, Enterobacteriaceae, moulds and yeasts will be assessed. Additionally, the most common pathogenic bacteria in cheese, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enterica*, will be investigated. (A3.2) Chemical, physical, and physicochemical characterisation - This will involve assessment of proximate composition, pH, acidity, degree of proteolysis, degree of lipolysis, volatile aroma profile, and texture profile analysis (TPA). (A3.3) Sensory characterisation - Sensory analyses will include descriptive sensory analyses (QDA) and consumer sensory tests.

A4) **Packaging and Shelf-Life of Developed Dairy Products.** (A4.1) Development of suitable packaging materials and systems - Assessment of the most appropriate packaging materials and systems (e.g., vacuum, MAP) for the type of cheese produced. (A4.2) Definition of Product Shelf-Life - The shelf-life of the packaged product will be determined by evaluating its microbiological and sensory quality through the storage period.

A5) **Writing and Editing** of the PhD Thesis, Scientific Papers, and Oral and/or Poster Communications.

Table 1 Gantt diagram for this PhD thesis project.

	Activity Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Vegetable Curd Production***																								
	1) Plant selection																								
	2) Standardisation of extraction processes																								
	3) Characterisation of plant coagulants																								
	4) Definition of stability under optimised storage conditions.																								
A2)	***CMP Development and Optimisation***																								
	1) Selection of CMP																								
	2) Optimisation of CMP																								
A3)	***Characterisation of Developed Dairy Products.***																								
	1) Microbial characterisation																								
	2) Chemical, physical, and physicochemical characterisation																								
	3) Sensory Characterisation																								
A4)	***Packaging and Shelf-Life of Developed Dairy Products***																								
	1) Evaluation of packaging materials and systems																								
	2) Shelf-Life Definition																								
A5)	***Thesis and Paper Preparation***																								

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Severina Pacifico, Emilia Caputo, Simona Piccolella and Luigi Mandrich (2024) Exploring New Fruit- and Vegetable-Derived Rennet for Cheese Making.

Recovery of bioactive compounds and fiber from citrus wastes (P-I-55)

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The PhD project aims to contribute to reducing citrus processing waste through the recovery, purification and valorisation of these by-products, whose current destination represents a significant environmental problem or, alternatively, a reckless destruction of resources still available to the community. The work aims at industrial-scale application for the recovery of bioactive compounds such as flavonoids and the production of dietary fibre, which would guarantee greater profits for the entrepreneur compared to other uses.

Recupero di composti bioattivi e fibra da scarti degli agrumi.

Il progetto di dottorato si propone di contribuire a ridurre il problema della gestione degli scarti della lavorazione agrumaria, attraverso il recupero, la purificazione e la valorizzazione di tali sottoprodotti la cui attuale destinazione rappresenta un problema ambientale significativo o in alternativa una sconsiderata distruzione di risorse ancora disponibili per la collettività. Il lavoro mira alla messa a punto su scala industriale, di procedure per il recupero di composti bioattivi come i flavonoidi e la produzione di fibra alimentare, che garantirebbero un maggiore profitto all'imprenditore rispetto ad altri usi.

1. State-of-the-Art

The industrial processing of citrus fruits produces natural juice, concentrated juice, essential oils, aromas, and by-products such as citrus molasses, biomass (50% fruit waste) as citrus peels, pulp, and seeds. Molasses come from an intermediate stage in the processing of the peel. At this stage, the peels are treated with lime and then pressed; the liquid obtained from pressing is then concentrated at least at 35.5 °Brix. This process leads to a high concentration of many secondary metabolites, including flavonoids. Nowadays, especially in the circular economy, the valorization of by-products, as added value for companies, is emerging as a symbol of a sustainable and economically advantageous approach, reducing, or eliminating waste and increasing profits with the planning of secondary processing lines. Citrus peels are commonly utilized for pectin extraction, as feedstock for biogas plants or livestock, or in the production of candied fruit. However, their richness in bioactive compounds sets them apart, making them a sustainable source of phenolic substances and a valuable alternative source of dietary fiber. Citrus fiber demonstrates excellent water-holding capacity, a high pectin content, and notable apparent viscosity, properties that enhance its functionality in food formulations. Upon hydration, these fibers swell, contributing to texture modification and satiety (Lundberg et al., 2014). Many flavonoids have been identified in nature, and they differ depending on the type of citrus cultivar and matrix we are going to consider as: flowers, fruits, seeds and peels. Among these phenolic compounds, eriocitrin, a flavonoid found mainly in lemons, is particularly important due to its excellent health properties as shown in *Table 1*.

Table 1. Eriocitrin properties.

Application	Target	Mechanism of action
Pharmaceutical	- Muscle atrophy - Prediabetes	Reduction of reactive oxygen species that cause activation of the FOXO1 transcription factor. Metformin 500 mg + Eriomin 200 mg/day produced the best results, modulation of the gut microbiota with production of short fatty acids
Cosmetic	- Enzyme tyrosinase (vitro)	Citrus peel extract acts on skin spots by inhibiting the enzyme tyrosinase, thereby reducing the synthesis of melanin responsible for hyperpigmentation.
Industrial	- Enzyme tyrosinase (vitro)	Anti-browning agent, especially in the fresh-cut industry.

R: (Cesar et al., 2023 ; Fang et al.,2023 ;; Takase et al., 2021).

Many studies in the literature describe methods for extracting flavonoids from dried citrus peels or their concentrated forms. These methods aim to preserve the bioactivity of these compounds for functional food applications. Qualitative and quantitative analyses are predominantly carried out using High Performance Liquid Chromatography (HPLC), a technique well-suited to accurately profile flavonoid content due to its high sensitivity and resolution (Modica et al., 2022), Wang et al., (2025) recovery flavonoids from dried peels of citrus reticulata with the help of a deep eutectic solvent (DES, choline chloride and ethylene glycol 1:4 at 80°C) and adsorption in specific HD-1 resin. Alonso-Vázquez et al., (2025) extracted flavonoids by ultrasound-assisted extraction (UAE)

for 10 minutes with water (solid-liquid ratio 1:10), followed by centrifugation to remove solid residues. UAE is effective because it breaks down cell walls, facilitating the release of compounds. During the purification phase, a polyethersulfone (PES) membrane was used in an ultrafiltration process, which is useful for separating and concentrating bioactive compounds based on molecular size. This PhD thesis project will focus on the recovery of dietary fiber, eriocitrin and other flavonoids by optimizing the extraction method, first on a lab-scale and after on an industrial-scale. Moreover, the PhD project will focus on obtaining high-purity flavonoids, which are in large-scale demand in the pharmaceutical, industrial and cosmetic markets due to their significant antioxidant, antidiabetic, antitumoral properties. The presence of these compounds in the peel offers opportunities for developing functional ingredients such as dietary fiber and nutraceuticals application as bioflavonoid supplements.

2. PhD Thesis Objectives and Milestones

This PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in *Table 2*:

1) **Bibliographic research**: To optimise the extraction yield of dietary fiber, eriocitrin and other bioflavonoids, it is essential to study the scientific literature in depth to develop sustainable extraction methods with low environmental impact, which guarantee a final product free of impurities that can be produced on an industrial scale.
2) **Methodological development**: of an efficient and specific extraction method for bioflavonoids and dietary fiber with validation of the instrumental method for characterization and quantification.
3) **Recovery, production and purification of samples:** the production of citrus lemon fiber containing high concentrations of acid galacturonic and recovery and purification of bioflavonoids from lemon wastes with high concentration of eriocitrin
4) **Process optimisation with the design of a pilot plant for industrial applications**: tests with a pilot plant adapted to industrial volumes with attached ultrafiltration for bioflavonoids. Another test with different instruments to produce industrial dietary fiber.
5) **Dissemination of results** of the PhD thesis, publication of scientific articles and reviews. Presentation of results at conferences and seminars.

Table 2 Gantt diagram for this PhD thesis project.

Activity · Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1)	***Bibliographic research***																								
1A	Bioflavonoids																								
1B	Dietary fiber																								
2)	***Methodological development***																								
2A	Profile of lemon peel and molasses																								
2B	Profile of dietary fiber																								
3)	***Recovery, production and purification***																								
3A	Recovery and purification eriocitrin																								
3B	Production of dietary fiber																								
4)	***Pilot plant for industrial applications***																								
4A	Pilot plant for bioflavonoids																								
4B	Pilot plant for dietary fiber																								
5)	***Thesis and Paper Preparation***																								

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Sustainability Aspects in the Reformulation of Products for the Confectionery Industry: A Case Study on Gummy Candies (P-I-56)

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This PhD project aims to develop a new formulation of gummy candies, more sustainable and healthier, characterized by a reduced content of sugars and enriched with high nutritional value ingredients, such as dietary fibers and plant-based proteins. Sustainability will be achieved both by low environmental impact ingredients and by optimizing the production process to reduce energy consumption and waste. To assess the overall environmental impact of the product, Life Cycle Assessment (LCA) will be applied, with the goal of developing a replicable model for the confectionery industry.

Aspetti di Sostenibilità nella Riformulazione di Prodotti per l'Industria Dolciaria: Un Caso Studio sulle Caramelle Gommose

La presente tesi di dottorato si propone di sviluppare una nuova formulazione di caramelle gommose più sostenibili e salutari, caratterizzate da un ridotto contenuto di zuccheri e arricchite con ingredienti ad alto valore nutrizionale come fibre e proteine vegetali. La sostenibilità sarà perseguita sia attraverso l'impiego di ingredienti a basso impatto ambientale, sia mediante l'ottimizzazione del processo produttivo per ridurre consumi energetici e scarti. Per valutare l'impatto ambientale complessivo del prodotto verrà impiegata l'analisi del ciclo di vita (LCA) con l'obiettivo di sviluppare un modello replicabile per l'industria dolciaria.

1. State-of-the-Art

The global gummy candy market is experiencing significant expansion, due to changes in consumer behaviour and continuous innovation in product offering (Tarahi et al., 2024), with forecasts indicating a total value of USD 53.5 billion by 2030, supported by a compound annual growth rate (CAGR) of 9.3 % (Polaris Marker Research, 2024). This phenomenon is driven by the growing demand for products with healthier and more transparent formulations, such as candies with reduced sugar content, free from synthetic additives, and enriched with functional ingredients (Gunes et al., 2022). These dynamics highlight the urgency for the confectionery industry to innovate processes and formulations to keep up with new trends. Among the factors determining consumer acceptance of confectionery products, texture plays a central role, significantly influencing purchasing decisions. In the gummy candy segment, key sensory attributes such as softness, elasticity, cohesiveness, and non-stickiness are critical to consumer perception. An increase in viscosity (semi-solid systems) or hardness (gelled systems) globally reduced both perceived taste and aroma intensities (Saint-Eve et al., 2011). A well-balanced texture enhances the overall eating experience the impacts flavor release and taste perception. Therefore, the development of new formulations must consider the effect of ingredients on product structure and rheological behaviour, as changes in composition can alter gelling properties and, consequently, the final texture. The texture of gummy candies, typically obtained through gelation techniques, is influenced by several factors, including the type and concentration of hydrocolloids, water content, pH, temperature, and humidity during processing (Tirek et al., 2023). In the context of sustainable and inclusive reformulation - which also meets the needs of vegan, Halal, and Kosher consumers - there is a growing opportunity to replace animal-derived gelatine with plant-based alternatives which offer advantages in terms of availability, cost, and environmental impact. In response, the scientific literature (Song et al., 2022; Mariz de Avelar and Efraim, 2020) has explored plant-based alternatives such as pectin, agar-agar, carrageenan, plant gums, and modified starches, which are capable of partially replicating the functional properties of gelatine. However, these solutions present technological challenges in terms of texture, stability, and sensory acceptability. There remains significant potential for the development of plant-based gummy candies that are nutritionally suitable, sensorially appealing, and competitively effective compared to conventional products. For example, exploring other ingredients as the range of dietary fibres and vegetable proteins.

Based on these considerations, this PhD project aims to develop innovative and sustainable gummy candies based on plant-based proteins and fibers. In particular, the research will investigate the gelling and rheological properties of pea and or soy proteins alone or coupled with soluble dietary fibers to understand their behaviour under the different manufacturing processing conditions of the gummy candies, that is extrusion and moulding. The optimization of the formulation of the innovative gummy candies, made by both manufacturing processes, will be reached using the Design of Experiments (DoE) approach, which will return a predictive mathematical model on the influence of the quantitative effect of each ingredient on the main mechanical and sensory properties that make the quality of the gummy candy. The resulting innovative products will be evaluated through sensory analysis to assess consumer acceptance in comparison with commercial products. To meet sustainability

goals, the project will include a Life Cycle Assessment (LCA) of the entire production chain to quantify the environmental impact at each stage.

2. PhD Thesis Objectives and Milestones

Based on overall objective mentioned above and supported by a literature review conducted during the preliminary phase, the activities of this PhD project are organized as follows, according to the Gantt chart shown in Table 1: A1) **Bibliographic research:** Literature collection and analysis (A1.1)

A2) **Non-conventional Ingredients Screening:** Identification and characterization of fibres and plant-based proteins (A2.1)

A3) **Formulation Development using Molding Process**

- Identification of independent variables to set experimental plan by DoE (A3.1)
- Optimization of the formulation using DoE (A3.2)
- Validation of DoE results through molding trials, rheology and Texture Profile Analysis (TPA) analysis on products (A3.3)

A4) **Formulation Development using Extrusion Process**

- Identification of independent variables to set experimental plan by DoE (A4.1)
- Optimization of the formulation using DoE (A4.2)
- Validation of DoE results through extrusion trials rheology and Texture Profile Analysis (TPA) analysis on products (A4.3)

A5) **Sensory Evaluation and Shelf life of molded and extruded gummies**

- Panel test to assess the acceptance of new products, ensuring changes made do not compromise consumer preference and satisfaction (A5.1 on molded gummies; A5.3 onextruded gummies)
- Shelf-life study: monitoring and data analysis of physicochemical and sensory properties during the storage (A5.2 of molded gummies; A5.4 of extruded gummies)

A6) **Life Cycle Analysis (LCA)** of the molding process (A6.1) and extrusion process (A6.2) to assess and compare the environmental impact of reformulated vs. traditional gummy production

A7) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 *Gantt diagram for this PhD thesis project*

	Activity Months - 2nd and 3rd year										1	1	1	1	1	1	1	1	1	2	2	2	2	2
A1)	***Bibliographic research***																							
	1) Literature collection and analysis																							
A2)	***Non-conventional ingredients Screening***																							
	2.1) I dentification and characterization of fibers and plant-based proteins																							
A3)	***Formulation Development using Molding Process***																							
	3.1) Identification of independent variables to setting experimental plan by DoE																							
	3.2) Optimization of the formulation using DoE																							
	3.3) Validation of DoE analysis																							
A4)	***Formulation Development using Extrusion Process***																							
	4.1) Identification of independent variables to setting experimental plan by DoE																							
	4.2) Optimization of the formulation using DoE																							
	4.3) Validation of DoE results																							
A5)	***Sensory Evaluation and Consumer Acceptance***																							
	5.1) Panel test on molded gummies																							
	5.2) Shelf-life study of molded gummies																							
	5.3) Panel test on extruded gummies																							
	5.4) Shelf-life study of extruded gummies																							
A6)	***Life Cycle Analysis (LCA)***																							
	6.1) Molding Process LCA																							
	6.2) Extrusion Process LCA																							
A7)	***Thesis and Paper Preparation***																							

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From food biofortification to probiotic: exploring biotechnological applications of B2-overproducing LAB (P-I-57)

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Increasing consumer awareness is driving the development of new strategies for functional foods production. The selection and characterization of riboflavin-overproducing lactic acid bacteria can unveil the potential to unlock promising, innovative, and sustainable biotechnological applications. This project aims to optimize a fermented plant-based beverage through a comprehensive investigation of the genotypic and physiological traits underlying riboflavin overproduction, as well as to elucidate the interactions with the host and the gut microbiota.

Dalla biofortificazione di alimenti ai probiotici: applicazioni biotecnologiche di batteri lattici sovraproduttori di vitamina B2

La crescente consapevolezza dei consumatori sta incoraggiando lo sviluppo di nuove strategie per la produzione di alimenti funzionali. La selezione e la caratterizzazione di batteri lattici sovraproduttori di riboflavina possono svelare promettenti applicazioni biotecnologiche, innovative e sostenibili. Questo progetto ha lo scopo di ottimizzare una bevanda vegetale fermentata, investigando i tratti genotipici e fisiologici alla base di questa caratteristica, oltre alle interazioni con l'ospite e il microbiota intestinale.

1. State-of-the-Art

Riboflavin (vitamin B2) is an essential vitamin that plays a crucial role as a precursor of FMN and FAD, involved in numerous flavoprotein-catalyzed reactions. Riboflavin-overproducing Lactic Acid Bacteria strains, natural mutants selected using roseoflavin (Ripa et al., 2022), are gaining attention due to their potential applications in innovative functional foods and probiotics. Studies suggest that riboflavin-overproducing bacteria can ameliorate induced colitis symptoms in murine models (Levit et al., 2018). Moreover, efficacy of several strains in enriching various food matrices with riboflavin, including bread, pasta, and soy milk has been demonstrated (Russo et al., 2016). The increasing demand for non-dairy functional products further highlights the relevance of these riboflavin-overproducing strains in developing innovative and healthy food options.

B2 deficiency remains a significant health concern in humans, particularly in developing countries or when inflammatory diseases are present (Mosegaard et al., 2020). Riboflavin could mitigate oxidative stress, typically found in various chronic diseases, including inflammatory bowel diseases (IBD) (LeBlanc et al., 2020). Additionally, riboflavin has been suggested to modulate the gut microbiota (Bedani et al., 2024) and ameliorate serum levels of inflammatory markers in Chron's disease. In fact, riboflavin's ability to reduce luminal reactive oxygen species (ROS) concentrations in the gut might support the growth of beneficial anaerobic bacteria that rely on dietary sources or mutualistic interactions for B vitamins (von Martels et al., 2020). However, this potential effect remains controversial since vitamins are normally absorbed in the proximal small intestine limiting their reach to the distal gut, and in-situ production and cross-feeding interactions have never been studied (Steinert et al., 2016). However, regarding the physiological role and potential advantages that overproducing strains may derive in stress conditions related to the food industry or the GIT, literature remains limited. Preliminary results also indicate that the efficiency of riboflavin production varies with different carbon sources and their concentrations. Moreover, overproducing strains seem to show a better resilience to some stressors such as NaCl or hydrogen peroxide (data not shown).

In this context, this project aims to evaluate the potential application of riboflavin-overproducing lactic acid bacteria (LAB) to create bio-enriched plant-based beverage. This application, other than a more sustainable alternative for riboflavin bio-fortification, can be considered an innovative strategy for gut delivery thanks to the in-situ production by the overproducing bacteria, potentially achieving a positive modulatory effect towards beneficial gut microorganisms.

Designing rational approaches requires an in-depth investigation of the pathway, the genes involved, their regulation, the effect of mutations and impact of culture conditions. The results of this project will provide valuable insights into the genetic and physiological basis of riboflavin overproduction in LAB.

2. PhD Thesis Objectives and Milestones

The project will be structured into four sequential work packages (WP), each one addressing the above-mentioned aims. Critical points that could have a negative impact on the project progress are being addressed as reported in Table 1. Deliverables and milestones are identified to keep track of the project progress and deadlines are shown in the Gantt chart (Table 2).

WP1) **Selection and analysis of riboflavin-overproducing strains. Task 1**: Comparative genomic analysis, to

assess the intra- and inter-specific occurrence of the *rib* operon (M1.1). **Task 2**: Selection of new riboflavin-overproducing strains (D1.1) and optimization of the selection protocol (M1.2). **Task 3**: Analysis of mutations to determine correlations with the phenotypic trait (D1.2).

WP2) **Characterization and physiological significance of overproduction. Task 1**: Growth performance and effect on response against abiotic stressors (M2.1). **Task 2**: Study of involved pathways (M2.2) and the mechanism of action in response to stress conditions with omics approach (D2.1).

WP3) ***In vitro* analysis of the interactions among overproducers and gut conditions/microbiota Task 1**: Effect of riboflavin overproduction and gut microbiota modulation (M3.1) by co-cultivation assay and fecal small batch fermentation (D3.1). **Task 2**: Effect of riboflavin overproduction on GIT transit survival (D3.2).

WP4) **Development of a plant-based fermented beverage and evaluation of its performance in a murine model Task 1**: Beverage optimization (M41.; D4.1). **Task 2**: Adhesion and reactive oxygen species measurement in intestinal epithelial cells after simulated digestion (D4.2). **Task 3**: Performance in murine model of colitis (D4.3).

Table 1 Mitigation plan of the project.

Risk	Impact	Mitigation
Delay of preliminary WPs (selection of strains)	Cause overlaps of work and lacks preliminary data	Use already isolated strains
Similar response to stress	Difficulties in detecting differential expressions of pathways	Use riboflavin non-producing strains (WCFS1)
Fermentation optimization failure	Impossible to evaluate the activity of the product	Enrichment of a commercial plant-based beverage

Table 2 Gantt chart of the project with milestones and deliverables deadlines.

Activity Description	1st semester	2nd semester	3rd semester	4th semeter	5th semester	6th semester
WP1 Selection and analysis of ribofalvin-overproducing strains						
Comparative genomic analysis		M1.1				
Selection and optimization of selection protocol		M1.2	D1.1			
Analysis of mutations			D1.2			
WP2 Characterization and physiological significance of overproduction						
Growth kinetics			M2.1			
Omics approach				M2.2	D2.1	
WP3 In vitro analysis of the interaction among overproducers and gut conditions/microbiota						
Co-cultivation assays and faecal fermentations				M3.1	D3.1	
GIT transit survival				D3.2		
WP4 Development of a plant-based fermented beverage and evaluation of its performance in a murine model						
Beverage optimization and characterization			M4.1	D4.1		
Adhesion assays and ROS measuremement					D4.2	
Performance in a murine model of ulcerative colitis						D4.3
Dissemination						
Data analysis and thesis writing						

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Design of bioprotective microbial consortia to improve the shelf life and overall quality of meat products (P-I-58)

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The aim of this PhD research project is to enhance the safety and quality of perishable Mediterranean food products, particularly focusing on meat products, by first investigating their microbial composition and then applying innovative and sustainable strategies. Specifically, the project focuses on the use of bio-protective microbial cultures to inhibit spoilage and pathogenic microorganisms, thereby improving shelf life and ensuring microbial safety.

Progettazione di consorzi microbici bioprotettivi per migliorare la durata di conservazione e la qualità complessiva di prodotti a base di carne.

L'obiettivo di questo progetto di ricerca di dottorato è migliorare la sicurezza e la qualità dei prodotti alimentari deperibili del Mediterraneo, ponendo attenzione su prodotti a base di carne, attraverso un'indagine preliminare sulla loro composizione microbica e l'applicazione di strategie innovative e sostenibili. In particolare, il progetto si concentra sull'utilizzo di colture microbiche bio-protettive per inibire i microrganismi deterioranti e patogeni, migliorando così la durata di conservazione e garantendo la sicurezza igienico-sanitaria

1. State-of-the-Art

Food has long been fermented and bio-preserved using a variety of microorganisms, including molds, coagulase-negative, catalase-positive cocci (CNCPC), and lactic acid bacteria (LAB) (Lücke, 1998). Most notably, LAB are widely regarded as safe (GRAS) and have numerous positive health impacts, making them ideal for use in food biotechnology (Leuschner et al., 2011; Stiles & Holzapfel, 1997). Specifically, the production of a wide range of compounds (such as organic acids, ethanol, hydrogen peroxide, bacteriocins, and antibiotic-like peptides) by LAB and CNCPC helps to increase the shelf life of different fermented foods by preventing or reducing the growth of spoilage or pathogenic microorganisms (Ibrahim et al., 2021).
Studies have been conducted to investigate the effect of LAB strains on Mediterranean foods such as traditional sausages (García-López et al., 2023), on fruits and vegetables (Siroli et al., 2015a) and meat (Vermeiren et al., 2004). LAB have demonstrated great potential as biocontrol agents of a lot of non-fermented foods, as well as widespread use in fermented foods, long history of safe use, and GRAS status. Strains of different genera of LAB have been identified as bacteriocin producers, and their action against pathogenic bacteria such as *Listeria monocytogenes* in fermented meat has been deeply investigated (Franciosa et al., 2018).
However, while there has been a surge in research in recent years regarding the use of biocontrol agents in foods, a critical review of the literature reveals that factors such as the inoculation level, background microbiota presence, physico-chemical and compositional features of the products, and storage conditions all impact the effectiveness of biocontrol agents, regardless of the species and strains employed. For this reason, in this project, research will investigate the microbial diversity of both spoiled and sound meat products through culture-dependent (traditional microbiology) and independent methods (PCR, amplicon sequencing). This combination of methods has been commonly used in other studies to describe the microbial population at species level (Chaillou et al., 2015; Pennacchia et al., 2011; Rantsiou et al., 2005)
Overall, detailed knowledge of the microbiota of meat products will be essential in the design of bioprotective microbial consortia that holds significant potential for enhancing their shelf life and quality. This research will provide valuable insights and practical solutions for food preservation, aligning with consumer demand for safe minimally processed food products.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Description and characterisation of the composition of microbial communities in spoiled/non spoiled meat products**. Meat microbiota will be characterized in relation to supply chain features, physicochemical characteristics, and conservation approaches available. Data will be collected from the literature and requested by companies providing meat products and will be integrated with data on culture-based methods.
A2) **Isolation of LAB and characterization as Bioprotective microorganisms**. An evaluation of antagonistic activity against several foodborne bacteria, but also against spoilage microorganisms from A1.
A3) **Microbial communities of meat products and their functionality characterisation.** A metataxonomic approach will be followed to describe the composition of the microbiota of both fresh and spoiled meat. In

this way, specific taxa or networks of taxa of the microbiota associated with each type of product will be identified. In addition, temperature and atmospheric conditions in packaging, among other abiotic factors, will be recorded. Further, a shot-gun sequencing approach will provide insights into the functional properties of microbial communities.

A4) **Validating shelf-life enhancing microbiota candidates.** Promising isolates/consortia based on outcomes from A2, showing a modulating power and having the potential to act as bioprotective agents, will be tested on meat, aiming at the extension of shelf life and maintaining the quality of meat products.

A5) **Writing and Editing** of the PhD thesis, scientific papers, and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity \ Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Characterisation of microbial communities***																								
	1)Literature review																								
	2) Microbial sampling/isolation of LAB																								
A2)	***Characterisation of microorganisms as Bioprotective***																								
A3)	***Metataxonomic analysis***																								
	1 Shot-gun sequencing																								
A4)	***Application and validation of microbial consortia***																								
	1) microbial sampling																								
	2) sensory analysis																								
A5)	***Thesis and Paper Preparation***																								

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Coffee bean microbiome as a suitable source for new microbial starter cultures (P-I-59)

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This PhD project aims to deepen scientific understanding and knowledge of postharvest coffee processing, especially fermentation, by focusing on *Coffea arabica* and *Coffea canephora* (Robusta). The goal is to enhance fruity, floral, and winey notes in Arabica, and reduce bitterness and astringency in Robusta while promoting sweeter sensory profiles, by using selected strains of yeasts and bacteria. The research will investigate native Brazilian microbiota involved in fermentation and assess its impact on coffee quality.

Il microbioma della drupa del caffè come fonte di nuovi starter microbici

Questo progetto di tesi di dottorato mira ad approfondire la comprensione scientifica della lavorazione del caffè dopo il raccolto, concentrandosi su *Coffea arabica* e *Coffea canephora* (Robusta). L'obiettivo è quello di esaltare le note fruttate, floreali e vinose dell'Arabica e di ridurre l'amarezza e l'astringenza della Robusta, promuovendo al contempo profili più dolci e morbidi attraverso l'utilizzo di ceppi microbici selezionati. La ricerca studierà il microbiota autoctono brasiliano coinvolto nella fermentazione e valuterà il suo impatto sulla qualità del caffè.

1. State-of-the-Art

Coffee is one of the most widely consumed beverages in the world. The final quality of coffee, particularly its flavor and aroma, is influenced by a range of factors, including region, climate, species, cultivation and harvesting practices, processing methods, and especially postharvest processing and fermentation (Seninde and Chambers, 2020; Freitas et al., 2024). While many of the scientific research studies have focused on identifying the aromatic compounds formed during roasting, especially those formed due to Maillard reaction, less attention has been given to the critical role of coffee processing and fermentation (Haile and Kang, 2019).

Coffee processing begins immediately after harvest, with the primary goal of converting ripe cherries into green beans suitable for storage, transport, and roasting. The choice of processing method has a substantial impact on the final beverage's flavor, aroma, and overall quality (Freitas et al., 2024). The most used processing techniques are wet, dry, and semi-dry (honey) methods (Janne Carvalho Ferreira et al., 2023). In the wet process, cherries are depulped and afterwards usually fermented in water tanks. The dry process involves sun-drying of whole cherries on the platforms. The semi-dry or honey method includes pulping the cherries while retaining some mucilage during drying. Depending on other factors, such as producer strategy and infrastructure, coffee processing can include also other steps. In all methods, the fermentation usually occurs between the initial fruit treatment and the drying phase (Janne Carvalho Ferreira et al., 2023).

Fermentation itself is a microbial metabolic process, during which microorganisms (yeasts and bacteria) convert sugars, mainly from the fruit's mesocarp, into simpler compounds such as ethanol, acetic acid, and lactic acid, while releasing energy (Elhalis et al., 2023; Janne Carvalho Ferreira et al., 2023). In the context of coffee processing, fermentation refers to all microbial activity naturally occurring during processing, which aids in mucilage breakdown, but also contributes significantly to the development of flavor precursors. However, traditional fermentation relies on spontaneous, uncontrolled microbial activity (Haile and Kang, 2019). Such fermentations can lead to the formation of undesirable compounds, especially butyric and propionic acids and harmful mycotoxins (Janne Carvalho Ferreira et al., 2023; Elhalis et al., 2023), posing food safety concerns and complicating efforts to meet global market standards. Therefore, a shift towards controlled fermentation processes by using starter cultures is needed to ensure predictability, improved sensory quality and increased final product quality. Starter cultures are preselected microorganisms, either single strains (yeast or bacteria) or mixed cultures, which can outcompete the naturally occurring microbial populations throughout the fermentation process (Haile and Kang, 2019; Janne Carvalho Ferreira et al., 2023). The starter cultures selection for coffee fermentation is often carried out from strains identified in coffee fruit or spontaneous fermentative process (Haile and Kang, 2019).

The microbial dynamics of coffee fermentation also notably vary between the two main species: *Coffea arabica* and *Coffea canephora* (Robusta) (Dos Santos et al., 2021). Arabica, typically cultivated at higher altitudes, is valued for its delicate, mild and sweet flavors, while Robusta, grown at lower altitudes, is known for its lower acidity and stronger, more bitter profile (Dos Santos et al., 2021; Seninde and Chambers, 2020). This PhD project focuses on exploring these dynamics within Brazil, the world's largest coffee producer (Dos Santos et al., 2021), by investigating the native microbiota associated with postharvest processing. Through microbial characterization, found in coffee beans, and the development of starter cultures, the project aims to standardize the process of coffee fermentation and enhance the coffee quality. Therefore, the use of selected microbial strains should lead to more consistent and predictable sensory outcomes. For Arabica the goal is to emphasize fruity, floral and wine-like

sensory notes. For Robusta the focus is on reducing astringency and bitterness and promoting more neutral and sweeter sensory profile.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Study of coffee cultivation system ecology and fermentation process:**
   1) Focus on the microbiota/microbiome of the coffee cultivation system using molecular, cultural, and metabolic analyses, along with chemical parameter studies.
   2) Study of current fermentation processes on Robusta and Arabica, defining microbial populations and their dynamics, with chemical and sensory characterization of the product.

A2) **Physiological characterization of isolates and selection of candidates based on predefined organoleptic goals:**
   1) Genetic identification and physiological screening of isolates, focusing on metabolic and enzymatic activities relevant to fermentation
   2) Study of the most interesting strains under controlled conditions, with chemical and sensory characterization of the final product, and data analysis using machine learning to identify key aromatic compounds.

A3) **Validation of processes on a pilot scale:**
   1) Pilot-scale tests of selected microbial strains, monitoring the fermentation process, and characterizing the effect of the inoculum through chemical and sensory analyses on green coffee.

A4) **Writing and editing**
   1) Writing and editing of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
A1)	***Study of coffee cultivation system ecology and fermentation process***																																				
	1) Characterization of coffee cultivation system microbiota																																				
	2) Study of fermentation processes on Arabica and Robusta																																				
A2)	***Characterization and selection of candidates***																																				
	1) Screening of isolates, based on metabolic and enzymatic activities																																				
	2) Evaluation of promising candidates under controlled conditions																																				
A3)	***Validation of process***																																				
	1) Pilot scale tests of selected microbial strains																																				
A4)	***Thesis and paper preparation***																																				

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A green approach to the synthesis and extraction of bioactive compounds from *vitro*-derived hop plants (P-I-60)

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This PhD project aims to develop new treatments to improve the production of secondary metabolites in hop plants (*Humulus lupulus* L.) *in vitro* cultured, given their richness in bioactive compounds. For the extraction of bioactive compounds with antioxidants and antimicrobial properties, green extraction methods will be applied, particularly using Natural Deep Eutectic Solvents (NaDES) as a sustainable alternative to conventional solvents. The resulting extracts will be tested for their antioxidant, antimicrobial and antibiofilm activities, primarily against pathogenic and spoilage microorganisms.

Un approccio green alla sintesi e all'estrazione di c0omposti bioattivi da piante vitro-derivate di luppolo
Questo progetto di dottorato ha l'obiettivo di sviluppare nuovi trattamenti per migliorare la produzione di metaboliti secondari nelle piante di luppolo (*Humulus lupulus* L.) coltivate *in vitro*, data la loro ricchezza in composti bioattivi. Per l'estrazione di queste molecole con proprietà antiossidanti e antimicrobiche, verranno applicati metodi di estrazione green, in particolare utilizzando i NaDES (Natural Deep Eutectic Solvents) come alternativa sostenibile ai solventi convenzionali. Gli estratti ottenuti saranno testati per valutarne l'attività antiossidante, antimicrobica e antibiofilm, principalmente contro microrganismi patogeni e alterativi.

1. State-of-the-Art

Hop (*Humulus lupulus* L.) is a widely cultivated plant renowned for its richness in bioactive compounds across its various parts. In recent years, it has garnered growing interest from both the food and non-food industries, thanks to its potential as a source of natural extracts with antioxidant, antimicrobial, and antiviral properties (Astray *et al.*, 2020, Keskin *et al.*, 2019). Traditionally grown in open fields, hop cultivation is subject to seasonal and environmental variations, which can impact the consistency of its phytochemical profile. To overcome these limitations, plant tissue culture (PTC) techniques offer a promising alternative. *In vitro* cultured plant organs can produce the same bioactive compounds as those found in conventionally grown plants, but with the added benefit of a controlled, stable, and season-independent environment. This approach enables more predictable and standardized production, which is critical for industrial applications (Dias *et al.*, 2016). While several researchers have investigated the extraction of valuable compounds from hop, most studies have focused on field-grown plants. Only a limited number have explored the use of *in vitro* cultured hop explants. Moreover, although the influence of external stressors on polyphenol content in field-grown hops has been studied, no research to date has examined how *in vitro* cultured hop tissues respond to such stimuli (Abram *et al.*, 2015). Until now, the extraction of bioactive compounds from hop has predominantly relied on conventional solvents. However, there is a growing shift towards the use of environmentally friendly, or "green," solvents. Analytical chemistry is increasingly embracing innovative extraction techniques aligned with the Twelve Principles of Green Analytical Chemistry, aiming to develop safer, cleaner, and more sustainable methodologies (Carbonell-Rozas *et al.*, 2024). One of the most promising developments in this area is the emergence of Natural Deep Eutectic Solvents (NaDES). These solvents, which form through the eutectic combination of natural, biodegradable components, share key physical properties with ionic liquids—such as low melting points, low volatility, and high thermal stability—while being safer, cost-effective, and easy to prepare. Due to these advantages, NaDES are gaining recognition as efficient green solvents, particularly well-suited for the extraction of bioactive compounds from plant materials (Peng *et al.*, 2016). This PhD project aims to analyse a collection of different hop genotypes cultured *in vitro* and to characterize them in order to assess and highlight genotypic differences in antioxidant activity and total polyphenol content. The project will also evaluate the effectiveness of various elicitors in stimulating the biosynthesis of bioactive compounds *in vitro* derived hop plantlets, using the previously selected genotypes. For the first time, NaDES will be employed as a green and eco-friendly extraction method for *vitro*-derived hop plantlets. The resulting extracts will be used to determine their antimicrobial and antibiofilm activities against foodborne pathogenic and food spoilage microorganisms, such as *Listeria monocytogenes*, *Salmonella spp.*, *Escherichia coli*, *Bacillus cereus*, and *Pseudomonas spp.* This will lead to the identification of the extracts' minimum inhibitory concentrations (MICs) effective in inhibiting specific microbial targets and bacterial biofilms. Additionally, the project will investigate intraspecific variability in microbial sensitivity to the different hop extracts. All experiments will be designed using a Design of Experiments (DoE) mathematical approach through the MODDE® Pro software. An efficient DoE strategy offers several advantages: significantly reducing experimental costs, minimizing project-related risks, increasing success rates, and optimizing the use of valuable samples, raw materials, and human resources.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in *Table 1*:

A1) **Micropropagation**: *In vitro* establishment and proliferation of hop cultivars and testing of different abiotic elycitors

A2) **Extraction of bioactive compounds with green techniques** as the use of NaDES and ASE (Accelerated Solvent Extraction); chemical assays to test the polyphenolic content and the antioxidant activity will be performed on the extracted bioactive compounds. A chemical characterization will follow with different techniques (LC-MS/MS, GC-MS)

A3) **Antimicrobial activity evaluation** will include the use of different techniques (agar well diffusion assays, microbial growth monitoring, determination of MIC values) to value effectiveness of hop extracts against pathogen and spoilage microorganisms. The antibiofilm activity will be evaluated as well and then challenge tests will be conducted to test the extracts antimicrobial activity in food products.

A4) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

ACTIVITIES		MONTHS																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1	**Micropropagation**																								
	1) *In-vitro* establishment and proliferation of hop cultivars																								
	2) Testing of different abiotic elycitors																								
A2	**Extraction of bioactive compounds with green techniques**																								
	1) NaDES, ASE																								
	2) Chemical assays (poliphenolic content, antioxidant activity)																								
	3) Chemical characterization (LC-MS/MS, GC-MS)																								
A3	**Antimicrobial activity evaluation**																								
	1) Antimicrobial activity evaluation against pathogens and spoilage microorganisms																								
	2) Antibiofilm activity evaluation																								
	3) Testing extracts on food products (challenge tests)																								
A4	**Thesis and Paper preparation**																								

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Application of targeted lactic acid bacteria for the revalorization of food matrices and by-product (P-I-61)

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This PhD thesis project aims to reduce or eliminate the presence of undesirable compounds in plant-based matrices by leveraging the metabolic activity of lactic acid bacteria strains belonging to the University of Parma's strains collection (UPCCO). Special attention will be given to the reduction of antinutritional factors commonly found in cereals, legumes, and their derivatives, with the goal of producing improved flours suitable for use as food ingredients.

Rivalutazione di matrici vegetali e sottoprodotti alimentari utilizzando le potenzialità di differenti ceppi di batteri lattici

Questo progetto di tesi di dottorato si pone l'obiettivo di ridurre o eliminare la presenza di composti indesiderati nelle matrici vegetali sfruttando l'attività metabolica di batteri lattici più adatti tra quelli che appartengono alla collezione dell'Università di Parma (UPCCO). Particolare attenzione sarà dedicata alla rimozione di antinurienti presenti in cereali, legumi e i loro derivati con l'obiettivo di ottenere ingredienti con un ridotto contenuto di antinutrienti.

1. State-of-the-Art

The agro-food sector is increasingly facing numerous challenges to obtain a product that is safe for the consumers. Moreover, over the past decades, there has been a growing demand for plant-based products, which, however, may contain compounds potentially harmful to human health.

According to the World Health Organization (WHO), toxic ingredients in food may be classified as toxicants derived from microorganisms, toxic chemicals such as pesticides and heavy metal and antinutrients derived from plant material before processing. Toxic substances can have different origins within products; they can be originated by bacteria, such as toxins and mycotoxins, chemical like pesticides and heavy metals, or antinutritional like phytates, oxalates, and glycosides.

These elements can cause different human diseases, for example gastrointestinal trac disorders (GIT), neurological, cardiovascular, immunological and psychological damage (Pretova *et al.,* 2022).

To mitigate this issue, a range of treatment strategies have been explored, that are divided in three different categories: chemical, physical and biological treatment. Chemical and physical methods have some disadvantages such as high cost, loss of nutritional quality and often required multiple treatments to achieve optimal efficacy (Lu *et al.,* 2022).

Biological method involves the use of different microorganisms including yeast, fungi and bacteria (Armenova *et al.,* 2023). Fermentation, which represents one of the most widely used strategies for food preservation, employed microorganisms to enhance sensory, nutritional, and safety properties of food matrices. Latic acid bacteria (LAB) are a group of microorganisms recognized as GRAS (Generally Recognized as Safe) by the FDA and QPS (Qualified Presumption of Safety) by EFSA. This group includes different species/strains that possess specific characteristics enabling them to degrade, absorb or metabolize undesirable compounds alongside other functions (Pretova *et al.,* 2022).

Antinutritional elements represent an extremely broad, heterogeneous, and controversial group. They can be classified into two major groups: i) based on their chemical structure, such as protease inhibitors, glycosides, phenol and salts and ii) based on functional significance, such as anti-enzymes, anti-vitamins and anti-minerals. These elements can be found in plant-based matrices, such as cereal, legumes fruits and vegetables and can be the cause of incorrect absorption of vitamins, minerals, proteins etc. Despite their potential adverse effects on human health, the strategies to mitigate them are still poorly investigated (Arsov *et al.,* 2024), however recent study, identify the fermentation with LAB as one of the most promising strategies for the reduction of antinutritional elements (Pham *et al.,* 2023).

For these reasons, this research proposal aims to investigate the potential of various LAB strains to reduce or eliminate antinutritional factors in plant-based matrices, particularly in legumes, cereals, and their processed or semi-processed derivatives. The strategy involves analyzing the genomes of selected LAB strains to identify gene sequences encoding enzymes associated with the degradation of antinutritional compounds. Subsequently, the antinutritional factor degradation will be evaluated through *in vitro* assays, followed by fermentation in cereal and legume products.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) Review of Existing Literature on Antinutritional Factors and Their Microbial Degradation

(A1.1) Identification of the main antinutritional factors present in cereals and legumes

(A1.2) Identification of bacterial enzymes involved in the degradation of antinutritional factors

(A1.3) Identification of known genes encoding enzymes responsible for antinutritional factors degradation

A2) Identification of LAB Strains with Potential for antinutritional factors **Degradation**

(A2.1) Genomic analysis of lactic acid bacteria (LAB) strains belonging to the University of Parma Culture Collection (UPCCO) to detect genes encoding for enzymes involved in the antinutritional factors degradation

(A2.2) Selection of LAB strains with potential antinutritional factors degradation capabilities based on genomic data

A3) In Vitro Screening of antinutritional factors **Degradation by Selected LAB Strains**

(A3.1) In vitro evaluation of antinutritional factors degradation by selected LAB strains

(A3.2) Selection of the most promising strains based on their detoxification performance

A4) Fermentation and Evaluation of antinutritional factors **Degradation in Food Matrices and By-products**

(A4.1) Evaluation and optimization of antinutritional factors degradation in cereals and cereal-based products through fermentation with selected LAB strains

(A4.2) Evaluation and optimization of antinutritional factors degradation in legumes and legume-based products through fermentation with selected LAB strains

A5) **Writing and Editing** of the PhD thesis, scientific publications, and oral and/or poster communications

Table 1 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	Literature Review																								
	1)Antinutrient compounds in legumes and cereals matrices																								
	2) Identification of bacterial enzymes involved in detoxification																								
	3) Genomic analysis to identfy enzymes involved in detoxification																								
A2)	Strains Identification																								
	1) Genomic analysis of strains from UPCCO																								
	2) Selection of the most promising strains																								
A3)	Screening of Detoxification Activity																								
	1)Evaluation of activity in vitro usign kits or analytichal methods																								
	2)Selection of the most high-performing strains																								
A4)	Evaluation in Food Matrices																								
	1) Evaluation of detoxification activity in cereals and cereal-based products																								
	2) Evaluation of detoxification activity in legumes and legumes-based products																								
A5)	***Thesis and Paper Preparation***																								

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Study of the Antimicrobial Activity of Natural Extracts Obtained from Olive Trees on Food-Borne and Spoilage Bacteria (P-I-62)

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This PhD thesis research project is aimed at investigating the antimicrobial properties of natural extracts from olive trees against foodborne and spoilage bacteria. By exploring the antimicrobial and antioxidant efficacy of phenolic compounds with these natural extracts as eco-friendly alternatives to synthetic preservatives, this approach promotes food safety and shelf-life extension. By employing advanced molecular analyses to uncover the mechanisms driving their antimicrobial activity, which establishes sustainable practices, green-label food products align with growing consumer demands.

Studio dell'attività antimicrobica degli estratti naturali ottenuti dagli ulivi sui batteri di origine alimentare e di deterioramento

Questo progetto di ricerca di tesi di dottorato mira a studiare le proprietà antimicrobiche degli estratti naturali di ulivo contro i batteri di origine alimentare e quelli che causano alterazioni. Esplorando l'efficacia antimicrobica e antiossidante dei composti fenolici presenti in questi estratti naturali come alternative ecocompatibili ai conservanti sintetici, questo approccio promuove la sicurezza alimentare e l'estensione della shelf-life. Utilizzando analisi molecolari avanzate per scoprire i meccanismi che guidano la loro attività antimicrobica, che a sua volta promuove pratiche sostenibili, i prodotti alimentari a marchio verde rispondono alle crescenti esigenze dei consumatori.

1. State-of-the-Art

The global food industry faces a significant challenge in ensuring the safety, quality, and nutritional value of food products, as they are inherently vulnerable to microbial contamination. The presence of microorganisms not only diminishes food safety but also accelerates spoilage, resulting in reduced shelf life and potential health risk. To address these issues, synthetic preservatives have been widely used in the food industry to counteract microbial growth. These chemical additives, which include both antimicrobial and antioxidant agents, play a pivotal role in extending the shelf life of food products. Despite their widespread application, concerns have been raised regarding the potential long-term health risks associated with these synthetic substances. However, increasing consumer concern regarding the long-term health implications of chemical additives has driven a search for safer natural alternatives (Roila et al., 2024).

In response to these concerns, increasingly turning to plant-based solutions, with olive tree extracts emerging as a promising source of natural preservatives. Olive products and byproducts, particularly olive leaf extract, have gained attention due to their high phenolic content, which is responsible for their potent antimicrobial, antioxidant, and antiproliferative properties (Mitsagga et al., 2021). Olive leaves, a traditionally underutilized part of the olive tree, contain a diverse array of bioactive compounds such as oleuropein, hydroxytyrosol, luteolin-7-glucoside, epigenin-7-glucoside, and verbascoside have a remarkable efficacy against a wide range of foodborne pathogens, including *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella*. These compounds not only possess significant antimicrobial activity but also contribute to the preservation of food by preventing oxidation, thus enhancing food safety and nutritional quality in the food industry (Sar & Akbas, 2023).

Table 1: Phenolic compounds and their properties.

Phenolic compound	Primary Source	Concentration	Mode of action
Hydroxytyrosol	Olive leaves and fruits	Moderate	Antioxidant by scavenging free radicals Antimicrobial activity by disrupting their cell membranes
Tyrosol	Olive leaves	Moderate	Antioxidant properties Enhance the immune response Antibacterial
Oleuropein	Olive leaves, fruits, and oil	High	Antioxidant activity Inhibit the growth of various bacteria, including *Helicobacter pylori* Disruption of bacterial cell walls and metabolic processes
Oleocanthal	Oil oil	High	Anti-inflammatory properties Inhibit the activity of cyclooxygenase enzymes Antimicrobial activity
Luteolin-7-	Olive leaves	Moderate	Antioxident

glucoside			Antinflammatory
Epigenin-7-glucoside	Olive leaves	Low	Antioxident Anticencer

Recent studies have highlighted the antimicrobial potential of olive mill waste products, such as olive mill water, which has shown activity against various bacterial genera, including *Pseudomonas*, *Staphylococcus*, and Enterococcus. This antimicrobial property has been linked to the high concentration of phenolic compounds present in discarded olive mill waste. Such findings underscore the versatility of olive-derived phenolic compounds as a natural antimicrobial agent, offering an environmentally sustainable solution to the growing demand for safer food preservation methods (Caballero Guerrero et al., 2022). Thus, this PhD thesis project will focus on assessing the antimicrobial potential of olive extracts by conducting in vitro and in vivo studies, including MIC and MBC testing, while employing a food model to evaluate a scientific basis for their real-world application. Advanced omics techniques will be utilized to unravel molecular mechanisms underlying the antimicrobial activity, with the goal of establishing olive extract as a sustainable and effective alternative to synthetic preservatives in food safety and preservation.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 2:

A1) **Determination of Optimal *Bacillus* strain for Solid-state fermentation (SSF) of olive leaves,** assessing their enzymatic profile (cellulase, amylase, and peptidase activity) (A1.1), along with their synergistic or antagonistic interaction between Bacillus and Lactic Acids bacteria (A1.2) to enhance the SSF process and efficiently extract the desired phenotic compounds.

A2) **Assessment Quantification, and characterization of phenolic compounds in fermented Olive leaves** to extract (A2.1) and quantify the phenolic compounds (A2.2) by the Folin-Ciocalteu Method for their potent antimicrobial and antioxidant properties, enabling a comprehensive analysis of their bioactive potential for natural preservatives.

A3) **Comprehensive evaluation of antimicrobial properties** to evaluate the antimicrobial properties of olive extract both in vivo using a food model (A3.1) and in vitro by measuring the Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) (A3.2) to determine the optimal concentration of olive extract required to inhibit and eradicate microbial growth, and assessing their effectiveness against foodborne and spoilage pathogens.

A4) **Mechanisms of bacterial growth inhibition** to elucidate the molecular mechanisms underlying microbial growth inhibition at the genetic, protein, and metabolic level by olive extracts through the application of high-throughput omics approaches, including proteomic (A4.1), transcriptomic (A4.2), and metabolomic (A4.3).

A5) **Writing and Editing** of the PhD thesis, scientific papers, and oral and/or poster communications.

Table 2 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Solid-State Fermentation of Olive Leaves***																								
	1) Enzymatic Activity																								
	2) Interactional Behaviour																								
A2)	***Extraction and Quantification of Extract***																								
	1) Extraction of Phenolic Compounds																								
	2) Folin-Ciocalteu Method																								
A3)	***Evaluation of Antimicrobial Properties***																								
	1) In vitro (MIC and MBC)																								
	2) In vivo Food Model																								
A4)	***Mechanisms by Omics***																								
	1) Proteomics																								
	2) Transcriptomics																								
	3) Metabolomics																								
A5)	***Thesis and Paper Preparation***																								

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Microbial biodiversity as a sustainable strategy for innovative plant-based fermented products (P-I-63)

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This PhD thesis research project is aimed at the development of sustainable strategies, based on the valorization of microbial biodiversity, to enhance the quality and nutritional properties of innovative fermented products obtained from unconventional matrices (nut by-products or lower-grade nuts not suitable for retail sale), using tailored fermentation processes.

Biodiversità microbica come strategia sostenibile per prodotti fermentati innovativi a base vegetale

Questo progetto di ricerca per la tesi di dottorato è finalizzato allo sviluppo di strategie sostenibili, basate sullo sfruttamento e valorizzazione della biodiversità microbica, per migliorare la qualità e il valore nutrizionale di prodotti fermentati innovativi ottenuti da matrici non convenzionali (sottoprodotti della lavorazione della frutta a guscio o frutta a guscio di qualità inferiore non adatta alla vendita al dettaglio), utilizzando processi di fermentazione mirati.

1. State-of-the-Art

In recent years, global population growth and rapid urbanization have significantly increased the demand for protein-rich foods in emerging and developing countries. Adopting dietary habits that include functional or multifunctional foods can help maintain and preserve good health. The Mediterranean diet, known for its versatility and adaptability across cultures, promotes overall health and well-being. Healthier diets involve reducing the intake of saturated and trans fats, sugar, and salt, while increasing the consumption of plant-based foods.

Over the past 50 years, the demand for animal-sourced proteins has more than doubled, posing environmental challenges such as land use, water eutrophication, and substantial greenhouse gas emissions. As awareness grows about the negative effects of meat on both the environment and health, plant-based meat substitutes are emerging as healthy and sustainable alternatives to meet the rising demand for meat products. Nuts and legumes, rich in nutrients, trace elements, and vitamins, also contain functional compounds such as phenols and bioactive peptides, making them valuable resources for developing multifunctional foods. Consumer interest in reducing meat consumption and choosing alternative proteins is increasing, driving food market innovations and offering promising opportunities for stakeholder investment.

From this perspective, process innovation and the implementation of green solutions tailored to specific plant-based raw materials and target food models are essential. Fermentation can be a sustainable approach in innovative plant-based processes for creating value-added products, thanks to its low costs, low energy consumption, and minimal wastewater generation. Currently, plant-based products are produced using ultra-processed and thermally treated methods. Fermentation, on the other hand, is a sustainable biotechnological tool because it does not require high-impact or energy-demanding processes. This is an innovative aspect because there are currently not many fermented substitutes available. Typically, substitutes found include frozen hamburgers, extruded products, etc.

Most food fermentations rely on diverse species mixtures capable of performing complex activities with high robustness and reliability, crucial for achieving desired product characteristics. These microbial consortia primarily include Lactic Acid Bacteria (LAB) and Staphylococcus. Thanks to obtaining a master's degree in Food Safety and Food Risk Management, with a particular focus on Food Microbiology, the study and research of safe microorganisms that can be used in the food industry is of great importance.

2. PhD Thesis Objectives and Milestones

The objective of this project is to valorize the microbial biodiversity associated with spontaneous fermentations of matrices of different origins through the characterization of microbial populations and the study of specific strains and their technological and functional performance both *in vitro* and in food systems. The aim is to develop nut-based fermented products with enhanced safety, quality and nutritional properties using sustainable strategies based on tailored fermentation processes.

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities

according to the Gantt diagram given in Table 2:

A1) Literature review and dissemination activities at conferences or/and general audience/stakeholders

A2) Isolation and characterization of lactic acid bacteria (LAB) and autochthonous staphylococci from naturally fermented matrices. This phase aims to provide a comprehensive overview of the microbial biodiversity present in populations isolated from spontaneously fermented foods. These spontaneously fermented products, obtained without the use of starter cultures, will be used as sources of indigenous LAB and staphylococci. Once microbial collection has been established, the strains will be evaluated for safety characteristics (including antimicrobial resistance (AMR) and biogenic amine production) and technological properties.

A3) Design of microbial consortia for the fermentation of plant-based substrates (e.g., nuts and legumes). This phase will focus on exploring different combinations of microbial strains to create consortia capable of efficiently fermenting specific plant-based substrates, including their by-products or waste materials (e.g., broken, aesthetically defective, or undersized nuts).

A4) Optimization of the fermentation process, characterization of the obtained prototypes, and improvement of safety and quality features. The fermentation process will be initiated on a laboratory and/or pilot scale, with the goal of characterizing the resulting prototype, optimizing product safety, aroma profile, and potential shelf life.

A5) Thesis writing.

Table 2 Gantt diagram for this PhD thesis project.

Activity	Month	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**	**26**	**28**	**30**	**32**	**34**	**36**
A1)	***Literature review***																		
A2)	***Isolation and characterization of microbial strains***																		
	1) Isolation and purification of isolates (LAB and staphylococci)																		
	2) Molecular identification of strains																		
	3) Characterization of strains for safety traits, technological properties, and antimicrobial activity																		
A3)	***Design of microbial consortia for fermentation***																		
	1) Fermentation of a model plant-based substrate using pairs of LAB strains																		
	2) Addition of selected staphylococci to LAB consortia and characterization of the aroma profile																		
	3) Optimization of fermentation for different substrates																		
A4)	***Optimization of the fermentation process***																		
	1) Analysis of biological risks associated with innovative fermented products from nuts or legumes																		
	2) Improvement of the functionality and quality of the obtained innovative fermented products																		
	3) Sensory evaluation of innovative and unconventional fermented products																		
A5)	**Thesis writing**																		

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Sustainable Production of Bio-Based Surfactants (P-I-64)

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This PhD project investigates a sustainable synthesis pathway for alkyl polyglucosides (APGs) using glucose and fatty alcohol. It integrates laboratory and industrial pilot-scale activities to establish scalable processes under green chemistry principles. The research focuses on catalytic system optimization, mathematical modeling, and real-time industrial applicability of bio-based surfactant production.

Produzione sostenibile di tensioattivi bio-based

Questo progetto di dottorato mira a sviluppare un processo sostenibile per la sintesi di alchil poliglucosidi (APGs) utilizzando glucosio e alcoli grassi. Le attività si svolgeranno sia in scala di laboratorio sia in scala pilota industriale, seguendo i principi della chimica verde. La ricerca si concentrerà sull'ottimizzazione dei catalizzatori, modellazione matematica e applicabilità realedei tensioattivi bio-based.

1. State-of-the-Art

Alkyl polyglucosides (APGs) are non-ionic surfactants synthesized from renewable resources such as glucose and fatty alcohols, offering excellent biodegradability, low toxicity, and dermatological compatibility [1]. Their growing use in detergents, cosmetics, and food applications reflects the increasing demand for eco-friendly alternatives to petrochemical surfactants. Industrial APG production traditionally relies on acid-catalyzed transglycosylation to address the immiscibility between glucose (hydrophilic) and fatty alcohols (hydrophobic). While effective, this method requires multiple purification steps, operates under harsh conditions, and consumes significant energy [2]. Direct glycosylation is a simpler one-step alternative that reduces intermediate costs but presents challenges such as water removal, glucose degradation, increased viscosity, and difficulty in controlling selectivity [3]. A major challenge in APG synthesis is the formation of polyglucose by-products, which increase the viscosity of the reaction mixture, complicated product separation, and hinder recycling of unreacted components [4].

Catalysis plays a central role in addressing these limitations. Common acid catalysts include H_2SO_4, HCl, and sulfonic acids, but these often compromise environmental performance [5]. Recent innovations have explored heterogeneous and green catalysts. Karam et al. introduced Aquivion, a solid superacid that achieved up to 85% yield under emulsion conditions [6]. Van Es et al [7]. demonstrated the use of 5-furandicarboxylic acid as a co-catalyst to enhance phase transfer and reduce acid dosage. Zeolites have also proven effective due to their high selectivity and environmental compatibility [8]. Process intensification strategies have further improved APG synthesis. Roy and Mukhopadhyay [9] developed a H_2SO_4–silica hybrid catalyst that improved efficiency and reduced alcohol use. Additionally, ionic liquids and triflate-based catalysts have shown promise for selective glycosylation with lower reagent consumption [10]. These advancements indicate strong potential for developing a sustainable and scalable APG production process, making use of modern catalysts and integrated modeling to optimize performance and minimize environmental impact.

2. PhD Thesis Objectives

- Design and construction of a dual-scale synthesis setup for APGs.
- Study the reaction behavior of glucose and dodecanol across process variables.
- Evaluate catalyst efficiency and develop a reaction kinetics model.
- Characterize final products using rheology, spectroscopy, and chromatography.
- Transfer laboratory findings to pilot-scale implementation.

3. Methodology and Activities

The research is structured into five phases:
A1) Preliminary design and literature review
A2) Laboratory setup development and process optimization
A3) Catalyst research abroad with integration and advanced characterization
A4) Pilot-scale industrial validation
A5) Thesis writing and dissemination

4. Expected Results

The project aims to deliver a robust and scalable method for APG production, with improved catalyst systems, validated reaction models, and strong industrial relevance. It will contribute to scientific knowledge in green surfactant production and foster sustainable process development in collaboration with industry.

Table 1: Gantt diagram for this PhD thesis project.

ACTIVITIES	1nd Year						2nd Year						3rd Year					
	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1 – Preliminary design & literature review																		
A2 – Lab setup & process optimization "analytical validation with commercial APG samples"																		
A3 – Catalyst study & advanced characterization (abroad)																		
A4 – Pilot-scale validation																		
A5 – Thesis writing & dissemination																		

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Exploring plant-microbiome synergy for hydrocarbon bioremediation of polluted soils (P-I-65)

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This PhD project explores how plants and soil microbes can work together to clean up hydrocarbon-polluted soils. It investigates the plant's ability to attract helpful bacteria through root exudation - a process known as the "*cry for help"* - with the final aim to enhance rhizoremediation. Using multiomics tools, it will study how microbial communities in polluted soil respond to biostimulation by plants and bioaugmentation practices, identifying the bacteria involved in hydrocarbon degradation and key root exudates in the plant-microbe crosstalk. The goal is to build sustainable solution based on plant-microbe interactions to clean up polluted soil preserving ecosystem health.

Studio della sinergia pianta-microbioma per il biorisanamento degli idrocarburi nei suoli inquinati

Questo progetto di dottorato studia l'interazione pianta-microbioma per il biorisanamento di suoli contaminati da idrocarburi. Studiando la capacità delle piante di attrarre batteri benefici, mediata dall'essudazione radicale (i.e., "*cry for help*") il progetto ha l'obiettivo finale di potenziare il "*rizorisanamento*". Utilizzando un approccio multi-omics, il progetto studierà come il microbioma nei suoli contaminati risponda alla biostimolazione da parte delle piante e alle pratiche di bioaugmentation, identificando i batteri coinvolti nella degradazione degli idrocarburi e gli essudati radicali rilevanti nel dialogo pianta-microrganismo. L'obiettivo è sviluppare soluzioni sostenibili basate sulle interazioni pianta-microbioma per bonificare i suoli contaminati, preservando la salute degli ecosistemi.

1. State-of-the-Art

Soil is a vital resource supporting life and ecosystem services. However, this precious resource faces severe threats and according to the Food and Agriculture Organization (FAO, 2021), over half of global agricultural land is already degraded through erosion, salinization, desertification, and pollution. As world population grows, degraded soils must be reclaimed for critical needs like food and energy production (Sekhohola-Dlamini et al., 2022). Soil pollution from anthropogenic activities, especially petroleum hydrocarbons (PHCs), poses severe natural ecosystem. These contaminants disrupt microbial soil communities and harm ecosystem health, affecting both human and plant well-being (Truskewycz et al., 2019; Haider et al., 2021)

Traditional methods cannot be applied to clean up historically large hydrocarbon-contaminated sites since they can further harm soil health (Petruzzelli et al., 2016), hence it is crucial to develop Nature-Based Solutions. Phyto-rhizoremediation represents a sustainable alternative that leverages the powerful partnership between plants and their associated microbes (Vergani et al., 2017), i.e. the plant holobiont. This approach works through the fascinating "*cry-for-help*" phenomenon, where plants under stress from contaminants release specific root exudates involved in the recruitment of bacteria capable of degrading the pollutants (Rolli et al., 2021). Indeed, certain bacterial species exhibit remarkable proficiency in degrading a range of hydrocarbon substrates through different pathways, possessing enzymes like *alkB* and *xylE* (Varjani, 2017). Additionally, plant growth-promoting bacteria (PGPB) support plants by decreasing environmental stress and boosting the development of larger root systems, which is crucial for the rhizoremediation success (Correa-Garcia et al., 2018).

The EU project 'Nymphe' targets different highly polluted sites, including an industrial site located in Italy, historically polluted by PHCs. In this project, which is part of 'Nymphe', my aim is to discover optimal plant-microbe combinations for PHCs-contaminated soils to clarify the plant holobionts' perform in terms of growth and degradation activity. Moreover, I will clarify how native soil microbiomes of the target polluted soil respond to the supplement of degrading bacteria (bioaugmentation) and to biostimulation by plants. I will use synthetic microbial communities (SynComs) to design simplified, well-defined systems for the identification of causal relationships in plant–microbiome interactions (Großkopf and Soyer, 2014). SynComs will be designed using rhizobacteria isolated from historically polluted sites and then administered to plant species selected for soil bioremediation, e.g. *Helianthus annuus,* under outdoor conditions. This research aims to develop practical applications for rhizoremediation, deepening the understanding of the complex factors, including root exudation patterns, that rule beneficial relationships between plants and microbes.

2. PhD Thesis Objectives and Milestones

The project focuses on: (i) selecting plant species and Petroleum hydrocarbons (PHCs) - degrading strains using historically contaminated soil; (ii) investigating plant–microbiome interactions under biostimulation and bioaugmentation; (iii) designing and applying SynComs to assess holobiont-based HC degradation; and (iv) unravelling root exudation in response to pollution. These objectives will be pursued through molecular techniques (e.g., 16S rRNA sequencing, qPCR), culturomics, and metabolomics, according to the activities scheduled as detailed in the Gantt diagram (Table 1). For each activity, milestones are also indicated.

A1) **Study of plant-microbe interactions in hydrocarbon polluted soil** from a target polluted site to evaluate the growth performance of candidate plant species and to understand ecological shifts in the rhizosphere microbiome **(**M1.1: plant species candidate made available for the rhizoremediation approach, M1.2: description of the changes in the soil bacterial community).

A2) **Culturomics approach and biodegradation performance** in terms of isolating and characterizing bacteria from rhizosphere soils of plants grown in hydrocarbon-contaminated environments, evaluating their plant-growth-promoting and degradative abilities. Selected strains will be assembled into synthetic communities (SynComs) and tested for their effectiveness in enhancing plant performance and hydrocarbon biodegradation. (M2.1: Novel collection of candidates strains, M2.2: Test of five different holobionts)

A3) **Metabolomics to identify molecules of relevance for HC rhizoremediation** aims to identify root exudate metabolites released by plants in hydrocarbon-contaminated soils using untargeted metabolomics. These compounds will be analysed for their role in shaping microbial communities and enhancing hydrocarbon degradation. (M3.1: Identification of root exudates, M3.2: Activity of the identified root exudates on the structure and degrading potential)

A4) **Dissemination and thesis preparation:** scientific research outputs will be disseminated pursuing the open diffusion of scientific results. Participation in public engagement activities is foreseen. (M4.1: participation every year to national/international conferences, public events, and publication of open-access articles).

Table 1. *Gantt diagram for this PhD thesis project*

Activity/month		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Study of plant-microbe interactions in hydrocarbon polluted soil***																								
	1) Identification of candidate plant species																								
	2) Investigate changes in the structure of rhizosphere																								
A2)	***Culturomics approach and biodegradation performance***																								
	1) Novel collection of candidates strains																								
	2) Test of five different holobionts																								
A3)	***Metabolomics to identify molecules of relevance for HC rhizoremediation***																								
	1) Identification of root exudates																								
	2) Role of identified root exudates on the microbiome structure and degrading potential																								
A4)	***Dissemination and thesis preparation***																								

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Development of sustainable strategies for effective delivery of probiotics and bioactive substances (P-I-66)

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This PhD thesis project aims to develop microcapsules based on chitosan, pectin, and possibly inulin to effectively deliver probiotics to the colon, with the goal of improving their viability and efficacy in subjects with irritable bowel syndrome (IBS). Microencapsulation aims to protect probiotics from various factors such as temperature, humidity, and especially the harsh conditions present in the gastrointestinal tract, thereby promoting beneficial effects and supporting sustainable and functional solutions for intestinal health.

Sviluppo di strategie sostenibili di delivery efficace di probiotici e sostanze bioattive

Questo progetto di tesi di dottorato si propone di sviluppare microcapsule a base di chitosano, pectina ed eventualmente inulina per veicolare probiotici in modo efficace fino al colon, con l'obiettivo di migliorare la vitalità e l'efficacia nei soggetti con sindrome dell'intestino irritabile (IBS). La microincapsulazione, infatti, mira a proteggere i probiotici da diversi fattori quali temperatura, umidità ma soprattutto dalle condizioni ostili presenti nel tratto gastrointestinale, favorendo così un effetto benefico e promuovendo soluzioni sostenibili e funzionali per la salute intestinale.

1. State-of-the-Art

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder characterized by chronic abdominal pain or discomfort and disturbances in intestinal homeostasis. Although the incidence of IBS is increasing, its etiology and pathogenesis remain poorly understood (Kim et al., 2017). IBS symptoms are partly due to increased intestinal mucosal permeability, which exposes the underlying layers to abnormal contact with irritants normally present in the intestine, making it hypersensitive. Therapeutic approaches for IBS aim to reduce localized inflammation, promote mucosal healing, and prevent disease progression (Kotla et al., 2019). One of the most commonly adopted strategies to combat IBS involves the use of probiotic microorganisms, which help restore intestinal eubiosis and repair the damaged and inflamed intestinal barrier. Probiotics are defined as live and viable microorganisms that, when administered in adequate amounts, confer a health benefit to the host. An important criterion for a potential probiotic strain is its ability to adhere to the mucosal surfaces of the human intestinal tract (Verdenelli et al., 2009). Probiotics are sensitive to external factors such as temperature and humidity and must also withstand the harsh conditions of gastric acid and bile acids in the gastrointestinal tract. Therefore, it is essential to improve the stability and viability of probiotic strains during their passage through the gastrointestinal tract to ensure effective delivery to the target site in the colon, where they can exert positive effects. Microencapsulation of probiotic cells is an increasingly popular approach that offers an innovative solution to the challenges of probiotic stability and survival. This technique provides a physical barrier to probiotic cells, helping maintain their viability during production, storage, and transit through the gastrointestinal tract, with controlled and targeted release. The biopolymers used must be biocompatible, non-toxic, and ensure effective protection of probiotic cells (D'Amico et al., 2024). Lactic acid bacteria are of particular interest due to their abundance and presumed benefits for intestinal health, especially Lactobacillus. Most Lactobacillus species are common, non-pathogenic inhabitants of the human and animal intestine, and their presence is important for maintaining the intestinal microbial ecosystem (Verdenelli et al., 2009). To ensure effective activity of probiotic microorganisms, they must survive the adverse conditions of the preceding sections— the stomach and small intestine—alive and viable. Therefore, this project proposal aims to develop a method to encapsulate probiotics to improve their viability and enable targeted delivery to the colon.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 2:

A0: Literature search

A1: Development of an innovative solution: Characterization of raw materials (chitosan, pectin, and inulin) to establish a protocol for microcapsule formulation. Characterization of the cell culture and evaluation of probiotic viability through plate counting, followed by tests for gastro-resistance, shelf-life, and heat shock tolerance.

A2: In vivo study: Preparation of ethical documentation and experimental protocols, followed by the selection, enrollment, and monitoring of clinical study participants before and after the administration of probiotic microcapsules.

A3: Analysis of fecal samples: Real-Time PCR analysis to quantify the viability and integrity of the encapsulated strain, 16S rRNA gene sequencing to characterize the composition of the intestinal microbiota, and fecal metabolome analysis to identify and quantify key metabolites.

A4: Writing and Editing of the PhD thesis, scientific papers and oral and/or poster communications.

Table 2. Gantt diagram for this PhD thesis project.

Activity	**Months**	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**	**26**	**28**	**30**	**32**	**36**
A0)	***Literature search***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
A1)	***Development of an innovative solution***				■	■	■	■	■	■	■							
A2)	***In vivo study***											■	■	■				
A3)	***Analysis of fecal samples***														■	■	■	
A4)	***Thesis and Paper Preparation***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

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Functional foods: set-up of biotechnology processes, design and market strategies (P-I-67)

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This PhD project aims to develop biotechnological processes for exploiting plant-based matrices to obtain functional ingredients for use in formulating baked products, thereby creating products with enhanced nutritional profiles and extended shelf life. Food products with better technological and nutritional performance on a pilot scale will then be transferred to an industrial scale.

Alimenti funzionali: set-up di processi biotecnologici, design e strategie di mercato

Questo progetto di dottorato mira a mettere a punto dei processi biotecnologici, atti a valorizzare matrici di origine vegetale, al fine di ottenere ingredienti funzionali da inserire nella formulazione di prodotti lievitati da forno, al fine di creare prodotti con un profilo nutrizionale migliorato e con una shelf life prolungata. I prodotti con migliori performance tecnologiche e nutrizionali su scala pilota verranno in seguito trasferite su scala industriale.

1.State of the Art

In recent years, the perception of food has evolved beyond taste and nutrition to encompass its role in improving human health. Both consumer and scientific communities have increasingly paid attention to the development of functional foods that, in addition to their basic nutritional value, offer health benefits (Pinto *et al*., 2014). The bakery industry is actively pursuing innovation, aiming to enhance nutritional quality, food safety, and product sustainability.The integration of biotechnological processes such as fermentation (e.g., sourdough-like) not only enriches baked products with bioactive compounds that promote human health, but also improves microbiological stability, successfully extending shelf-life (Gobbetti *et al.,* 2020). In this context, bio-preservatives derived from microbial fermentation of plant matrices present a viable alternative to synthetic preservatives commonly used in this sector (Coda *et al*., 2011). This approach aligns with consumer demand for "clean-label" products, while maintaining product quality, hygiene and self-life.

A major concern in the grains and baking sector is mycotoxins contamination, which poses public health risk due to their presence in cereal grains intended for bread making. The impact of baking on different mycotoxins is minimal, as these compounds are highly resistant to heat. Additionally, pesticide residues in grains represent another source of toxic exposure with long-term adverse effects. Biotechnology is emerging as an innovative tool for mitigating these contaminants. A promising strategy for reducing mycotoxin content includes fermentation with selected lactic acid bacteria (Zadeike *et al*., 2021) and grain germination, which activates endogenous enzymes capable of reducing toxicological load (Pakfetrat *et al*., 2019).

Beyond reducing contaminants, germination enhances the bioavailability of nutrients and bioactive compounds in grains and the resulting flour, by improving protein quality and increasing amino acids, sugars, and vitamins. These combined effects contribute to producing safer, more functional, and sustainable baked goods. Baked goods have long been a staple in the human diet and fortifying them with functional ingredients is an effective strategy to provide healthy and safe nutrition. Legumes, for example, are valuable sources of protein, dietary fibre, vitamins, minerals, carbohydrates and phytochemicals essential for human health. However, their use in bakery products is limited due to poor rheological properties from the absence of gluten. Moreover, many plant-based matrices contain functional compounds in a non-bioavailable form, which may not be sufficiently utilized (Gobbetti *et al.,* 2020). The full potential of these unconventional ingredients can be harnessed through biotechnological processes such as fermentation.

Another opportunity to improve the nutritional profile and quality of foods, while enhancing sustainability, is the valorization of plant-based by-products. A wide range of food residues and by-products of plant origin are generated during the processing of fruits, vegetables, cereals, legumes, and plant-based beverages. Their characteristics vary depending on source; nevertheless, they represent a rich and sustainable source of bioactive compounds with health benefits including prevention of coronary heart disease, obesity, diabetes, and cancer. Microbial metabolism during fermentation can form novel nutritionally active compounds, such as peptides and amino acid derivatives (e.g., γ-aminobutyric acid), exhibiting various functional properties, as well as potentially prebiotic exopolysaccharides.

Moderate doses can improve certain rheological and nutritional properties by increasing protein solubility and enzymatic activity. Fibres and polyphenols present in these matrices affect starch texture and retrogradation, helping maintain bread freshness. This suggests that natural ingredients could replace chemical additives with similar technological functions (Gobbetti *et al.,* 2020).

The synergistic integration of fermentative biotechnology, germination techniques and bio-preservation are promising innovative approaches for developing functional foods in the baking industry. These strategies, in addition to improving nutritional profile and food safety, respond to the needs of a market increasingly oriented towards natural products with a positive impact on consumer health and agri-food sustainability.

2. PhD Thesis Objectives and Milestones

Within the overall objective outlined above, this PhD thesis project can be subdivided into the following activities, as detailed in the Gantt chart presented in Table 1:

A1) **Combination of lactic bacteria and vegetable flours as bio preservatives in the baking industry:** A1.1. Development of legume-based sourdoughs using selected lactic acid bacteria and non-Saccharomyces yeast strains, both in combination and individually; A1.2. Assessment of the antifungal activity and identification of bioactive antimicrobial compounds in sourdough extracts; A1.3. Pilot- and industrial-scale production of sandwich bread incorporating the bio-preservative and evaluation of consumer acceptability.

A2) **Effect of the sprouting and/or fermentation with lactic acid bacteria on the content of mycotoxins and pesticides in contaminated wheat:** A2.1. Fermentation of contaminated flours with mycotoxins and pesticides using 20 lactic acid bacteria strains previously selected for their antifungal properties and followed by quantitative analysis of residual contaminants; A2.2. Implementation of fermentation and germination processes under the most effective conditions identified, with investigation of the biochemical mechanisms responsible for contaminant reduction.

A3) **Production of nutrient-enriched Apulian bakery products and pasta using protein fractions derived from cereals, legumes, and oat/tomato okara:** A3.1. Microbiological characterization of plant-based matrices and selection of starter cultures and enzymes with pro-technological traits and the ability to modulate nutritional and functional properties; A3.2. Development, production, and industrial-scale validation of biotechnological protocols for the valorization of plant-based matrices. Evaluation of consumer acceptability.

A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity \ Month		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Bio-preservatives in bakery***																								
	1																								
	2																								
	3																								
A2)	***Sprouting and LAB fermentation effects on contaminants in wheat***																								
	1																								
	2																								
A3)	***Nutrient-enriched bakery and pasta from vegetable by-products***																								
	1																								
	2																								
A5)	***Thesis and paper preparation***																								

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Beyond Probiotics: Unravelling the Secret Weapons of VSL#3 as a Postbiotic (P-I-68)

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Based on preliminary data obtained in mouse studies that have highlighted the probiotic potential of the postbiotic version of VSL#3, this PhD thesis project aims to perform a comparative characterization at subcellular level between the inactivated strains of VSL#3 compared to their viable counterparts. Specifically, we want to obtain the maximum possible characterization of the bacterial cells constituting VSL#3 following the thermal inactivation process to which they are subjected in view of their commercialization as postbiotics.

Oltre i probiotici: svelare le armi segrete di VSL#3 come postbiotico.

Sulla base di dati preliminari ottenuti in studi su topo che hanno evidenziato le potenzialità probiotiche della versione postbiotica del VSL#3, questo progetto di tesi di dottorato mira a effettuare una caratterizzazione a livello subcellulare comparativa tra i ceppi inattivati di VSL#3 rispetto ai corrispettivi vitali. Nello specifico, si vuole ottenere la caratterizzazione massima possibile delle cellule batteriche costituenti il VSL#3 a seguito del processo di inattivazione termica a cui sono sottoposte in previsione di una loro commercializzazione come postbiotici.

1. State-of-the-Art

The concept of probiotics—as bacteria that confer health benefits to their host—can be traced back to the early 20th century, when Ilya Ilyich Metchnikoff observed that regular consumption of lactic acid bacteria, particularly through fermented dairy products like yogurt, was linked to improved health and increased longevity among residents of Bulgarian villages. Nowadays, probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill *et al*., 2014). They are usually ascribed to a narrow range of genera, mainly including *Pediococcus*, *Lactococcus*, *Enterococcus*, *Streptococcus*, *Propionibacterium*, and *Bacillus* but also yeasts such as *Saccharomyces* spp. On the market there are mainly two different forms of probiotics preparations, single-strain probiotics or multi-strain and multi-species probiotic mixtures. The current trend favors multi-strain and multi-species probiotics, as they may be more effective than single-strain probiotics in restoring gut dysbiosis and enhancing immunomodulation, thanks to the synergistic effects of different strains in inhibiting pathogens and increasing adhesion to the gut epithelium. VSL#3 is considered as one of the most extensively researched multi-strain and multi species probiotic formulations, consisting of four strains of *Lactobacillus* (*Lactobacillus acidophilus*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus paracasei*, and *Lactobacillus helveticus*), three strains of *Bifidobacterium* (*Bifidobacterium breve,* two strain of *Bifidobacterium animalis* subsp. *lactis*), and one strain of *Streptococcus* (*Streptococcus thermophilus*). Many studies have proved its efficacy in improving intestinal barrier functions, which makes VSL#3 a proper medical food for treating specific conditions such as ulcerative colitis (UC), pouchitis, and irritable bowel syndrome (IBS) (Cheng *et al*., 2020). Other studies have been carried out to detail the genomic data and the safety of VSL#3 strains and to propose reliable criteria for the evaluation of the quality of this product (Duillard *et al.*, 2018; Mora *et al.*, 2019). As a probiotic formulation, viability of all the eight strains is an unavoidable prerequisite and in the case of VSL#3 it must be keep by applying cold chain all over the commercialization and storage step. This necessity might limit the accessibility and commercial scalability of VSL#3 in comparison to other probiotics that are stable at room temperature. In this context, postbiotics—defined as "a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" (Salminen *et al.*, 2021)—offer several advantages, including greater stability during industrial processing, enhanced safety and predictability, longer shelf life, and easier transport and storage. There are growing number of evidence according to which these heat-inactivated microorganisms retain their functionality despite the lack of viability thanks to specific cellular structures and metabolites such as teichoic acids, exopolysaccharides, peptidoglycan and organic acids. Mechanisms driving postbiotic efficiency main consist in three types: protective modulation against pathogens, fortification of the epithelial barrier and modulation of immune response (Ma *et al*., 2023). As far as heat-inactivated VSL#3 is concerned, it has been demonstrated that heat-killed VSL#3 ameliorate DSS-induced colitis in SD rats through its immunomodulatory and anti-inflammatory activities (Sang *et al*., 2013). Another study proved that heat-inactivated VSL#3 can still induce dendritic cell maturation in vitro and modulate cytokine production, supporting immunoregulatory functions (Mastrangeli *et al*., 2009). Despite such promising results, little is known about the role of each inactivated strain in determining the protecting effect on gut barrier of VSL#3 as a postbiotic. Moreover, the detailed characterization of specific cellular components responsible for the efficacy of inactivated VSL#3 and the exact molecular mechanisms involved remain less explored and are an emerging area of research. Thus, this PhD project will be directed to conduct a deep characterization of inactivated VSL#3 strains at sub-cellular level in comparison to viable strains, developing a scientific background knowledge needed for

commercializing a multi-species postbiotic product with reduced transport and storage costs.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Comparison between viable and inactivated freeze-dried formulations (whole product and single strains)** in terms of comprehensive proteome (A1.1) and quantification of organic acids and sugars (A1.3).

A2) **Comparison between viable and non-active freeze-dried single strains** in terms of potential differences in cells' integrity of viable and inactive single strains together with morphological differences (A2.1) and in vitro resistance to gastrointestinal transit (A2.2). Isolation of cell walls and specific cellular components (A2.3) will be also performed for each viable and inactive strain to perform A3 activities.

A3) **In vitro screening of bioactivities of viable and inactive lyophilized cells of VSL#3 single strains, isolated cellular components and supernatants** intended as improvement of gut barrier permeability (A3.1), anti-inflammatory activity (A3.2) and immunomodulation (A3.3).

A4) **In vivo cross-over study for the evaluation of viable and inactive lyophilized cells of VLS#3 on healthy subjects** to assess their modulatory effect on the gut microbiota of healthy subjects (A4.1).

A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
A1)	***Comparison between viable and inactivated freeze-dried formulations (whole product and single strains)***																																				
	1) Proteomic analysis																																				
	2) Quantification of organic acids and sugars																																				
A2)	***Comparison between viable and non-active freeze-dried single strains***																																				
	1) Cells' integrity and morphological differences																																				
	2) In vitro analysis of cell's integrity after gastro-intestinal transit (INFOGEST)																																				
	3) Isolation of cell wall and cellular components																																				
A3)	***In vitro screening of bioactivities of viable and inactive lyophilized cells of VSL#3 single strains, isolated cellular components and supernatants***																																				
	1) Gut barrier permeability																																				
	2) Anti-inflammatory activity																																				
	3) Immunomodulation																																				
A4)	***In vivo cross-over study for the evaluation of viable and inactive lyophilized cells of VLS#3 on healthy subjects***																																				
	1) Gut microbiota's modulation by viable and inactive lyophilized cells of VLS#3																																				
A5)	***Thesis and Paper Preparation***																																				

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Biotechnological innovations for the reduction of microbiological risk in the dairy supply chain (P-I-69)

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This PhD thesis research project is aimed to develop and transfer innovation to the dairy industry, supporting it in the creation of innovative solutions that can reduce the microbiological risk and/or counteract the development of deteriorating microorganisms that lead to the production of cheeses of unacceptable quality and which as such become waste. The application of biotechnological processes, through the use of selected indigenous microorganisms and/or from dominant microbial consortia found in artisanal cheeses, will aim to characterize and determine the microbiota of whey starters and, where applicable, to isolate protechnological microorganisms, protective cultures (biopreservative), and probiotics, evaluating their ability to act as native starter cultures for the production of fresh and maturated cheeses for a better management of microbial resources through controlled fermentation processes in order to reduce overall food losses.

Innovazioni biotecnologiche per la riduzione del rischio microbiologico nella filiera lattiero-casearia

Questo progetto di ricerca per la tesi di dottorato ha l'obiettivo di sviluppare e trasferire l'innovazione all'industria lattiero-casearia, supportandola nella creazione di soluzioni innovative in grado di ridurre il rischio microbiologico e/o contrastare lo sviluppo di microrganismi deterioranti che portano alla produzione di formaggi di qualità inaccettabile e che, come tali, diventano rifiuti. L'applicazione di processi biotecnologici, attraverso l'uso di microrganismi selezionati autoctoni e/o provenienti da consorzi microbici dominanti nei formaggi artigianali, avrà lo scopo di caratterizzare e determinare il microbiota dei siero-innesti ed isolarne eventualmente microrganismi protecnologici, colture protettive (biopreservanti) e probiotici, valutandone la capacità di fungere da colture starter autoctone per la produzione di formaggi freschi e stagionati per una migliore gestione delle risorse microbiche attraverso processi di fermentazione controllata al fine di ridurre a sua volta le perdite alimentari complessive.

1. State-of-the-Art

Food safety is a critical component in dairy products, especially when they are made from raw milk as is the case for some artisanal cheeses, which are often associated with microbiological risks related to the presence of pathogens such as *Listeria monocytogenes, Salmonella spp. ed Escherichia coli spp.* (EFSA, 2014a; EFSA Journal, 2022; EFSA and ECDC, 2023). Microbial contamination of dairy products can occur at various points along the production and processing continuum and includes organisms such as Gram-negative bacteria, Gram-positive bacteria, and a wide range of fungal organisms (Martin et al., 2021). Currently, microbial spoilage of dairy products is one of the main causes of worldwide food waste (FAO, 2011). Psychrotrophic microorganisms can increase their cell densities and produce hydrolytic enzymes causing rancidity, softening, defects in odor and flavor, and pigment production (Quinteri et al., 2019), eventually rendering dairy products inedible (Martin et al., 2021). Indeed, especially fresh cheeses represent an ideal substrate for microbial growth and are therefore susceptible to spoilage organisms depending on their physico-chemical characteristics such as high moisture content (50-60%), relatively low NaCl concentration, high water activity (Aw), and moderately acidic pH (5.2-5.5); thus, when spoilage occurs, the entire product or part of it necessarily becomes waste, increasing food loss. Another major cause of economic losses for dairy companies is associated with microbiological spoilage due to the presence of clostridia, Gram-positive anaerobic spore-forming bacteria responsible for late blowing defects (Le Bourhis et al., 2007; Gómez-Torres et al., 2015; Bermúdez et al., 2016). When advantageous environmental conditions occur in cheese, clostridial spores contaminating silage used to feed livestock enter the raw milk through the food chain and may germinate into vegetative cells producing acetic acid, butyric acid, carbon dioxide, and hydrogen through the fermentation of lactate (Garde et al., 2012), thus causing gas-blown cheese with eyes and fractures in the structure of hard cheeses with long ripening times (Bassi et al., 2020). Therefore, effectively counteracting the development of spoilage organisms and their persistence in the dairy sector does not represent a simple challenge, as it would require the development of different strategies to prevent their growth without affecting the quality of the finished product. Using microbial starter cultures is fundamental for production purposes, but they may cause the loss of typical organoleptic characteristics of artisanal cheeses (Leboš Pavunc et al., 2012). Using natural whey starters, on the other hand, allows both the activation of lactic fermentation, acidification, and the inhibition of undesired microorganisms, contributing to the definition of sensory and structural typical properties of cheeses. This approach is emblematic in the production of Italian spun paste cheeses, where the whey starter favours the selection of a thermophilic and aciduric lactic microflora (Beresford et al., 2001; Santarelli et al., 2008; Parente and Cogan, 2004). However, the microbial composition of whey starters can vary depending on seasonal and geographical factors, related to the microflora of the original raw milk (Fortina et al., 1998; Giraffa et al., 2004; Rossetti et al., 2008). Therefore, the aim of this research is to isolate and characterize the dominant lactic acid bacteria (LAB) in artisanal fresh cheeses and to evaluate their capacity to function as autochthonous starter cultures

to produce fresh and ripened cheeses, selecting protechnological microorganisms, protective (biopreservative) cultures, and probiotics, thus enhancing the value of whey starters.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Chemical and microbiological characterization for determination of the whey microbiota and qualitative analysis of fresh cheeses (Mozzarella, Burrata, Primosale) and Caciocavallo obtained from them:** microbiological characterization by colture-dependent methods.

A2) **Isolation and selection of Lactic Acid Bacteria and optimization of fermentation protocol:** isolation of "autochthonous" LAB starting from the same matrix or after a spontaneous fermentation process of dairy products; LAB selection with strong fermentative (growth and acidification curve) and proteolytic activity. Development and optimization of the fermentation process by modulating temperature, duration and percentage of inoculum.

A3) **Characterization of isolates:** study of protechnological, probiotic, and antimicrobial activity.

A4) **Creation of biobank, reconstitution of whey starters and their validation by micro-cheesemaking trials analysis by:** culture methods (using selective culture media and mass spectrometry), metagenetic (partial sequencing of 16S genes), metagenomics (sequencing of genomes present in the whey microbiota), metatranscriptomics (sequencing of microbial transcripts under different technological conditions) in order to isolate the strains present (dominant and sub-dominant) and to understand the role of each of them both in the stability of the whey starter and in the enzymatic contribution in the production of cheeses.

A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 *Gantt diagram for this PhD thesis project.*

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A0)	***Literature search***																								
A1)	***Chemical and microbiological characterization of the whey microbiota and qualitative analysis of fresh cheeses***																								
A2)	***Isolation and selection of Lactic Acid Bacteria and optimization of fermentation protocol***																								
A3)	***Characterization of isolates***																								
A4)	***Creation of biobank, reconstitution of whey starters and their validation by micro-cheesemaking trials***																								
A5)	***Thesis and Paper Preparation***																								

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Hybrid nature-inspired metal chelators as antifungal agents for crop protection (P-I-70)

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The PhD research project aims at the design and synthesis of nature and nature-inspired multitarget agents with high antimicrobial activity for crop protection. These compounds will be obtained by merging optimized hydroxycinnamic acid dimers with siderophoric moieties, aiming at exploiting a multitarget approach to overcome the insurgence of resistant strains.

Agenti chelanti ibridi d'ispirazione naturale come antifungini per la difesa delle colture

Questo progetto di ricerca è finalizzato alla progettazione e sintesi di agenti multi-target, naturali e analoghi, dotati di elevata attività antimicrobica per la protezione delle colture. Questi composti saranno ottenuti combinando dimeri di acidi idrossicinnammici più attivi con gruppi sideroforici, al fine di sviluppare un approccio multi-target capace di contrastare efficacemente l'insorgenza di ceppi resistenti.

1. State-of-the-Art

Research, development and innovation in agricultural and food sectors are the keywords to address many of the Sustainable Development Goals (SDGs) established by the UN Agenda 2030. Among them "Zero Hungher", which represents a priority, requires both improvement in agricultural productivity and reduction of food waste. However, crop diseases caused by a heterogenous group of phytopathogens, continue to pose a serious threat to food security, worsening poverty and malnutrition worldwide.

Despite their known adverse effects on health and environment, chemical agents remain indispensable for agricultural development and production. The widespread of pesticides has facilitated the emergence of multi-drug-resistant microorganisms (MDR), highlighting the need to discover and develop novel, high-efficient and low-risk compounds. In this context, natural products (NPs) are an important source of pesticide active ingredients (AIs).

Among these, phytoalexins and phytoanticipins, which are specialized metabolites biosynthesized by plants under biotic and abiotic stress involved in growth and defense mechanisms, gained great interest.

For example, hydroxycinnamic acid dimers identified in barley and beer are described as promising antifungal compounds. One of the most studied ferulic acid dimer is poacic acid. Its antifungal properties are believed to be related to multiple modes of action, including interference with chitin production, disruption of fungal cell wall β-1,3-glucan production and assembly and metal homeostasis.

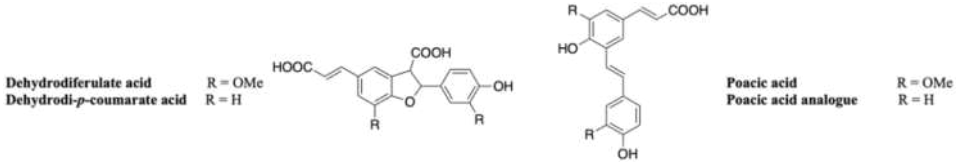


Figure 1 *Examples of natural hydroxycinnamic acid dimers*

Metal ions play a pivotal role in a plethora of physiological processes across all forms of life. Iron is in fact involved in redox systems, regulation of protein stability and participates as a cofactor in numerous enzymatically catalyzed reactions. In plants, iron controls growth and development processes, and in microorganisms it can trigger pathogenicity-related mechanisms. Fungi virulence is promoted by an iron-mediated programmed cell death of conidia which leads to the formation of a specialized infection structure called appressorium. Sequestering iron in this early stage of fungal life cycle represents a promising strategy to inhibit ferroptosis, and therefore the infection mechanism. Siderophores, a class of low-molecular-weight organic natural compounds, owe their metal-chelating abilities to electron-donating functional groups that form tight and stable complexes with Fe (III). These iron-chelating moieties can be exploited in the design of synthetic antimicrobial conjugates in an approach called "Trojan horse".



Figure 2 *Natural siderophores belonging to three different families: hydroxamates, catechols and citric acid derivatives.*

In the light of these interesting findings, this PhD project aims at the design and synthesis of novel hybrid

antifungal compounds by combining hydroxycinnamic acid (HA) dimers with siderophores moieties. In particular, the antifungal activity of natural HA dimers will be investigated on phytopathogen fungi, and a collection of structurally modified analogues will be synthesized to enhance their biological activity through Structure-Activity Relationship (SAR) studies. The most promising dimers will be coupled with selected siderophoric moieties to create novel chelating agents with potential dual mechanism: i) direct antifungal effects, thanks to optimized HA dimeric portion; ii) disruption of fungal iron uptake, thanks to iron chelating side chains. This approach holds promise for developing novel antifungal agents in overcoming resistance issues.

2. PhD Thesis Objectives and Milestones

Within the overall objective outlined above, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 2:

A1) **Rational design and synthesis of natural and novel fungicides dimers:** desired *p*-coumaric and ferulic acid dimers and derivatives will be synthesized exploiting chemoenzymatic synthetic procedures. Horseradish peroxidase (HRP) and laccases (from *T. versicolor*), commercial redox enzymes, will be employed for the oxidative dimerization steps. Through a SAR study, different types of chemical modifications will be performed: for example, methylation/demethylation of phenolic groups, modification of carboxylic acid groups into amides and hydroxamic acids, insertion of additional -OH groups on aromatic rings.

A2) **Evaluation of dimers antifungal activity** by *in vitro* mycelium inhibition test on different phytopathogenic fungi strains and *in vitro* germination inhibition test

A3) **Synthesis of hybrid chelating compounds:** inspired by three natural siderophores, rhodotorulic acid, mixochelin and rhizoferrin, siderophoric molecules will be synthesized. The hybrid compounds will be obtained by coupling the chelating moieties with the most active dimers, taking advantage of the carboxyl groups.

A4) **Evaluation of the chelating ability and antifungal activity** of hybrid compounds will highlight if this approach can improve biological activity. Additionally, the results could help to elucidate possible different mechanisms of action.

A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 2 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Hydroxicinnamic acid dimers***																								
	1) Rational design and molecular modelling																								
	2) Synthesis of natural dimers																								
	3) Chemical modifications (SARs)																								
A2)	***Dimers biological activity***																								
A3)	***Hybrid chelating compounds***																								
	1) Identification of sidereophore pharmacophoric moieties																								
	2) Synthesis of siderophore chains																								
	3) Synthesis of hybrid compounds																								
A4)	***Hybrid compounds activity***																								
A5)	***Thesis and Paper Preparation***																								

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Enzymatic prenylation of waste-derived polyphenols to enhance their biological activity and/or physico/chemical properties (P-I-71)

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This PhD project aims at the production, characterization and implementation of new aromatic prenyltransferases which will allow the selective biocatalyzed transformation of polyphenols. Green and innovative extraction processes will be designed to recover natural phenolic compounds from agri-food residues through environmentally friendly and innovative extraction processes, i.e. grape and wine by-products, enriched in stilbenoids. The prenylation of these compounds increases their lipophilicity and theoretically their bioactivity. A collection of prenylated polyphenols will be produced, and the resulting compounds will be evaluated for their antifungal and antibacterial properties.

Prenilazione enzimatica di polifenoli derivati da scarti per aumentarne l'attività biologica e/o le proprietà fisico/chimiche

Questo progetto di tesi di dottorato mira alla produzione, caratterizzazione ed implementazione di nuove preniltransferasi aromatiche in processi enzimatici per la trasformazione selettiva di polifenoli. Grazie allo sviluppo di approcci estrattivi sostenibili ed innovativi, polifenoli naturali verranno recuperati da scarti agro-alimentari, come i sottoprodotti della filiera dell'uva e del vino, arricchiti in stilbenoidi. La prenilazione di questi composti aumenta la loro lipofilia e potenzialmente la loro bioattività. Verrà prodotta una collezione di polifenoli prenilati, e i composti saranno testati per le loro proprietà antifungine e antibatteriche.

1. State-of-the-Art

Waste produced by the agri-food industry wweighheavily on the environment, especially for their management and disposal. These still contain valuable compounds, such as polyphenols which can exert a protective function against chronic diseases like cardiovascular, neurodegenerative disorders and osteoporosis. (Berenguer *et al.*, 2023) Therefore, their recovery prior to the waste disposal is important. Apart from the extraction through conventional methods like maceration, new more efficient and eco-friendly techniques as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), accelerated solvent extraction (ASE), together with enzymatic pre-treatments and the use of novel green solvents (i.e., ionic liquids (ILS), natural deep-eutectic solvents (NADES) and biomass-derived solvents (BDS)) are catching on. These innovative methods can increase the efficacy of the extraction while saving time, energy and solvents. (Berenguer *et al.*, 2023) Among the most cultivated fruit trees in the EU, there are vineyards (28%) (second after olive orchards) for a total of 8 million hectares. Italy is the third European country for grapevine production. These plants undergo the pruning process each year to improve quality and quantity of the produced fruits. (Aliaño-González *et al,* 2022) It is estimated that 1 hectare of fruit tree cultivation produces between 0.5 and 2 tons of wood. The extracts derived from these residues have shown to contain several bioactives. Among them, *trans*-resveratrol (11–66.200 mg/kg dw), ε-viniferin (47–40.600 mg/kg dw), catechin (101–8320 mg/kg dw), caftaric acid (424–5488 mg/kg dw) and quercetin-3-*O*-glucoside (191–2651 mg/kg dw) are the most important. (Aliaño-González *et al,* 2022)

Prenylated flavonoids are secondary metabolites widespread in the plant kingdom. With respect to non-prenylated parent compounds, these molecules possess better lipophilicity thus enhancing the gastrointestinal absorption and the interactions between cell membranes and P-glycoproteins. (Shi *et al*, 2021) Prenylated resveratrol derivatives have been synthesized and have shown important properties both as antioxidants (4-prenylresveratrol in particular) and as neuroprotective agent for Alzheimer's disease. (Puksasook *et al*, 2017) Their biosynthesis starts from the basic skeleton of flavonoids or isoflavonoids, to which the lipophilic prenyl sidechain is added by a prenyltransferase enzyme which can be membrane-bound (Ubi-a like) or cytosolic (dimethyl allyl tryptophan synthases, DMATs). These enzymes attach pyrophosphate-activated terpenoids (dimethyl allyl alcohol, geraniol and farnesol pyrophosphates) to aromatic molecules. (Mori, 2020) An example of aPTase is the ABBA-like AtaPT, which was found in the *Aspergillus terreus* genome and cloned in *Escherichia coli*; after purification it was used to prenylate a variety of different aromatic substrates with DMAPP, GPP and FPP, giving products with different yields. Among the accepted substrates can be found: lignanoids, tryptophan-containing cyclic dipeptides, quinoline alkaloids, xanthones, benzophenones, flavonoid aglycons and glycosides, a phenylethyl chromone, a curcuminoid, a stilbene, coumarins, hydroxynaphthalenes and 4-hydroxybenzaldehyde can be found. (Chen *et al*, 2020)

An interesting possibility is the immobilization of enzymes on supports for their reutilization for different reaction

cycles. Immobilized enzymes present different advantages: starting from the possibility to reuse the catalyst (impacting on the process costs) to an augmented stability and ease of enzyme removal (simplified workflow procedures). Moreover, the immobilization of enzymes allows their utilization in packed bed reactors for continuous flow processes, enhancing mass and heat transfer, increasing local concentration of (bio)catalyst, limiting the product inhibition effect and improving the control of the reaction parameters, resulting in higher space-time yields. (Ruscoe *&* Cosgrove*,* 2024)

2. PhD Thesis Objectives and Milestones

This PhD project can be subdivided into the following work packages (WPs) (*Table 1*):

WP1) **Recovery of polyphenols** from agri-food waste through green and sustainable extraction methodologies (done during the first year).

WP2) **Production and characterization of aromatic prenyltransferases** through heterologous expression in *E. coli,* both known (WP2.1) and novel (WP2.2) aPTases will be expressed (started during the first year).

WP3) **Set-up and optimization of batch reactions** with the prenyltransferases produced and the molecules recovered from agri-food waste (started during the first year).

WP4) **Scale-up to continuous processes** in flow systems with enzymes immobilized onto support (started during the first year).

WP5) **Biological evaluation** of antifungal activities of the compounds recovered and the prenylated ones.

During the PhD, manuscripts regarding scientific activity and the final thesis will be written.

Table 1 Gantt diagram for the 2nd and 3rd years.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
WP2)	***Enzymes production***																								
	1) known aPTases																								
	2) novel aPTases																								
WP3)	***Batch reactions***																								
WP4)	***Continuous processes***																								
WP5)	***Biological evaluation***																								
	Thesis and Paper Preparation																								

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Sustainable bioprocesses for plant secondary metabolite biotransformation (P-I-72)

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This PhD thesis research project is aimed at developing a cost-effective, industrially scalable and environmentally friendly process for the selective demethylation and subsequent glycosylation of methoxy-containing natural products. This approach involves the use of both whole cells and purified and immobilized enzymes for the generation of bioactive compounds with improved properties.

Bioprocessi sostenibili per la trasformazione di metaboliti secondari di origine vegetale

Questo progetto di tesi di dottorato mira a sviluppare processi a basso costo, scalabili industrialmente ed ecosostenibili per la demetilazione selettiva e la successiva glicosilazione di prodotti naturali contenenti gruppi metossilici. Questo approccio impiega sia cellule intere che enzimi purificati e immobilizzati per l'ottenimento di composti bioattivi con proprietà migliorate.

1. State-of-the-Art

The last decade has seen a renewed interest in pharmacologically active natural products, bioactive compounds that are produced by plants, microorganisms, or animals and that are extensively used in medicine, agriculture, food and cosmetic industry due to their low toxicity, chemical diversity, and sustainability. However, a common feature of many natural products, that can limit their use as pharmaceuticals, is the presence of methoxy (-OCH_3) groups, that are susceptible to oxidation reactions, leading to the quick excretion of the drug from the human body (Chiodi D et al., 2024).

The demethylation of natural products has the potential to generate derivatives with an enhanced bioactivity and to valorise aromatic compounds such as those derived from lignin depolymerization during biofuel production, but chemical methods suffer from poor chemo- and regioselectivity, undesirable side products formation and high costs (Shen C et al., 2019).

A possible alternative is the use of biocatalysts, that often provide simplified synthetic routes to the assembly of complex targets, since they operate under mild conditions, often with fewer synthetic steps and reduced waste (O'Connell A et al., 2024). In the last two decades, the improvement of biocatalysts guided by bioinformatic tools in combination with rational design or directed evolution and high-throughput screening tools have led to the development of several bioprocesses to produce high value chemicals and to their successful industrial application. The annual growth rate of the bioprocessing industry has been estimated at 8.6% annually and the global market for bioactive compounds is expected to increase to USD 727.1 billion by 2025 (Ade N et al., 2019).

Particularly, the demethylation of -OCH_3 groups can be obtained by using selective monooxygenases [Figure 1], which operate by hydroxylation of the methoxy group, generating a hemiacetal that is spontaneously hydrolysed to the corresponding hydroxyl group with the release of formaldehyde.

The resulting hydroxy-containing compound can then be further functionalized to improve the pharmacological properties of the molecule. Indeed, many natural compounds with an interesting biological activity have a limited applicability due to their poor solubility in aqueous medium, that determines a low concentration of these molecules in the bloodstream, with a consequent decrease in their biological activity (Andreu A et al., 2023). The presence of sugar residue can enhance the polarity of these molecules, therefore improving their bioactivity and bioavailability.

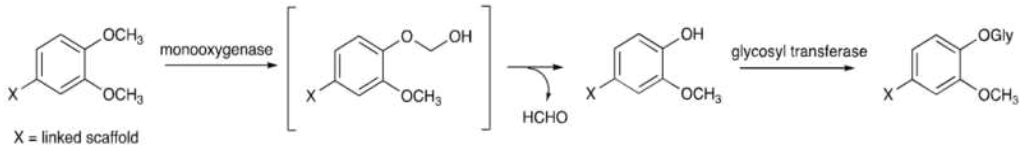


***Figure SEQ Figure * ARABIC 1**: Selective demethylation and glycosylation of a methoxy compound.*

Among the enzyme classes known to catalyze aromatic O-demethylation, the cytochrome P450 (CYP or P450) superfamily is one of the most extensively studied. Its high catalytic turnover, efficient expression in heterologous hosts, low production costs, and ease of engineering make it particularly well-suited for biotechnological applications.

Interestingly, the CYP102 family is characterized by the presence of both a catalytic heme domain (BMP) and a diflavinal reductase domain (BMR) fused in a single peptide chain. A well-known member of this family is BM3 (CYP102A1) from *Priestia megaterium* (formerly *Bacillus megaterium)*, a self-sufficient monooxygenase which catalyses the regio- and stereoselective hydroxylation of long-chain fatty acids at a high turnover rate of >3000 min^{-1}.

A strain of *Priestia megaterium* was reported to also be capable of further glycosylating alkaloids, although the enzymes responsible for this second step have not been identified (Ponzone C. et al., 2014). The use of this microorganism as whole cells therefore enables demethylation and subsequent glycosylation to occur in a single step. Moreover, since *Priestia megaterium* can grow on many carbon sources including wastewater from the meat processing industry, corn syrups and a wide range of sugars, it would be possible to use agri-food waste(s) as fermentation feedstock, for a more sustainable production process (Biedendeck R. et al., 2021). In this project, different approaches for improving the biocatalytic O-demethylation and glycosylation of selected natural compounds, such as flavonoids and alkaloids, will be developed following two distinct strategies. The first consists in process implementation of wild type *P. megaterium* whole cells, firstly by growing the strain on different waste residues to optimize biomass production and secondly by immobilizing the cells for their reuse in flow and rotating bed reactors. The second strategy aims at the characterization and expression of the enzymes with the desired activity in host cells, their study for strategic mutations and their final use as isolated immobilized enzymes in flow reactors. These process advancements will not only enhance the bioactivity and bioavailability of active compounds but also promote sustainable and scalable biotechnological methods.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be divided into the following activities according to the Gantt diagram given in Table 1:

A1) **Process improvement using *Priestia megaterium* wild-type cells expressing monooxygenases and glycosidases/glycosyltransferases:** this will be done firstly through optimization of *P. megaterium* fermentation using waste-derived culture medium and secondly by testing the cells on a wide range of molecules for the substrate screening.

A2) **Immobilization of *P. megaterium* whole cells and their use in flow reactors and in rotating bed reactors** to allow their recycling for multiple reactions, an easy recovery and the scale-up in continuous systems.

A3) **Expression of the key enzymes in host strains** using the deposited sequence of BM3. The glycosyltransferase involved in the glycosylation step will be identified using bioinformatic tools and transcriptomic analysis.

A4) **Immobilization of the produced enzymes for their use in flow reactors and set-up of cascade reaction** to further increase their stability in operational conditions and to allow the recycling of the catalysts. Kinetic studies will be conducted to design and optimize tandem flow reactions involving multiple enzymatic steps, to automatize and implement the whole biotransformation process.

A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 2 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Process improvement with whole cells***																								
	1) Agrifood waste as culture medium																								
	2) Demethylation screening																								
A2)	***Use of immobilized cells***																								
	1) Immobilization of the cells																								
	2) Flow reactors and rotating bed reactors																								
A3)	***Expression and study of the purified enzymes***																								
	1) Identification of glycosyltransferases																								
	2) Expression in *E. coli*, purification and characterization of the enzymes																								
A4)	***Use of immobilized enzymes***																								
	1) Immobilization of the enzymes																								
	2) Design of tandem flow reactions																								
A5)	***Thesis and Paper Preparation***																								

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Chemical and biological characterization of pomegranate seed oil: a novel functional ingredient (P-I-73)

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This PhD project aims to characterize and evaluate the chemical profile and biological activity of ethanol-extracted pomegranate seed oil (PSO). We will investigate its potential to reduce inflammation and oxidative stress in LPS-stimulated macrophage-differentiated THP-1 cells (M0). Cell viability, cytokine levels, ROS, and nitrite production will be measured in presence or absence of PSO, or its fractions. The molecular mechanisms involved in inflammatory and antioxidant responses will be also elucidated. Using gas chromatography-mass spectrometry (GC-MS), the chemical composition of PSO—focusing on polyphenols, phenolic compounds, and fatty acids—will be determined. PSO may serve as a functional ingredient in anti-inflammatory food applications.

Caratterizzazione chimica e biologica dell'olio di semi di melograno: un nuovo ingrediente funzionale

Questo progetto di dottorato mira a caratterizzare e valutare il profilo chimico e l'attività biologica di un estratto etanolico diolio di semi di melograno (PSO). Verrà studiato il suo potenziale nel ridurre l'infiammazione e lo stress ossidativo in cellule THP-1 differenziate in macrofagi (M0), stimolate con LPS. Saranno analizzati vitalità cellulare, livelli di citochine, ROS e produzione di nitriti, in presenza e assenza di PSO o delle sue frazioni. La composizione chimica del PSO, con particolare attenzione a polifenoli, composti fenolici e acidi grassi, sarà determinata tramite GC-MS. Il PSO potrà essere utilizzato come ingrediente per la realizzazione di alimenti funzionali.

1. State-of-the-Art

The pomegranate (*Punica granatum* L.) is a deciduous, fruit-bearing shrub native to Iran (historically Persia), whose cultivation has spread extensively across the Mediterranean basin, as well as to regions including Afghanistan, Spain, Italy, Egypt, Russia, China, Japan, and California in the United States. The fruit processing industry, including the processing of pomegranates, converts raw agricultural materials into higher value-added products. However, this industrial transformation also generates significant amounts of solid waste. On a global scale, around one-third of all food produced is either wasted or lost annually. The Food and Agriculture Organization (FAO) reports that 40% of this loss occurs in developed countries (M.A. Andrade et al., 2019). According to Aviram et al. (2000), nearly half a kilogram of a typical pomegranate comprises inedible components such as the peel, while the remaining portion yields approximately 400 g/kg of juice and 100 g/kg of seeds.

Pomegranates are rich in a diverse array of bioactive compounds, including alkaloids, ellagic acid, punicalagin and other ellagitannins, anthocyanins, flavonoids, tannins, and other phytochemicals. These compounds have been associated with numerous health-promoting properties and are being actively investigated for their potential roles in the prevention and treatment of various diseases (Setiadhi and Sufawati, 2017). Among the by-products of pomegranate processing, pomegranate seed oil (PSO) constitutes approximately 12–20% of the total seed weight. PSO is particularly notable for its high content (approximately 80%) of conjugated linolenic acids (CLnAs), particularly punicic acid (cis9, trans11, cis13), the predominant fatty acid in the oil. These CLnAs have demonstrated several potential therapeutic effects, including anti-inflammatory, anti-diabetic, anti-obesity, anti-proliferative, and anti-carcinogenic activities, as well as regulatory effects on lipid metabolism (Boussetta et al., 2009; Johanningsmeier & Harris, 2011).

Inflammation, in the detail, is a complex biological response to injury or infection, mediated by signaling pathways activated by microbial endotoxins such as lipopolysaccharides (LPS). These molecules stimulate immune cells—particularly macrophages—to produce nitric oxide and pro-inflammatory cytokines including TNF-α, IL-6, and IL-1β, thereby initiating the acute inflammatory response. While this response is crucial for limiting tissue damage and promoting repair, excessive or unresolved inflammation can lead to chronic degenerative conditions such as asthma, diabetes, rheumatoid arthritis, inflammatory bowel disease, and cancer (Abdulkhaleq et al., 2018). Consequently, there is an ongoing scientific effort to discover natural compounds capable of modulating inflammatory responses without adverse effects.

In this context, the present Ph.D. project aims to investigate the anti-inflammatory potential of pomegranate seed oil extracts using an *in vitro* model of inflammation. Specifically, the study evaluates the effects of these extracts on LPS-stimulated macrophage-differentiated THP-1 cells, a human monocytic cell line widely used to model macrophage activity. Additionally, chemical characterization of pomegranate seed oil (PSO) using GC-MS will identify the main phytochemical constituents responsible for its anti-inflammatory activity. The final goal is to

elucidate whether pomegranate seed oil, and/or its single fractions, can modulate pro-inflammatory signaling and contribute to the development of sustainable, plant-derived beneficial agents, useful for the management of inflammation-related disorders and as a functional ingredient in innovative food applications.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 2:

A1) **State of the Art and Biological Activities of Pomegranate Seed.** Literature review on chemical characterization of pomegranate seed, extraction methods, *in vivo* and *in vitro* studies about anti-inflammatory activities of PSO and food applications.

A2) **Cell Analysis.** THP-1 cell differentiation process, MTT Assay for cell viability, ELISA for cytokines level determination and western blot analysis for the evaluation of gene involved in anti-inflammatory and antioxidant response, reactive oxygen species (ROS) and nitrite determination.

A3) **Chemical Characterization of PSO.** GC-MS for chemical composition of PSO and HPLC for identification of total flavonoids.

A4) **Potential Food Applications.** Based on the biological activities of PSO, pomegranate seed oil will be used as functional ingredients for innovative food applications.

A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 2 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***State of the art and biological activities of pomegranate seed***																								
	1) Literature review on extraction methods of PSE extracts, chemical characterization of PSO																								
	2) Literature review on *vivo* and *vitro* studies about anti-inflammatory and anti-cancer effects of PSO.																								
A2)	***Cells Analysis***																								
	1) Cell Viability (MTT assay)																								
	2) ELISA assay for cytokine production																								
	3) ROS level and nitrite level																								
	4) Western blot analysis																								
A3)	***Chemical Characterization of PSO***																								
	1) HPLC for identification of total flavonoids																								
	2) GC-MS analysis for chemical composition																								
A4)	***Potential food applications***																								
A5)	***Thesis and Paper Preparation***																								

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Bioactive compounds in convenience foods and valorisation of their processing by-products and waste (P-I-74)

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Company tutors: Dott.ssa Rosanna Doris & Dott.ssa Lisa Masiero (Ghisetti 1870)

This PhD project focuses on the characterization of plant-based convenience foods developed by Ghisetti 1870, with particular attention to the content and profile of bioactive compounds. The project also aims to valorize company by-products, waste, and surpluses through upcycling strategies and green technologies, leading to the production of circular functional ingredients, such as powders and extracts. These ingredients will be nutritionally and techno-functionally characterized and used in the formulation of food prototypes. All within a framework of sustainability and circular economy.

Composti bioattivi nei *convenience food* e valorizzazione dei loro sottoprodotti di lavorazione e scarti

Questo progetto di dottorato mira a caratterizzare alimenti *convenience* a base vegetale sviluppati da Ghisetti 1870, con particolare attenzione al contenuto e al profilo dei composti bioattivi. In questo contesto, diventa un obiettivo prioritario anche la valorizzazione di sottoprodotti, scarti ed eccedenze aziendali attraverso strategie di *upcycling* e tecnologie *green*, per ottenere ingredienti funzionali circolari sotto forma di polveri ed estratti. Questi ingredienti saranno caratterizzati dal punto di vista nutrizionale e tecno-funzionale e impiegati nella formulazione di prototipi alimentari, in un'ottica di sostenibilità ed economia circolare.

1. State-of-the-Art

In recent years, convenience foods have assumed a central role in the dietary patterns of developed countries, driven by lifestyle changes, limited time availability, and growing attention to health and food quality (Stranieri et al., 2017). Within this category, a variety of products has been developed to meet different consumer needs in terms of preparation time and ease of use, including ready-to-eat, ready-to-heat, ready-to-end-cook, and ready-to-cook options (Roascio - Albistur & Gámbaro, 2018). Despite their popularity, a major concern associated with convenience foods is their ability to deliver a nutritionally complete and balanced diet. Among innovative food processing technologies, sous-vide cooking has gained substantial momentum since the 2010s. This method—based on vacuum-sealing raw ingredients in heat-stable pouches followed by prolonged cooking at precisely controlled low temperatures—ensures accurate thermal regulation, extended shelf life, and superior preservation of vitamins, minerals, and antioxidant activity. Moreover, it enhances sensory attributes, offering both nutritional and qualitative advantages over traditional cooking methods (Cui et al., 2004; Kathuria et al., 2022). Concurrently, the agri-food sector is under increasing pressure to reduce food loss and waste. According to the FAO, approximately one-third of all food produced for human consumption—amounting to roughly 1.3 billion tons annually—is lost or wasted globally. Fruits, vegetables, roots, and tubers account for 45–50% of this total (Casson et al., 2024; FAO, 2011, 2019). These discarded materials are often rich in dietary fiber, polyphenols, antioxidants, and other valuable bioactive compounds, positioning them as promising raw materials for the development of functional ingredients (Socas-Rodríguez et al., 2021). In this context, upcycling has emerged as an effective strategy to combat food waste by converting surpluses, by-products and food waste into high-value food ingredients—such as powders and extracts—with enhanced nutritional and functional profiles (Benvenutti et al., 2025). Building on these considerations, the aim of this Ph.D. project is to bridge current gaps in integrated upcycling strategies for the valorization of the company's surpluses, by-products, and waste streams, while also exploring innovative solutions for the development of plant-based convenience foods with high nutritional and sensory quality.

2. PhD Thesis Objectives and Milestones

The present PhD project is funded through a thematic scholarship awarded under the Smart PhD 2024 initiative, supported by Fondazione Cassa di Risparmio di Padova e Rovigo, Intesa Sanpaolo S.p.A., and UniSMART – Fondazione Università degli Studi di Padova, and co-founded by the industrial partner Ghisetti 1870.
Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in **Table 1**:
A1) Comprehensive literature review and methodological framework. A critical review of scientific literature will be conducted to define the research framework, identify current knowledge gaps, and support the methodological planning of experimental activities.

A2) Selection and preliminary screening of plant-based matrices. In collaboration with *Ghisetti 1870*, plant-based fifth-range convenience foods will be developed, and compositional screening will be carried out (A2.1). Upcyclable by-products, surpluses and waste will be identified and functionally characterized (A2.2).
A3) Development and assessment of circular functional powders. Selected matrices will be processed to obtain upcycled powders (A3.1), which will be evaluated for nutritional composition, bioactive content and techno-functional properties (A3.2).
A4) Green extraction and characterization of bioactive compounds. Green extraction methods will be optimized for the recovery of bioactive compounds (A4.1), followed by their chemical and functional characterization (A4.2).
A5) Design and development of plant-based enriched food prototypes. Prototypes enriched with the obtained powders and extracts will be formulated and optimized at lab scale (A5.1), and promising candidates scaled up in collaboration with *Ghisetti 1870* (A5.2). Selected prototypes will be assessed for techno-functional and nutritional characterization, consumer acceptability, sensory and comprehensive *shelf-life* evaluation (A5.5).
A6) PhD thesis and papers preparation. The final phase will involve integration of results into the doctoral thesis and dissemination through publications and conference presentations.

Table 1 Gantt diagram for this PhD thesis project.

Year		**1**				**2**				**3**			
Activity	***Trimester***	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
A1)	***Comprehensive literature review and methodological framework***												
A2)	***Selection and preliminary screening of plant-based matrices***												
	1) Development and compositional screening of plant-based fifth-range convenience foods produced with Ghisetti 1870.												
	2) Identification and functional characterization of upcyclable by-products, surpluses and waste.												
A3)	***Development and assessment of circular functional powders***												
	1) Production of upcycled powders												
	2) Compositional, bioactive and techno-functional profiling.												
A4)	***Green extraction and characterization of bioactive compounds***												
	1) Green extraction and its optimization												
	2) Chemical and functional profiling of extracts												
A5)	***Design and development of plant-based enriched food prototypes***												
	1) Set up food formulations at laboratory scale using upcycled powders/extracts as functional ingredients												
	2) Pilot-scale validation of selected formulations in collaboration with Ghisetti 1870.												
	3) Techno-functional and nutritional characterization												
	4) Consumer acceptability and sensory evaluation												
	5) Comprehensive shelf-life evaluation												
A6)	***PhD thesis and papers preparation***												

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Innovative and sustainable methodologies for the extraction and characterization of bioactive and high value-added compounds obtained from agri-food supply chain by-products (P-I-75)

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This research project aims to identify by-products from the agri-food supply chain from which to extract, using sustainable, innovative, and efficient techniques, bioactive and high value-added compounds, and to characterize the extracts obtained through specific analytical investigations. The final goal is to incorporate them into formulations of products intended for human and animal nutrition (pet food).

Metodologie innovative e sostenibili per l'estrazione e la caratterizzazione di composti bioattivi e ad alto valore aggiunto ottenuti da sottoprodotti della filiera agro-alimentare

Questo progetto di ricerca mira a identificare sottoprodotti della filiera agro-alimentare dai quali estrarre, utilizzando tecniche sostenibili, innovative ed efficienti, composti bioattivi e ad alto valore aggiunto, e a caratterizzare gli estratti ottenuti attraverso specifiche indagini analitiche. L'obiettivo finale è incorporarli nelle formulazioni di prodotti destinati all'alimentazione umana e animale (pet food).

1. State-of-Art

The adoption of innovative circular practices for the management of agri-food by-products is of fundamental importance in promoting environmental, economic, and social sustainability. The agri-food sector generates a significant amount of waste and residue. Worldwide, the agri-food industry produces over 190 million tons of by-products each year. These by-products, mainly resulting from processing activities, include damaged raw materials, grape pomace, leaves, seeds, shells, bran, oilseed cakes, and molasses (Rațu *et al*., 2023).
By-products from the agri-food supply chain can play a key role in the formulation of new products, co-products, and functional and sustainable ingredients for both human and animal consumption (e.g., pet food), as they are generally rich in bioactive and high value-added compounds (Comunian *et al*., 2021). Compounds such as proteins, lipids, starches, vitamins, minerals, fibers, and antioxidants can be extracted from these by-products using various physical or chemical extraction techniques (Torres-León *et al*., 2018).
In recent years, innovative and sustainable technologies have been promoted and applied to optimize the extraction of high value-added components, improving their quality and functionality while increasing yields. These emerging technologies, some of which are already used in the food sector, include pulsed electric fields, ultrasound-assisted extraction, radio frequency drying, high hydrostatic pressure, and pressurized fluids (extraction with subcritical and supercritical fluids) (Pradal *et al*., 2016; Villacís-Chiriboga *et al*., 2021).
To assess the presence of bioactive and high value-added compounds obtained from agri-food by-products and to identify any undesirable substances, it is essential to carry out targeted analytical investigations. Techniques such as gas chromatography and liquid chromatography combined with various detectors such as mass spectrometry (MS), flame ionization detection (FID), and diode array detection (DAD) are suitable for characterizing extracts derived from agri-food by-products.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Investigation of the needs of the pet food and food sectors:** a study aimed at defining the bioactive and high value-added compounds that the pet food and food industries are interested in valorizing and incorporating into their products.
A2) **Selection of agri-food by-products:** identification of agri-food by-products from which to extract bioactive and high value-added compounds.
A3) **Selection and development of effective and sustainable extraction techniques:** study and application of the most suitable and sustainable extraction techniques for recovering bioactive compounds from agri-food by-products.
A4) **Characterization of the obtained extracts:** analytical characterization to verify the quality and safety of the obtained extracts.
A5) **Use of the extracts in food product formulations:** potential use of the extracts in formulations for products intended for human and animal (pet food) consumption.
A6) **Preparation of articles, scientific contributions, and the thesis.**

Table 1 Gantt diagram for this PhD thesis project

Activity	Months	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	**Investigation of the needs of the pet food and food sectors**																		
A2)	**Selection of agri-food by-products**																		
	1) Research on potential by-products to be used: by-products from extra virgin olive oil production such as olive pomace, olive mill wastewater, and olive stone impurities, as well as tomato pomace and exhausted hemp.																		
	2) Sampling and storage of by-products																		
A3)	**Selection and optimization of effective and sustainable extraction techniques**																		
A4)	**Characterization of the obtained extracts**																		
	1) Identification and quantification of bioactive and high value-added compounds present in the extracts using analytical techniques.																		
	2) Analysis of the quality and safety of the extracts																		
A5)	**Use of the extracts in the formulation of finished products**																		
A6)	**Preparation of articles, scientific contributions, and the thesis**																		

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Valorization of animal by-products: industrial production of hydrolyzed animal collagen and evaluation of its technological and nutraceutical properties (P-I-76)

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This industrial PhD project aims to study and valorize collagen obtained through enzymatic hydrolysis of agri-food by-products. The objective is to identify bioactive compounds with potential nutraceutical applications. The activities will include the physicochemical, biological, and functional characterization of the resulting peptides, as well as the development of microencapsulation techniques to improve their stability and bioavailability.

Valorizzazione dei sottoprodotti di origine animale: produzione industriale di collagene animale idrolizzato e valutazione delle sue proprietà tecnologiche e nutraceutiche

Questo progetto di dottorato industriale mira allo studio e alla valorizzazione di collagene animale ottenuto tramite idrolisi enzimatica di sottoprodotti dell'industria agroalimentare. L'obiettivo è identificare composti bioattivi con potenziali applicazioni nutraceutiche. Le attività includeranno la caratterizzazione chimico-fisica, biologica e funzionale dei peptidi, oltre allo sviluppo di tecniche di microincapsulazione per migliorarne la stabilità e la biodisponibilità.

1. State-of-the-Art

Valorization of animal by-products through the industrial production of hydrolyzed collagen is a rapidly evolving field that addresses both environmental sustainability and the production of high-value functional ingredients. Animal by-products such as skins, bones, and cartilage were traditionally considered waste or used for low-value applications. However, recent advances in extraction and hydrolysis technologies have enabled the efficient recovery of collagen, which, once hydrolyzed, possesses unique technological and nutraceutical properties (Cao *et al.,* 2022). Collagen, the most abundant protein in mammals, constituting approximately 30% of total body protein, is a key structural component in various tissues such as skin, tendons, bones, cartilage, and ligaments. Hydrolyzed collagen, also known as collagen peptides, refers to a group of low molecular weight peptides obtained by the hydrolysis of native collagen. The process flow generally includes raw material preparation, enzymatic hydrolysis, separation and filtration of the hydrolysate, concentration, drying, and packaging. Parameters such as temperature, pH, enzyme concentration, and hydrolysis time are carefully controlled to optimize yield and peptide characteristics. These approaches allow for the controlled breakdown of collagen into low molecular weight peptides, which are highly soluble, easily digestible, and rapidly absorbed by the human body. The resulting hydrolyzed collagen lacks the gelling properties of native collagen, making it suitable for incorporation into a wide range of food, beverage, and supplement products without altering their texture (Leon-Lopez *et al*., 2019). Hydrolyzed collagen has been shown to offer several health benefits, including support for joint, bone, and skin health, as well as antioxidant, anti-inflammatory, and anti-hypertensive effects. The bioactive peptides derived from collagen are of particular interest for their potential to deliver targeted physiological benefits. Oral intake studies in animal and human models have demonstrated the excellent absorption and metabolism of Hyp-containing peptides, particularly Pro-Hyp and Gly-Pro-Hyp, critical for various physiological functions. This has driven their widespread application in food, pharmaceutical, cosmetic, and biomedical industries (Gómez-Guillén *et al*., 2011). Despite its broad applications and documented benefits, a significant challenge for hydrolyzed collagen lies in its oral bioavailability, which is the rate and extent to which an active compound is absorbed and becomes available at its site of action. The bioactivity of collagen peptides is often compromised during oral administration due to susceptibility to enzymatic hydrolysis in the gastrointestinal tract, which can alter their structure and reduce biological activity. Small peptides, such as dipeptides and tripeptides, are rapidly absorbed in the small intestine, leading to transient spikes in blood levels that quickly decline, limiting their functional benefits. Conversely, larger peptides exhibit low oral bioavailability due to extensive enzymatic degradation in the gastrointestinal tract and limited absorption, even though they may offer unique biological effects. These challenges often necessitate higher doses and frequent intake, increasing costs and potential side effects. Recent research is increasingly focused on developing innovative strategies to enhance the delivery and effectiveness of collagen peptides, with particular emphasis on advanced encapsulation systems. These include microcapsules, water-in-oil-in-water double emulsions, hydrogels, nanoliposomes, and electrospun fibers. Such systems are designed to protect collagen peptides from gastrointestinal degradation, allow controlled release, and improve absorption, ultimately enhancing their bioavailability and functional benefits. This controlled release helps maintain consistent concentration of peptides in the bloodstream or target tissues, thereby maximizing their

physiological efficacy (Zhou et al., 2025). While these technologies show substantial promise and have yielded positive results in *in vitro* and animal models, further research is required for clinical validation and efficient industrial-scale production to ensure their broader implementation in food systems. To address these challenges and explore practical applications, this PhD project focuses on the comprehensive study and characterization of hydrolyzed collagen, investigating its diverse properties and potential applications in model food systems. A critical component of this research involves an *in vitro* investigation into the digestibility and overall oral bioavailability of hydrolyzed collagen. Recognizing the limitations of collagen peptides' transient absorption and their susceptibility to degradation, the project pioneers the application of microencapsulation strategies to enhance their bioavailability. Through the exploration of various encapsulation systems, this research seeks to protect hydrolyzed collagen from enzymatic breakdown in the gastrointestinal tract, enable its sustained release, and ultimately improve its functional efficacy *in vivo*. While this work lays a robust foundation for the valorization of animal by-products and the development of natural, health-promoting food ingredients, further clinical validation and scalable production methods remain key for bridging the gap between laboratory findings and industrial application.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Literature review** to identify recent advances and emerging trends in the research and development of hydrolyzed collagen, with particular focus on innovative production techniques, enhancement of bioavailability, and potential technological and nutraceutical applications.

A2) **Production and characterization of collagen peptides** in terms of molecular weight, amino acid sequence, degree of hydrolysis, biological activity, and physicochemical analysis.

A3) **Development of model foods containing collagen peptides.** The products will undergo physicochemical and structural characterization to assess their quality and stability. *In vitro* digestibility studies will be conducted to evaluate the release and potential bioavailability of collagen peptides within the food matrix.

A4) **Microencapsulation of Hydrolyzed Collagen and Characterization of Fortified Model Foods.** Different encapsulation systems will be evaluated to protect peptides during gastrointestinal digestion, ensuring their targeted release and functional activity. The resulting microcapsules will undergo thorough physicochemical characterization and will be then incorporated into a model food matrix. Finally, the fortified model foods will be physicochemically and sensorially characterized to evaluate the impact of microcapsule incorporation on product quality.

A5) **Writing and Editing** of the PhD thesis, scientific papers, and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Literature review***																								
A2)	***Characterization of collagen peptides***																								
	1) Evaluation of molecular and physicochemical properties (degree of hydrolysis, antioxidant activities)																								
A3)	***Model foods development***																								
	1) Food development																								
	2) Characterization analyses																								
	3) *In vitro* digestibility																								
A4)	***Microencapsulation of Hydrolyzed Collagen and Characterization of Fortified Model Foods***																								
	1) Collagen peptides microencapsulation																								
	2) Microcapsules characterization																								
	3) Fortification of model foods with microcapsules																								
	4) Physicochemical and sensory characterization of the model foods																								
A5)	***Thesis and Paper Preparation***																								

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Valorization of Fish in Healthy and Sustainable Dietary Patterns (P-I-77)

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This PhD project aims to evaluate the role of fish products within dietary patterns, with the goal of highlighting and enhancing their impact from both a nutritional and sustainability perspective. Fishery products play a pivotal role in public health and can be also integrated into nutritional strategies for population groups with specific needs. To this aim, a research and innovation strategy will be developed through collaboration with the industry, enabling the practical application of the proposed framework and supporting the improvement of existing market products.

Valorizzazione del Pesce all'interno di Modelli Alimentari Salutari e Sostenibili

Questo progetto di dottorato mira alla valutazione dei prodotti della pesca all'interno di modelli alimentari, con l'obiettivo di evidenziare e valorizzare il ruolo del pesce da un punto di vista nutrizionale e della sostenibilità. I prodotti della pesca svolgono un ruolo chiave nella salute pubblica e risultano utili anche per gruppi con bisogni specifici. A tale scopo sarà sviluppata una strategia di ricerca ed innovazione attraverso la collaborazione con l'azienda, favorendo l'applicazione pratica del framework proposto e supportando il miglioramento dei prodotti presenti sul mercato.

1. State-of-the-Art

Unhealthy and unsustainable dietary patterns pose a global risk for people and the planet. According to the latest Global Burden of Disease report, nutritional deficiencies contributed to 48.7 million disability-adjusted life years worldwide, covering 204 countries and territories. Although projections suggest that this trend is improving, food and diet still play a major role in the global burden of disease through two main pathways: nutritional deficiencies, which predominantly affect younger and low-income populations, and suboptimal diets, which contribute to overweight, obesity, and metabolic disorders [1]. In addition to the impact of poor diets on human health, the ongoing degradation of natural resources has heightened interest in assessing how food choices affect the planet's health. Today's food systems place significant pressure on the planet's ecosystems and play a major role in pushing several environmental limits beyond safe thresholds. Agriculture alone is responsible for about 25% of global greenhouse gas emissions, uses 40% of the Earth's ice-free land, consumes 70% of the available freshwater, and accounts for over 80% of global nitrogen and phosphorus use [2]. As a result, it is a leading driver of the current biodiversity loss. Given the significant environmental risks linked to food production, sustainable practices must function within the safe operating limits of food systems at all scales globally. In this context, the 2030 Agenda for Sustainable Development includes 17 goals seeking to end poverty, ensuring prosperity for all, and eradicating hunger and malnourishment [3]. Moreover, a transformative shift in global food systems is urgently aligned with the objectives of the Paris Agreement, in order to limit global warming to well below 2°C [4].

Diet can be considered a major link between human health and environmental sustainability. Unhealthy diets are not only responsible for the burden of obesity and diet-related non-communicable diseases but can also contribute to environmental degradation. Therefore, there is an urgent need to promote healthy diets with a low environmental impact. FAO and WHO define Sustainable Healthy Diets as "dietary patterns that promote all dimensions of individuals' health and well-being; have low environmental pressure and impact; are accessible, affordable, safe and equitable; and are culturally acceptable" [5]. The aim of the diet is to achieve optimal growth and development of all individuals and support physical, social and mental wellbeing.

The role of animal-source foods (ASFs) in the context of sustainable dietary patterns is currently subject of a widespread debate. Fish and seafood are often considered to have a lower environmental impact when compared to other ASFs. Seafood represents a diverse food category, with environmental impacts varying considerably depending on the species and the method of production (wild-caught vs. farmed) [6,7]. Moreover, fish is important from a nutritional point of view. It is rich in several essential nutrients and can help reduce nutrient deficiencies by contributing to the achievement of daily nutritional requirements (*e.g.*: high quality proteins, vitamin D, vitamin B12, omega-3 long-chain polyunsaturated fatty acids docosahexaenoic acid and eicosapentaenoic acid (DHA and EPA), iron, zinc and others). Addressing these deficiencies is particularly critical in vulnerable population subgroups, including young children, older adults, pregnant women, and women of reproductive age [8,9].

In this context, the role of fish and seafood will be investigated, in order to study their contribution to healthy and sustainable dietary patterns, with specific attention to strategies that allow to cover nutritional needs. Furthermore,

collaboration with the company will be fundamental for the practical application of the proposed framework through the identification and nutritional implementation of traditional and innovative products that can respond to the needs of different population targets.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1. **Literature research**: to better understand the role of fish in achieving both nutritional and environmental goals. This will lead to conducting a systematic review, also focusing on vulnerable population groups.
A2. **Evaluation of dietary habits of the Italian population**: to collect data about the current consumption and the dietary pattern of the Italian population, specifically focusing on fish and canned fish consumption.
A3. **Dietary pattern elaboration**: optimization of the existing MED_EAT-IT dietary pattern[10] through the inclusion of different portions of fish and canned fish, by developing different dietary scenarios. Then, there will be an evaluation in terms of nutritional composition and environmental impact of the newly created dietary pattern, with the aim of assessing its overall suitability, including for specific population groups.
A4. **Assessment/reformulation/implementation of food products**: evaluation of nutritional and environmental characteristics of fish products in the market and, in collaboration with the company, definition of strategies to improve products characteristics.

***Table 1**. Gantt diagram for this PhD thesis project*

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Literature research***																								
	1) Literature research																								
	2) Systematic review																								
A2)	***Evaluation of dietary habits***																								
	1) Data collection and analysis																								
	2) Elaboration of results																								
A3)	***Dietary pattern elaboration***																								
	1) Evaluation of the different scenarios																								
A4)	***Assessment of food products***																								
	1) Analysis of existing products																								
	2) Theoretical improvement																								
	3) Elaboration of results and application																								
A5)	***Thesis and Paper Preparation***																								

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Discovering the role of SEASOnal dietary choices on Mediterranean diet adherence and effect on gut microbiota (4-SEASOMed) (P-I-78)

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This PhD research project aims to investigate the role of in-season dietary choices in influencing the adherence to the Mediterranean Diet (MD), the gut microbiota composition, and the excretion of urinary (poly)phenol-related catabolites. A multicentre observational study will be conducted on 400 volunteers enrolled in three different Italian regions. Dietary and lifestyle habits will be monitored, and gut microbiota composition and diet-related metabolites will be analyzed in a subsample of subjects. By integrating dietary and biological data, the study will help to clarify the role of in-season food choices in promoting healthy and sustainable diets.

Scoprire il ruolo delle scelte alimentari sull'aderenza alla Dieta Mediterranea e il loro effetto sul microbiota intestinale (4-SEASOMed)

Questo progetto di dottorato ha l'obbiettivo di indagare il ruolo delle scelte alimentari stagionali nell'influenzare l'aderenza alla Dieta Mediterranea (MD), la composizione del microbiota intestinale e l'escrezione di cataboliti (poli)fenolici urinari. Uno studio osservazionale multicentrico sarà condotto su 400 volontari arruolati in tre diverse regioni italiane. Saranno monitorate le abitudini alimentari e di vita e saranno analizzati la composizione del microbiota intestinale e i metaboliti correlati alla dieta in un sottocampione di soggetti. Integrando dati dietetici e biologici, lo studio contribuirà a chiarire il ruolo delle scelte alimentari di stagione nel promuovere una dieta sana e sostenibile.

1. State-of-the-Art

Healthy dietary habits play a central role in preventing major chronic diseases and reducing disability worldwide (FAO&WHO, 2019). Among healthy patterns, the MD is associated with a reduced risk of cardiometabolic diseases, cancer, and overall mortality (Dinu et al., 2020). Beyond the beneficial effect in promoting health, the MD has been recognized as a sustainable dietary model for its lower environmental impact compared to other dietary patterns (Willet et al., 2020), also because of its emphasis on local and seasonal plant-based foods (Serra-Majem et al., 2020). In fact, in-season consumption of foods is often described as one of the key elements of the MD. Traditionally, fruit and vegetables were consumed locally and seasonally due to environmental and technological constraints (Macdiarmid, 2014). Nowadays, the market offers a wide variety of fruits and vegetables, and dietary habits have evolved accordingly (Régnier et al., 2022). However, the in-season consumption of fruits and vegetables seems to offer benefits from both sustainability and health perspectives (Vargas et al., 2021), even though these points remain unexplored and sometimes speculative. In-season fruits and vegetables are described as having higher polyphenol content than their off-season counterparts, and some authors have reported that the bioavailability of these compounds varies with both the season and individual traits (Cruz-Carriòn et al., 2021; Iglesias-Carres et al., 2019; Landberg et al., 2019). Metabotyping is a promising tool to identify individuals at risk of diet-related diseases and support targeted health strategies (Hillesheim et al., 2019). Although seasonal changes in the gut microbiome are documented (Davenport et al., 2014), the role of seasonal food intake, particularly fruits and vegetables, on metabotypes remains unexplored. Therefore, the present project aims to investigate the previously unexplored role of seasonal eating habits in influencing adherence to the MD, gut microbiota composition, and the production and excretion of health-related metabolites of dietary origin.

2. PhD Thesis Objectives and Milestones

The main objectives of 4-SEASOMed project are:
1. Assess the adherence to a seasonal diet focused on fresh fruits and vegetables across four seasons.
2. Evaluate the relationship between in-season food choices, MD, and lifestyle factors influencing dietary habits.
3. Investigate how seasonal diet adherence affects metabolic phenotypes (metabotyping) based on microbiota, urinary (poly)phenol metabolites, and faecal short-chain fatty acids across the four seasons.

This PhD thesis project can be divided into the following activities according to the Gantt diagram represented in Table 1:

A1) **Observational study (months 0-18)**: A 9-month multicentre observational study will be conducted for recruiting 400 healthy Italian adults from three regions (A1.1). Participants will complete a 3-days food diary and validated questionnaires (Seasonality Q, FFQ, Medi-Lite, IPAQ, PSQI, MEQ and Fito Q) for monitoring dietary habits and lifestyle factors during the 4 follow-ups (corresponding to each of the 4 seasons (0, 3, 6, 9 months) (A1.2). In a sub-cohort of 100 subjects, urine and faecal samples will be collected.

A2) **Sample and Data Analysis (months 9-21):** Data collected during the observational study will be analyzed (A2.1). Moreover, urine samples will undergo targeted and untargeted metabolomics (A.2.2) to profile dietary metabolites, while faecal samples will be analyzed for SCFAs (A.2.3) and microbiota composition through 16S rRNA gene sequencing and bioinformatics analysis (A.2.4).

A3) **Data integration and statistical analysis (months 21-27):** Data from 400 participants will be combined for statistical analysis, including descriptive statistics and statistical analysis based on data distribution. Associations between dietary habits, lifestyle factors and other variables will be examined through univariate and multivariate analyses (A.3.1). A sub-cohort will undergo metabotype classification using biological and anthropometric data, which will be analyzed through multivariate methods (PCA, cluster analysis, and PLS-DA) (A.3.2).

A4) **Writing and editing PhD thesis, scientific papers and oral and/or poster communications.**

Table 1 Gantt diagram for this PhD thesis project.

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
A1)	***Observational study***																																				
	1) Volunteer enrollment																																				
	2) Sample and data collection																																				
A2)	***Sample and Data Analysis***																																				
	1) Dietary/lifestyle habits and anthropometric data analysis																																				
	2) Targeted Urinary Metabolomics																																				
	3) Faecal metabolites																																				
	4) Gut microbiota composition analysis																																				
A3)	***Data integration and statistical analysis***																																				
	1) Association between seasonality and MD adherence																																				
	2) Metabotyping																																				
A4)	***Writing and editing PhD thesis***																																				

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No-alcohol, Low-alcohol (NoLo) and wines produced by sustainable and safe protocols: technological strategies and operative opportunities for new-frontier oenological productions (P-I-79)

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The project aims to evaluate innovative technologies and sustainable oenological practices to enhance the quality and health profile of wines. The focus is on developing and optimizing production methods that enable the creation of wines with reduced or zero alcohol content and limited, or ideally absent, use of sulfur dioxide, while ensuring stability, safety, and desirable sensory attributes. Experimental activities will be carried out in collaboration with wineries in the Lazio region, with particular attention to the use of autochthonous grape cultivars, to valorize local viticultural heritage and foster a regionally integrated approach to oenological innovation.

No-alcohol, Low-alcohol (NoLo) e vini prodotti secondo protocolli sostenibili e sicuri: strategie tecnologiche e opportunità operative per produzioni enologiche di nuova generazione.

Il progetto mira a sviluppare metodi di produzione sostenibili per la realizzazione di vini a ridotto o nullo contenuto alcolico, con un impiego limitato di anidride solforosa, garantendo al contempo stabilità del prodotto e qualità sensoriale. Particolare attenzione sarà rivolta agli effetti di tali pratiche sulla qualità (enotecnica, fenolica ed aromatica) e sulla shelf life del vino, al fine di assicurare elevati standard di sicurezza e gradimento del consumatore. Le attività sperimentali saranno condotte in collaborazione con aziende vitivinicole del territorio laziale, con un focus specifico sull'impiego di vitigni autoctoni, per valorizzare il patrimonio varietale locale e promuovere un'innovazione enologica integrata e territoriale.

1. State of the Art

Within this context, the development of NoLo (No- and Low-alcohol) wines represents a key area of innovation. One of the main technological challenges involves controlling yeast metabolism during alcoholic fermentation. Recent studies have explored the use of *non-Saccharomyces* yeast strains, either through sequential or co-inoculation with *Saccharomyces cerevisiae*, to modulate fermentation dynamics (Ciani et al., 2010; Varela et al., 2017). Certain strains, including *Torulaspora delbrueckii, Metschnikowia pulcherrima,* and *Lachancea thermotolerans*, have shown the ability to produce lower ethanol concentrations while enhancing the synthesis of favorable secondary metabolites such as glycerol, esters, and acetates (Benito, 2019). Nevertheless, the behavior of these yeasts remains highly context-dependent, varying according to grape matrix composition, environmental conditions, and microbial interactions. Common concerns include the risk of incomplete fermentation, excessive acetic acid production, and reduced stability of key metabolites under suboptimal nutrient availability or osmotic stress. After fermentation, physical dealcoholization processes such as vacuum distillation, reverse osmosis, and spinning column technology are widely applied to further reduce alcohol content. However, these techniques are frequently associated with undesirable modifications of the wine's volatile compound profile and phenolic equilibrium, leading to aroma losses and disruptions of sensory balance (Pickering, 2000; Liguori et al., 2023). Although lower processing temperatures have been proposed to mitigate degradation, current protocols are rarely optimized for native grape varieties, whose responses often differ from those of international cultivars. Moreover, the cumulative impact of dealcoholization on colloidal stability, oxidative resistance, and aging potential has not yet been fully elucidated, and the long-term consequences for structure, mouthfeel, and the perception of sweetness and acidity remain poorly understood (Ferreira et al., 2021). Another critical issue is the reduction or elimination of sulfur dioxide. While SO_2 remains a cornerstone of winemaking due to its antioxidant and antimicrobial properties (Waterhouse et al., 2016), its reduction is increasingly demanded by consumers and regulatory bodies. Several alternatives have been investigated, including plant-derived polyphenols, yeast derivatives with antioxidant activity, and oxidative treatments such as ozone, but most studies have been conducted on simplified systems or single varieties, and their interactions with reduced alcohol matrices are not yet well defined. A comprehensive understanding of these interlinked factors is therefore essential to design reliable, scalable, and sustainable protocols for NoLo wine elaboration that safeguard stability, quality, and consumer acceptance.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Application of low-ethanol fermentative protocols using non-Saccharomyces yeasts.** Micro-vinification trials using indigenous Vitis vinifera cultivars from the Lazio region will be conducted to test co-inoculation

and sequential fermentation strategies involving non-Saccharomyces strains, selected from autochthonous wine grape heritage, in combination with Saccharomyces cerevisiae ones (A 1.1). The alcoholic fermentations will be monitored to determine ethanol yield, secondary metabolite production, and fermentation kinetics, with the objective of characterizing fermentative efficiency, microbial interactions, and sensory impact of the resulting wines (A 1.2)

A2) **Evaluation of post-fermentation dealcoholizing technologies**. After fermentation (A2.1), three physical dealcoholizing methods, vacuum distillation (A2.2), reverse osmosis (A2.3), and spinning cone column technology (A2.4), will be tested at pilot scale to achieve partial or full alcohol removal. Treated wine fractions will undergo analytical profiling of volatile compounds, phenolic composition, acidity, and SO_2 levels to assess the impact on sensory attributes and stability (A2.5).

A3) **Blending and optimization of dealcoholized wine fractions.** Experimentally dealcoholized fractions will be recombined in various ratios (A3.1) and evaluated to identify optimized blends offering a favorable balance between sensory quality and chemical-physical stability. These blends will be validated through physicochemical analysis and sensory evaluation using trained panels (A3.2).

A4) **Assessment of sulfur dioxide alternatives for wine stabilization. A**dditives and processing aids with known or potential antioxidant properties—such as hydrolysable and condensed tannins, yeast extracts, and plant-derived polyphenols—will be tested on both NoLo and conventional wines (A4.1 and A4.2). The antioxidant capacity of each treatment will be measured, along with their effect on phenolic preservation, oxidative stability, and aromatic profile over time (A4.3), simulating shelf-life conditions (A4.4).

A5) **Literature review, writing and editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project. The following acronyms are used: E.P. = Enological Parameters, D.P.P. = Detailed Phenolic Profile VOC A. = Volatile Organic Compounds Analysis P.D. = Physical Dealcoholizing, S.A. = Sensory Analysis.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Use of wine starters for reduced ethanol***																								
	1) Grape harvest - vinification																								
	2) Analysis E.P. – D.P.P. – VOC A.																								
A2)	***Post-fermentation dealcoholization technologies***																								
	1) Grape harvest - vinification																								
	2) Vacuum distillation																								
	3) Reverse osmosis																								
	4) Spinning cone column technology																								
	5) Analysis E.P. – D.P.P. – VOC A.																								
A3)	***Blending and optimization***																								
	1) Blending																								
	2) Analysis E.P. – D.P.P. – VOC A. – S.A.																								
A4)	***Assessment of sulfur dioxide alternatives***																								
	1) Grape harvest - vinification																								
	2) Additives and processing aids																								
	3) Analysis E.P. – D.P.P. – VOC A.																								
	4) Evaluation of ageing potential																								
A5)	***Literature review, Thesis and Paper Preparation***																								

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Exploring the nature and mechanism of formation of red wine colloids: implications for stability and quality (P-I-80)

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Red wine can be seen as a complex colloidal system, with colloids influencing key sensory properties and stability. Despite their importance, the mechanisms underlying colloid formation and the role of macromolecules remain unclear. This PhD project aims to investigate the nature, formation, and stability of red wine colloids using analytical chemistry, spectroscopy, chromatography, and particle analysis. The study will assess how wine composition, winemaking practices, and storage conditions affect colloidal behavior. The goals are to advance understanding of colloid formation mechanisms and develop targeted strategies to manage colloidal phenomena, ultimately improving wine quality and production efficiency for the industry.

Analisi della natura e dei meccanismi di formazione dei colloidi del vino rosso: implicazioni per la stabilità e la qualità

Il vino rosso è un sistema colloidale complesso, in cui i colloidi hanno notevole influenza sulle proprietà sensoriali e la stabilità. Tuttavia, i meccanismi alla base della formazione dei colloidi e il ruolo delle macromolecole restano poco chiari. Questo progetto ha l'obiettivo di indagare la natura, la formazione e la stabilità dei colloidi nel vino rosso tramite l'impiego di chimica analitica, spettroscopia, cromatografia e caratterizzazione delle particelle. Lo studio valuterà come composizione del vino, pratiche enologiche e condizioni di conservazione influenzino il comportamento colloidale. L'obiettivo è approfondire la comprensione dei meccanismi e sviluppare strategie per migliorare la qualità ed efficienza produttiva.

1. State-of-the-Art

Red wine compounds such as proteins, polysaccharides, and phenolic substances are known to play a key role in defining several key wine characteristics (Pascotto et al., 2021). Oenological research has long tackled the issue of wine instabilities, which often result in haze formation, sedimentation, and color changes during the lifespan of wines, these being only some of the defects attributable to colloidal phenomena in red wines. Therefore, the presence of colloids directly impacts the winemaker's interventions aimed at clarifying and stabilizing wines (Vernhet, 2018), and eventually also consumers' perception.

Colloids show dynamic behavior, making them challenging to study, especially given the high complexity of red wine composition (Marassi et al., 2021), which evolves over time due to the multitude of chemical reactions occurring in wine. This is particularly true for red wines, where components extracted from grape skins contribute to wine evolution, affecting their stability and sensory properties such as clarity, color, taste, mouthfeel, and potentially also aroma partitioning (Jones-Moore et al., 2022; Marassi et al., 2021).

Despite the critical role colloids play in red wine quality, their formation, composition, and properties are only partly understood. Their large native size makes them more difficult to analyse than individual molecules. Additionally, the diverse range of interactions, both strong and weak, that hold colloid-forming molecules together can be disrupted during analysis, making it difficult to study colloids in their native state (Marangon et al., 2024). The complexity of red wine composition, which can vary according to environmental factors, production processes, climatic variations, and grape variety, makes it difficult to clearly define the dynamics of colloidal systems in wine. Furthermore, analytical challenges also arise due to the abundance of compounds that may interfere with the analysis of interest. Nevertheless, in recent years several studies have increased our knowledge of red wine colloids (Jones-Moore et al., 2022; Marangon et al., 2025, 2024; Marassi et al., 2021), confirming the idea that these structures result from the interaction of macromolecules. Initial studies focused on the role of phenolics interacting with polysaccharides, while recently, the key role of grape proteins in the colloidal assembly has been highlighted (Marangon et al., 2025, 2022), as well as the importance of the ratio between the different colloid-forming molecules, which depends also on technological factors (e.g. maceration conditions, fining, filtrations…).

Therefore, this PhD project will employ and validate experimental approaches to clarify the formation, structure, and evolution of colloids in red wine, with a particular focus on the role of macromolecules and the influence of winemaking conditions. The aim is to develop a comprehensive understanding of these phenomena and their impact on wine stability and quality, ultimately generating practical knowledge to support more effective management of colloidal properties in the wine industry.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following

activities, as detailed in the Gantt diagram in Table 1:

A1) **Bibliographic research** on the current state of knowledge on red wine colloids, including their formation, and stability, as well as identification of existing gaps in literature.

A2) **Characterization of the colloidal profile of representative commercial red wines** to provide an overview of the current wine colloidal composition. This activity will focus on using methods to identify and quantify the main colloid-forming components in red wine, providing a detailed profile of their composition and structural features.

A3) **Extraction and characterization of proteins, polysaccharides, and polyphenols from grape pulp and skin (including their aggregated forms)** to assess the state of colloid-forming molecules in grapes. This will be complemented by investigating how these molecules interact to generate colloidal structures and by determining the specific stages of the winemaking process in which colloids are formed and how they evolve over time.

A4) **Study of colloids' formation during model fermentation** to investigate the interactions between macromolecules and to identify potential synergies or antagonisms between them. Key variables such as ethanol content, temperature, and pH will be systematically tested to determine their influence on colloidal behavior.

A5) **Long-term stability study** to monitor the evolution of red wine colloids during barrel ageing.

A6) **Evaluation of different winemaking practices** to determine the most effective treatments for managing colloidal stability of red wines.

A7) **Dissemination activities** such as the preparation and submission of scientific papers, as well as oral and/or poster communications at national and international conferences.

A8) **Writing and Editing** of the PhD thesis.

Table 1 Gantt diagram for this PhD thesis project.

	Activity	Months																	
		2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Bibliographic research***																		
A2)	**Characterization of the colloidal profile of representative commercial red wines**																		
	1) Selection of different bottled wines																		
	2) Chemical characterization of colloids profile and colloidal stability of red wines																		
A3)	***Extraction and characterization of colloid-forming molecules in grapes***																		
	1) Sample preparation																		
	2) Macromolecule characterization																		
	3) Purification and aggregate study																		
A4)	***Study of colloids' formation during model fermentation***																		
	1) Settling model fermentation systems																		
	2) Characterization of colloidal particles																		
A5)	***Long-term stability study during wine ageing***																		
	1) Wine sampling																		
	2) Analysis of different wine parameters																		
A6)	***Evaluation of different winemaking practices***																		
	1) Wine treatment																		
	2) Wine analysis																		
A7)	***Dissemination activities***																		
A8)	***Writing and Editing of the PhD thesis***																		

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Bridging Agriculture to Food Technology for Sustainable Bakery Products (P-I-81)

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With the final objective of proposing strategies to limit the impact of climate change on wheat quality, this PhD project is aimed at evaluating the effect of regenerative agriculture strategies (i.e. cover crops, minimum tillage, and precision fertilization) and technological approaches (i.e. improvers and/or processes), on the quality of flour and final bakery products.

Prodotti da forno e sostenibilità: sinergia tra agricoltura e tecnologia alimentare

Con l'obiettivo finale di proporre strategie per contrastare l'impatto del cambiamento climatico sulla qualità del frumento, questo progetto di dottorato è finalizzato a valutare l'effetto di alcune strategie di agricoltura rigenerativa (ovvero colture di copertura, minima lavorazione del terreno e concimazione di precisione) e di approcci tecnologici (come miglioratori e/o processi) sulla qualità della farina e dei prodotti da forno.

1. State-of-the-Art

In the last decades, climate change has been threatening agriculture, limiting the sustainability of the system and affecting the quality of wheat and other crops. It is well known that changes in climate over time could be due to natural variability or to human activity, and cause rising temperatures, high concentration of carbon dioxide in the atmosphere and extreme events such as drought, heat waves, salinity stress, and changes in rainfall (Zahra *et al.*, 2023).

A possible solution to limit the problems caused by climate change could be the adoption of agronomic strategies belonging to regenerative agriculture. Indeed, regenerative agriculture could be defined as an approach to farming that uses soil conservation as the entry point and aims to enhance not only the environmental, but also the social and economic dimensions of sustainable food production (Schreefel *et al.*, 2020). Since the main objectives of regenerative agriculture are restoration of soil health, increasing biodiversity, carbon sequestration, mitigation of greenhouse gas emissions, and reduction in fertilizing input, practices like cover crops, minimum tillage, and precision fertilization seem to be promising solutions (Quintarelli *et al*., 2022; Liang *et al.*, 2025; Diacono *et al.*, 2013).

Most scientific publications appear to focus on protein content and crop yield. On the other hand, currently, there are limited studies aimed at evaluating the effects of the aforementioned agronomic practices on the technological quality of wheat. For this reason, it seems necessary to further investigate the impact of these agronomic strategies on the technological quality of wheat flour, in terms of gluten aggregation properties, starch gelatinization and retrogradation, mixing properties, and aspects related to food safety and quality of final baked products.

Moreover, since a deterioration in the quality of wheat cannot be excluded after the adoption of regenerative agriculture practices and given the need for companies to guarantee high quality standards, it becomes necessary to select improvers (e.g. enzymes, additives, ingredients) or (bio)technological processes (e.g. sprouting, fermentation, ozonation, high pressure process) to be applied to wheat flour (Tebben *et al.,* 2018; Dapčević-Hadnađev *et al.*, 2022).

Thus, this PhD thesis project will be focused on: 1) evaluating the impact of regenerative agriculture practices (cover crops, minimum tillage, and precision fertilization), proposed to mitigate climate change side effects, on the qualitative features of wheat flour and baked goods, 2) selecting flour improvers and/or processes to guarantee high and consistent quality of wheat flour and baked goods, in order to satisfy food industry demand and, 3) evaluating the effects of flour improvers and/or processes, identified as wheat flour enhancers, on qualitative features of wheat flour and baked goods.

2. PhD Thesis Objectives and Milestones

To achieve the objectives described above, this PhD thesis project can be divided into the following activities, according to the Gantt diagram given in Table 1:

A1) **Assessment of the effects of regenerative agriculture strategies on wheat quality.**

A1.1) Cover crops, A1.2) Minimum tillage, A1.3) Precision fertilization.

For each agronomic strategy gluten aggregation properties will be evaluated with GlutoPeak (Anton Paar GmbH, Graz, Austria), starch gelatinization and retrogradation through Micro-Visco-Amylograph (Anton

Paar GmbH, Graz, Austria), dough properties during mixing with Farinograph (Anton Paar GmbH, Graz, Austria; AACC 54-21.01), uniaxial with Extensograph (Anton Paar GmbH, Graz, Austria; AACC 54-10.01) and three-dimensional extension through Alveograph (Chopin, Villeneuve La Garenne, France; AACC 54-30.02).

In addition, the most suitable flours will be selected and adopted to prepare leavened products. Image analysis techniques will be used to assess physical features (e.g. size, color, porosity) and specific volume (AACC 10 05.01) and crumb softness (AACC 74-10.02) will be analysed.

A2) **Selection and assessment of technological strategies to improve wheat quality.**

A2.1) A detailed analysis of the existing literature and a selection of the most promising technological strategies (improvers and/or processes) will be conducted, also considering the specific needs of the company co-funding the PhD thesis project.

A2.2) Concerning the assessment of the impact of selected technological strategies, the properties reported at point A1) will be evaluated, following the methods above. The development of a leavened product will be the last step of the project and the methods reported at point A1) will be adopted to verify the quality of final bakery products.

A3) **Writing and dissemination.** Writing of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Regenerative agriculture strategies and wheat quality***																								
	A1.1) Cover crops																								
	A1.2) Minimum tillage																								
	A1.3) Precision fertilization																								
A2)	***Technological strategies and wheat quality***																								
	A2.1) Literature review and selection																								
	A2.2) Impact on wheat quality and product development																								
A3)	***Writing and dissemination***																								

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Integral Conversion of Industrial Fruit and Vegetable Waste into Extracts and Aerogels with Enhanced Functionality

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This PhD thesis research project is aimed at developing an integral valorization process for industrial fruit and vegetable waste by converting them directly into bioaerogels and bioactive extracts, to produce high-value-added ingredients and innovative biodegradable devices and packaging, aligning with the principles of a zero-waste circular economy.

Conversione Integrale di Scarti Industriali di Frutta e Verdura in Estratti e Aerogel con Funzionalità Avanzate

Questo progetto di tesi di dottorato mira a sviluppare un processo di valorizzazione integrale di scarti di frutta e verdura, generati industrialmente, mediante la loro conversione diretta in bioaerogel ed estratti bioattivi per produrre ingredienti ad alto valore aggiunto e dispositivi e packaging innovativi e biodegradabili, in linea con i principi di un'economia circolare senza sprechi.

1. State-of-the-Art

Nowadays, waste generated by the fruit and vegetable industry (fruit and vegetable waste, FVW) is becoming a serious problem, representing up to 60% of the world's yearly food waste (FAO, 2011). Although aligning with the circular EU policies (Fetting, 2020), current FVW management (e.g., anaerobic digestion, composting) lead to the downcycling of these residues, resulting in products (fertilizers, biogas, feed) characterized by a lower value as compared to the initial matrix. Nevertheless, FVW still contains significant amounts of nutritionally and technologically valuable components, with specific reference to fibers, other polysaccharides and proteins (Esparza et al., 2020), whose upcycling stands as a promising strategy to unlock the value of FVW. Despite this high potential, the upcycling of FVW into food ingredients and food contact materials (FCM) is still not widely applied, requiring the development of technological interventions for waste recovery and transformation into value-added products. Literature approaches to upcycle FVW typically fall under the biorefinery concept, which aims to the production of high-purity derivatives like cellulose, proteins, and starch. However, this strategy requires numerous sequential processing operations, with high costs, and significant solvent and energy consumption. Moreover, this approach results in low yields, associated with the generation of a significant amount of extraction residues spent, which still need to be managed as waste. For instance, this is the case of the extraction of proteins and other bioactive compounds from plant sources, which often results in low yields, due to the strong interactions among the target molecule and the vegetable fibrous matrix (Kumar et al., 2021; Martins et al., 2023). A way to overcome this problem could rely on development and optimisation of a process concomitantly allowing the integral valorization of FVW into: (i) extracts with technological function or nutritional value and (ii) aerogels to be used both as food ingredients and FCM. Aerogels are solid materials characterized by high porosity, low density, and high internal surface area (Fricke and Tillotson, 1997). When prepared from bio-based precursors, they are referred to as bioaerogels (Stergar and Maver, 2016). Bioaerogels are biocompatible, biodegradable, and food-grade, representing a promising material with versatile applications in the food industry. They can be used to encapsulate bioactive compounds, serve as fat replacers in food formulations, act as processing aids, and contribute to innovative food packaging solutions (Maleki, 2016). Bioaerogels are conventionally produced starting from a biopolymer aqueous solution, which is then gelled to produce a hydrogel. The latter must be then dried with a technique able to highly preserve the original biopolymeric network structure. This target is obtained using supercritical-CO_2-drying (SCD), which involves the preliminary substitution of hydrogel water with ethanol, followed by ethanol removal by a flow of CO_2 in the supercritical state. Interestingly, plant matrices such as FVW can be regarded as hydrogels, since they are made by a complex biopolymeric network, mainly structured by cell wall cellulosic fibers, embedding water within intra- and inter-cellular spaces.
The application of water-to-ethanol substitution followed by SCD to FVW could thus offer a double advantage. From one side, the use of high performing solvents, such ethanol and supercritical CO_2, would allow the efficient extraction of pigments and bioactive compounds from plant tissues, yielding high-purity extracts with significant upcycling potential. On the other hand, the direct treatment of FVW would simplify the conventional production process of bioaerogels, eliminating the need for FVW polymers extraction and gelling. Thus, this PhD thesis aims to develop a true zero-waste upcycling process for FVW, enabling the simultaneous preparation of extracts and advanced porous materials. The potential of these novel products will be demonstrated through their application as high-value food ingredients and innovative biodegradable active packaging solutions.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Development of extracts and bioaerogels:** FVW (e.g., fruit peels, substandard pea, and vegetable leaves) will be subjected to water-to-ethanol solvent exchange, followed by SCD to concomitant obtain bioactive ethanolic extracts (A1.1) and bioaerogels (A1.2).

A2) **Bioaerogel and extracts characterization:** the bioaerogels will be characterized by their physical features using different analytical techniques (e.g., scanning electron microscopy, BET analysis and FTIR spectroscopy) (A2.1), while the ethanolic extracts will be analysed for total phenolic content, antioxidant activity, antimicrobial activity and pigment profile using validated assays (A2.2)**.**

A3) **Loading bioaerogels with extracts:** the obtained bioaerogels will be loaded with the characterized extracts using different loading techniques (e.g., supercritical impregnation and wet impregnation), to obtain bioaerogels enriched with bioactive compounds (A3.1). The loaded bioaerogels will be tested for their ability to interact with food solvents and water vapor evaluating their adsorption and absorption capacity, release kinetics, antioxidants and antimicrobial activity (A3.2).

A4) **Prototyping:** loaded bioaerogels will be considered to produce active packaging (A4.1) able to inhibit oxidative phenomena and/or microbial growth with the final aim of obtaining shelf-life extending food contact materials. Bioaerogels will also be incorporated into various food formulations (A4.2) to assess the impact of their use as ingredients on food texture, stability and nutritional properties.

A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 2 Gantt diagram for this PhD thesis project.

Activity		Months																							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
A1)	***FVW conversion into extracts and bioaerogels***																								
	1) Ethanolic extracts production																								
	2) Bioaerogels development																								
A2)	***Extracts and bioaerogel characterization***																								
	1) Ethanolic extracts properties																								
	2) Bioaerogels characterization																								
A3)	***Loading of the bioaerogels with extracts***																								
	1) Loading optimization																								
	2) Loaded aerogels testing																								
A4)	***Prototyping of food grade ingredients and materials***																								
	1) FCM production																								
	2) Ingredients development																								
A5)	***Literature review and Thesis and Paper Preparation***																								

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Characterization and evaluation of food products using advanced statistical methods and models (P-I-83)

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This PhD thesis project aims to develop a structured analytical framework to jointly model consumer ordinal evaluations and objective food characteristics. The strategy involves the transformation of ordinal rating matrices into directional data to define individualized evaluative profiles and correct for subjectivity in rating scales. The project will proceed through phases of methodological development, model adaptation (including regularization and canonical correlation approaches), and possibly exploring the integration of mixed-type data under hierarchical structures, with the goal of identifying robust statistical models that capture both individual variability and product-specific sensory patterns.

Caratterizzazione e valutazione dei prodotti food mediante metodi e modelli statistici avanzati

Questo progetto di tesi di dottorato mira a sviluppare un quadro analitico strutturato per la modellizzazione congiunta delle valutazioni ordinali espresse dai consumatori e delle caratteristiche oggettive dei prodotti alimentari. La strategia prevede la trasformazione delle matrici di valutazione in dati direzionali, al fine di definire profili valutativi individuali e correggere la soggettività nell'uso delle scale di valutazione. Il progetto si articolerà in fasi di sviluppo metodologico, adattamento di modelli (inclusi approcci di regolarizzazione e analisi canonica), ed eventualmente esplorerà l'integrazione di dati misti all'interno di strutture gerarchiche, con l'obiettivo di individuare modelli statistici robusti in grado di rappresentare sia la variabilità individuale che le caratteristiche sensoriali specifiche dei prodotti.

1. State-of-the-Art

Consumer preference data, especially in the food sector, are often collected using ordinal scales (e.g., Likert-type ratings), which are intrinsically subjective and sensitive to individual differences in scale interpretation and usage. Classical methods in preference learning, such as Bradley-Terry models, typically handle pairwise comparisons but do not fully leverage the structure of multivariate ordinal evaluations or the interplay with objective product characteristics.

Recent advances in statistical learning from preference information (e.g., Bayesian and nonparametric models) and the increasing use of hybrid data (qualitative and quantitative) have highlighted the need for integrative models. In particular, representing ordinal ratings as directional data on the unit hypersphere allows one to model evaluative profiles while controlling individual differences in scale inflation or usage heterogeneity.

An innovative perspective involves treating the matrix of evaluations (judges × features) as structured across two analytical spaces: the row space, representing the evaluation behavior of each individual user through directional data transformation, and the column space, summarizing how each product feature is perceived across the panel. The row space captures a user's normalized preference vector across features, accounting for directionality and magnitude-free comparisons. The column space aggregates these directional judgments across users but must be corrected for individual scale usage to avoid biased interpretations.

Few methods have addressed the challenge of modelling hierarchical or structured data when both latent preference directions and objective features must be integrated. Lattice-based models, used to handle mixed-type data and incorporate feature hierarchies, offer a promising solution in this context. The work by Wang & Wang (2020) is a useful reference for structured modeling in multi-aspect evaluation settings.

2. PhD Thesis Objectives and Milestones

This PhD project aims to construct a novel statistical framework for the joint analysis of consumer ordinal ratings and objective food properties, grounded in directional data methodology. The research will proceed through the following activities:

A1) Exploratory Data Structuring and Directional Transformation

- Design a data matrix with rows as judges and columns as evaluated features.
- Develop a transformation strategy to represent each row (individual profile) as a directional vector, normalizing for individual rating inflation or compression.
- Investigate methods to extract interpretable feature profiles in the column space after controlling for user

heterogeneity.

A2) Modeling Preference Space

- Explore techniques to extract reliable feature profiles accounting for inconsistent scale usage.
- Apply normalization methods (e.g., percentile scaling, rank-centering) and assess their effectiveness in recovering latent evaluation structures.

A3) Modeling with Pairwise or Continuous Response Frameworks

- Explore the possibility of using ranking models suited for directional and multivariate representations.
- Evaluate the suitability of directional multi-aspect ranking and related models.

A4) Integration of Objective Product Attributes

- Investigate the contribution of intrinsic quantitative characteristics of food items to the structure of directional preferences.
- Develop analytical strategies based on regularization methods such as ridge- and lasso-type models, to evaluate the relationship between objective variables and latent directional profiles in both row and column spaces.
- Explore the application of Generalized Canonical Analysis, adapting it to directional data and assessing its performance under different normalization approaches of the rating scale.

A5) Mixed Data and Hierarchical Structures

- Explore lattice-based methods to model mixed-type data (ordinal + quantitative), especially in the presence of feature hierarchies.
- Propose extensions to integrate directional response variables in lattice structures.

A6) Writing and Dissemination

- Preparation of scientific papers and conference contributions.
- Final writing and editing of the PhD thesis.

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Formulation of new confectionery products based on functional compounds deriving from plant by-products (P-I-84)

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This PhD thesis research project is part of the circular economy and aims to valorize by-products of the agri-food industry, with particular focus to vegetable waste. Specifically, it proposes the recovery and transformation of citrus peels, prickly pear peels and carob pulp, in order to obtain functional flours to be used in the formulation of confectionery products, such as muffins, biscuits and panettone. The objective is twofold: on the one hand, to reduce the environmental impact resulting from the disposal of these wastes; on the other, to enrich the final products from a nutritional and functional point of view, contributing to the development of healthy foods with high added value.

Formulazione di nuovi prodotti dolciari a base di composti funzionali derivanti da sottoprodotti vegetali

Questo progetto di tesi di dottorato si inserisce nel contesto dell'economia circolare e mira alla valorizzazione di sottoprodotti dell'industria agroalimentare, con particolare riferimento agli scarti vegetali. Nello specifico, si propone il recupero e la trasformazione di bucce di agrumi, bucce di fico d'India e della polpa di carrubo, al fine di ottenere farine funzionali da impiegare nella formulazione di prodotti dolciari, come muffin, biscotti e panettoni. L'obiettivo è duplice: da un lato, ridurre l'impatto ambientale derivante dallo smaltimento di questi scarti; dall'altro, arricchire i prodotti finali dal punto di vista nutrizionale e funzionale, contribuendo allo sviluppo di alimenti salutistici ad alto valore aggiunto.

1. State-of-the-Art

The increase of the global population has led to greater food production and, consequently, to a significant increase in agri-food waste. These by-products, rich in nutrients such as carbohydrates, proteins, fibers, minerals and vitamins, represent potential functional ingredients. At the same time, the market of functional foods is rapidly expanding, driven by the growing interest of consumers in foods with health benefits. Confectionery products, which are widely consumed, do not often meet these nutritional needs, opening up to the use of alternative flours, increasingly used to produce low-calorie, high-fiber and low-glycemic index foods, aimed at health-conscious consumers. Numerous studies show that vegetable by-products, such as peels and inedible parts, can be transformed into flours rich in fibers and phenolic compounds, useful for improving the nutritional value, functional properties and shelf-life of confectionery products.

Citrus peels are one of the most abundant biomasses deriving from the processing industry. They are particularly rich in dietary fiber, with values ranging from 41.5% to 71.3%, and contain a good amount of protein, ranging from 4.0% to 8.8%. In addition to these nutrients, citrus peels are characterized by a rich presence of bioactive phytochemicals, such as hydroxycinnamic acids (including p-coumaric, gallic, caffeic, ferulic, and sinapic acids) and flavone glycosides (such as hesperidin, neo-hesperidin, narirutin, and naringin). They also contain essential oils, pigments such as carotenoids, vitamins including vitamin C, and soluble fibers like pectin. All these components can contribute positively to human health, thanks to their antioxidant, anticancer, antiviral, and anti-inflammatory properties. A study conducted by Abdelwahab et al. in 2018 demonstrated that the use of flour obtained from citrus peels in the preparation of functional flours used for confectionery products, in particular for cakes, not only brings benefits related to the high fiber content, but it also contributes to improve the nutritional composition, the functional properties of the finished product and its acceptability from a sensorial point of view.

Prickly pear peels, usually discarded during the industrial processing of the fruit, show also a remarkable nutritional potential. These peels contain 3.6% protein, 2.1% fat, 33.0% total dietary fiber, 17.1 mg/g total polyphenols, 16.9 mg/100 g flavonoids, 12 mg/100 g betaxanthins and 7.6 mg/100 g betacyanins, (Parafati et al., 2020). Several studies have shown that the partial replacement of wheat flour with prickly pear peel flour in the production of biscuits represents a promising strategy for the creation of functional baked products. Prickly pear flour offers also technological benefits thanks to the presence of fibers and polyphenols that promote kneading, flavor retention and the antioxidant capacity of the products. Furthermore, sensory tests showed that biscuits prepared with 20% and 30% of prickly pear flour were more appreciated by consumers in terms of aroma, taste, color and overall acceptability (Bouazizi et al., 2020). Carob peels, considered as a by-product of the carob bean processing industry, are also a valuable nutritional resource. They contain carbohydrates, mainly in the form of sugars and fiber, minerals, amino acids and vitamins. The secondary metabolites present in carob pulp, such as phenolic acids, flavonoids and tannins, take on particular importance. They are known for their functional properties and benefits for human health, including antioxidant, anti-inflammatory, and anti-aging effects. A study on the application of carob pulp flour in bread making showed that the addition of 15% of this flour improved the

nutritional value of bread by increasing the content of fiber and minerals, especially manganese and copper, as well as significantly increasing the antioxidant activity. Microbiological tests also confirmed that carob pulp flour contributes to prolonging the freshness and the shelf life of bread up to seven days. Consequently, it can be successfully used in the development of functional bread, in line with modern food trends (Zahorec et al., 2024). The project dealt with critical issues related to the optimization of formulations, the stability of bioactive compounds during processing, the safety and the nutritional functionality of products, as well as their sensorial acceptability, through a multidisciplinary approach oriented towards the development of functional confectionery from plant by-products. Further studies will be essential to optimize formulations, evaluate the stability of functional compounds during technological processes and ensure the safety and efficacy of the developed products.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Characterization of plant-based raw materials**: in the first phase of the project, the raw materials will be selected, and the waste will be transformed into flour through controlled drying and grinding processes. The flours obtained will then be subjected to chemical-physical and nutritional characterization to evaluate parameters such as residual humidity, dietary fiber content, polyphenols, sugars and other bioactive compounds. This phase is fundamental because it allows us to understand the technological and functional properties of the flour that will be used for the formulation of the product.

A2) **Formulation of recipes**: in the second phase, we will move on to the formulation of confectionery products in which functional flours will partially replace traditional flour. Different percentages of substitution will be tested for each type of plant-based flour, evaluating the impact on the technological characteristics of the doughs (such as water absorption capacity, workability, viscosity) and the final result in terms of taste, consistency and appearance.

A3**) Nutritional, sensory and shelf-life evaluation of finished products**: the confectionery products produced will be analyzed from a nutritional point of view (content of fiber, fats, proteins, phenols, flavonoids, anthocyanins, antioxidant activity), but also from a sensory point of view. Furthermore, the stability of the products over time will be monitored, evaluating shelf-life, resistance to oxidation, variations in texture and preservation of sensory properties.

A4) **Optimization of formulations and technical validation**: in this phase, the data collected in the previous phases will be used to identify the optimal combinations between the type of vegetable flour, the percentage of substitution, and the type of confectionery product. The best formulations will then be validated, considering both nutritional performance and consumer acceptability and industrial stability.

A5**) Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Flour characterization***																								
	1) flour selection and preparation																								
	2) chemical-physical and nutritional analysis																								
A2)	***Recipe formulation***																								
	1) flour substitution tests																								
	2) evaluation of dough properties																								
A3)	***Finished product evaluation***																								
	1) nutritional and sensory analysis																								
	2) Shelf life monitoring																								
A4)	***Formulation optimization***																								
	1) identification of optimal formulations																								
	2) technical validation																								
A5)	***Thesis and Paper Preparation***																								

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AI and emerging technologies driving innovation in functional and health-promoting biopeptides for enhanced development and efficacy of plant-based foods (P-I-85)

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This PhD project investigates strategies for producing bioactive peptides and enriching plant-based food products with health-promoting properties. Experimental designs will optimize peptide yield via fermentation, followed by identification of bioactive sequences through gold-standard and AI-based methods. In vitro assays will assess health functionality, and scalable production protocols will be validated at Barilla's pilot and industrial facilities

Intelligenza artificiale e tecnologie emergenti a supporto dell'innovazione nella selezione e nell'impiego di biopeptidi per lo sviluppo di alimenti funzionali di origine vegetale con accertata valenza nutrizionale e salutistica

Questo progetto di dottorato indaga strategie per la produzione di peptidi bioattivi e l'arricchimento di prodotti alimentari a base vegetale con proprietà salutistiche. Verranno utilizzati piani sperimentali per ottimizzare la resa peptidica tramite fermentazione, seguiti dall'identificazione delle sequenze bioattive mediante tecniche di riferimento e strumenti basati su intelligenza artificiale. La funzionalità salutistica sarà valutata tramite test in vitro e i protocolli produttivi saranno validati su scala pilota e industriale presso gli stabilimenti Barilla

1. State-of-the-Art

Peptides are short sequences of amino acids, typically 2 to 20 residues long, derived from animal, dairy, fish, and plant sources. With growing awareness of environmental pollution, animal welfare, and the role of diet in preventing chronic diseases, interest in plant-derived peptides has strongly increased in the last decade (Sun et al., 2024). Plant-derived peptides frequently exhibit bioactive properties such as antioxidant, antimicrobial, anticarcinogenic, hypocholesterolemic, antihypertensive, and immunomodulatory activities (Hajfathalian et al., 2018). These functionalities make them particularly appealing for the development of functional foods. However, given the complexity of chronic disease development in humans, more studies are needed to evaluate the therapeutic potential of these peptides under the expected condition for food consumption.

Industries face significant challenges in producing functional foods enriched with peptides. As reported by Sun et al. (2024), one of the greatest challenges lies in the production method. Peptides are mainly produced through enzymatic hydrolysis or microbial fermentation. Enzymatic hydrolysis is precise but expensive, whereas fermentation is more cost-effective but harder to standardize. Each method involves processing variables that can lead to structurally different peptides and, consequently, to differences in bioactivity. Currently, peptide identification and characterization remain costly, especially if purification and concentration protocols are a need, posing a challenge to the targeted optimization of industrial processes. In this context, achieving a high yield is also a key objective. Furthermore, the purification phase is the most expensive, representing a major bottleneck for industrial scalability.

Most studies have evaluated peptide efficacy in vitro but when added into food, additional variables should be taken into account. One of the main challenges is the complexity of the food matrix, that can lead to interaction between food components and peptides, especially during food production process. Rivero-Pino et al., (2023) reviewed that in some cases, the food matrix appears to hinder peptide bioavailability, while in others, it may have a favorable effect. They also reported that not only thermal processes generally don't have a negative impact on peptides bioactivity, but during Maillard reaction the bond between reducing sugar and peptides can enhance their functionalities. For instance, Valdez-Meza et al. (2019) observed a significant reduction in systolic blood pressure in an *in-vivo* study on rats within three hours of ingesting pasta enriched with amaranth hydrolysate as a functional ingredient. However, they observed a decrease in overall liking in enriched samples compared to control during sensory analysis. They also reported several changes in pasta quality parameters (especially firmness, cooking time, cooking loss, cooking loss, cooking loss, color).

Food as a peptides carrier could be more appealing compared to drugs from consumers prospectives but is generally reported that peptides have a major impact on technological properties and sensory acceptability. A greater understanding of the interactions between peptides and the food matrix, particularly during food processing, is essential not only to maximize the functional properties of peptides but also their technological and sensory attributes.

2. PhD Thesis Objectives and Milestones

This PhD thesis aims to explore different peptide production and food enrichment strategies of plant-based food

products with health properties. With this aim, the design of experiments will be carried out to maximize peptides production through fermentation. Gold standard techniques and AI-based tools will be performed to identify peptides with desirable health activities. For all food products of interest, health-promoting capacity will be tested *in vitro*. Finally, lab production protocols suitable for scale-up on pilot and industrial scales will be tested at Barilla's facilities.

Aiming to reach the PhD project objectives, the following milestones have been scheduled:

I. **Design of experiments** (DoE) combining mixed formulation-process variables aiming to obtain a wide range of peptides with different structures and molecular weight as well as to maximize the accumulation of classes of peptides with desirable health functionality

II. **Design of experiments** combining mixed formulation-process variables, aiming to obtain a wide range of food matrix interactions. Ingredients, doughs and final products will be characterized for their technological properties (emulsifying capacity and stability, water retention capacity, thermal, thermo-rheological properties). All food products of interest will be characterized for their sensory properties and health functionalities *in vitro (*ABTS+, DPPH, ACE inhibitory tests, anti-proliferative capacity on targeted cell lines, bioavailability on 3D organoids, prebiotic effect gut microbiota).

III. **Scale-up from lab scale (UNIVPM) to pilot line** (at Barilla facilities) both of peptide production and food enrichment.

IV. **Development and validation of AI tools based on physical-informed, rule-based and data driven models.** Such mathematical models will be validated using an experimental dataset different from that used for their development. Ruled-based models will be able to select the peptide profiles with a major contribution to a desirable functionality (output) as a function of formulation and process conditions (input). Physical-informed model could predict the degree of the extent of a desirable functionality as a function of the molecular size distribution properties (input). An attempt will be performed to use such models in prescriptive mode, i.e forecasting the formulation-process conditions (input) to maximize a desirable class of functional peptide (output).

V. **Result dissemination and communication** through publication of at least three original paper or review on peer reviewed journals: PhD Thesis editing and defending

Table1 Gantt diagram for this PhD project.

Activity \ Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
I)	**Peptide production (DoE)**																								
II)	**Food products production (DoE)**																								
	1) dough optimizing																								
	2) health capacity in vitro																								
III)	**Scale up on a pilot and industrial scale**																								
IV)	**Predictive and prescriptive modeling**																								
	1) development and training																								
	2) validation and simulations																								
V)	**Thesis and Paper**																								

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Valorization of Food By-products for the Development of Innovative Functional Snacks (P-I-86)

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Tutor: Prof. Paola Vitaglione

The present PhD research project aims to valorize by-products from the food industry through the characterization and recovery of the bioactive compounds they contain, with the aim of employing them in the development of functional food formulations. Particular focus will be placed on optimizing ingredients capable of modulating physiological responses related to metabolic homeostasis, through interactions with hormonal and sensory signaling pathways. The ultimate objective is the design of a functional snack that, in addition to exhibiting improved nutritional properties, may contribute to the prevention of metabolic disorders and the maintenance of overall health.

Valorizzazione di sottoprodotti alimentari per lo sviluppo di snack funzionali innovativi

Il presente progetto di tesi di dottorato mira a valorizzare sottoprodotti dell'industria alimentare attraverso la caratterizzazione ed il recupero dei composti bioattivi in essi contenuti, con l'obiettivo di impiegarli nello sviluppo di formulazioni alimentari funzionali. Particolare attenzione sarà data all'ottimizzazione di ingredienti in grado di modulare risposte fisiologiche correlate all'omeostasi metabolica, attraverso l'interazione con segnali ormonali e sensoriali. Lo scopo finale è la progettazione di uno snack funzionale che, oltre a presentare proprietà nutrizionali migliorate, possa contribuire alla prevenzione delle patologie metaboliche e al mantenimento di uno stato di salute generale.

1. State of the Art

Obesity, defined as an excessive accumulation of adipose tissue (BMI ≥ 30 kg/m²), represents one of the leading global public health challenges, with projections estimating over one billion cases by 2030 (Oladimeji and Adebo, 2023). This condition is closely linked to a wide range of non-communicable chronic diseases, including type 2 diabetes mellitus, cardiovascular diseases, hypertension, and certain types of cancer. The development of obesity is associated with genetic predispositions and adverse environmental factors, such as poor dietary habits and physical inactivity. In particular, maintaining a healthy lifestyle and adhering to balanced dietary patterns, such as the Mediterranean diet, are recognized as key strategies for preventing and controlling excessive caloric intake and its related complications (Konstantinidi et al., 2019). Within this framework, functional foods emerge as an important tool, defined as foods that, beyond their basic nutritional contribution, actively support health maintenance and disease prevention.

The current paradigm of the circular economy promotes the valorization of agri-food by-products, not only as a means to reduce food waste, but also to identify sustainable sources of bioactive compounds. Eurostat data (2024) estimate that approximately 19% of European food waste is generated by industrial processing. Improper management of this waste has substantial environmental impacts and entails costly treatments that further burden production sectors already operating with narrow profit margins.

Scientific evidence increasingly demonstrates that food industry by-products represent a valuable source of nutritionally relevant compounds, including polysaccharides, dietary fiber, proteins, lipids, vitamins, minerals, and polyphenols. When properly recovered, these components can be utilized to develop high value-added functional foods. Some of these compounds exhibit biological activities capable of positively influencing metabolic regulation, energy intake control, and, consequently, weigh management (Munekata et al., 2021). For instance, a study by Lumaga et al. (2024) reported that a croissant enriched with 4.8% fiber derived from plant matrices and produced through natural fermentation led to a significant 2.2% reduction in fasting blood glucose. This effect was accompanied by increased urinary excretion of urolithin A, a microbial metabolite known for its anti-inflammatory activity, and by decreased serum activity of DPP-IV, an enzyme involved in the degradation of incretins. These findings align with growing evidence supporting the role of dietary fiber in modulating satiety and improving glycemic and lipid metabolism, through both direct physical effects in the gastrointestinal tract and indirectly via microbial fermentation metabolites (e.g., short-chain fatty acids) produced in the colon (Vitaglione and Mennella, 2020). In addition to fiber and polyphenols, recent research has focused on the functional activity of bioactive peptides (BAPs), which are generated via enzymatic hydrolysis of food proteins or microbial fermentation. These peptides exhibit a wide range of biological functions, including inhibition of digestive enzymes, stimulation of incretin release, and modulation of insulin signaling. These effects contribute to improved glucose tolerance and reduced lipogenesis, making BAPs promising agents in the prevention and management of metabolic diseases (Wu and Jahandideh, 2022).

An important aspect in the development of functional foods lies in achieving a balance between the biological efficacy of bioactive compounds and the sensory appeal of the final product. Indeed, palatability is a key determinant of consumer acceptance and, consequently, of the product's commercial success. This implies that the formulation of functional foods must integrate not only physiological and nutritional considerations, but also

hedonic and psychological aspects related to the sensory experience of eating (Munekata et al., 2021).
In light of the current scientific evidence, enhancing the nutritional quality of food through the valorization of agri-food by-products and the strategic use of bioactive compounds represents a concrete and sustainable approach to preventing metabolic diseases associated with unhealthy lifestyles. This strategy, fully aligned with the principles of the circular economy, offers a tangible opportunity to innovate food systems, meet public health needs, and promote more responsible consumption models.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above, the present PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) Nutritional characterization and screening of food industry by-products to assess their content of potentially bioactive compounds. This activity includes extraction, quantitative analysis, and functional screening for polyphenols (A1.1), dietary fibers (A1.2), and peptides (A1.3), evaluated following *in vitro* simulated gastrointestinal digestion.

A2) Application of technological and biotechnological processes to maximize the functionality of by-products. This will be achieved through the use of proteolytic enzymes on protein fractions extracted from by-products (A2.1) and microbial fermentation of the by-products themselves (A2.2).

A3) Evaluation of the functionality of bioactive compounds related to metabolic health. This activity involves the use of *in vitro* models simulating gastrointestinal digestion to assess the stability of bioactive compounds (A3.1), enzyme inhibition assays on digestive and regulatory targets (A3.2), and studies on cellular models to investigate hormone-related responses (A3.3).

A4) Formulation of functional food prototypes incorporating the selected bioactive ingredients. The focus will be on food matrices optimized from a functional perspective (A4.1), as well as on technological and sensory aspects (e.g., processability, texture, sensory acceptability) relevant to product development (A4.2).

A5) Validation of the functional efficacy of the prototypes using *in vitro* models of simulated gastrointestinal digestion to evaluate functional activities related to metabolic health (A5.1).

A6) Writing and revision of the doctoral thesis, scientific articles, and oral and/or poster communications.

Table 1. Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Nutritional Charaterization***																								
	1) Polyphenol analysis																								
	2) Dietary fiber analysis																								
	3) Bioactive peptide analysis																								
A2)	***Functionality Enhancement***																								
	1) Proteolytic enzymatic treatment																								
	2) Microbial fermentation																								
A3)	***Functional Evaluation of Ingredients***																								
	1) Stability during digestion																								
	2) Enzyme inhibition																								
	3) Hormone response																								
A4)	***Prototype Development***																								
	1) Nutritional aspects																								
	2) Functional assessment																								
	3) Technological and sensory evaluation																								
A5)	***Functional Validation of Prototype***																								
	1) *In vitro* functional activity																								
A6)	***Thesis and Paper Writing***																								

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Regenerative recycling of Agro food waste for enhancing the Mediterranean food system's circularity (P-I-87)

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This PhD project aims to identify the different process steps (like pretreatment, volume reduction, phase change, solid removal, purification, and formulation) required to recover high-value products from agri-food residues. The conversion of agri-food side stream coming from Marche region crops into high-added-value and low-cost food ingredients is a challenge to formulate healthy and innovative foods and beverages.

Riciclo rigenerativo dei rifiuti agroalimentari per promuovere la circolarità del sistema alimentare Mediterraneo

Questo progetto di dottorato mira a identificare delle strategie ecosostenibili necessarie per recuperare composti bioattivi ad elevato valore aggiunto dai residui agroalimentari. La conversione dei sottoprodotti agroalimentari provenienti dalle colture maggiormente prodotte della regione Marche, in ingredienti alimentari ad alto valore aggiunto e a basso costo, rappresenta una sfida per la formulazione di cibi e bevande sani e innovativi.

1. State-of-the-Art

Every year, several Mediterranean food industries produce a significant amount of waste. Products from the food processing sector are produced in large quantities, and because of their undesirable qualities, they are frequently wasted.
The UN projects that by 2025, the Mediterranean population will reach nearly 580 million, 60 million more than in 2020, the equivalent of the population of Italy today.
As the population grows, a parallel increase in food waste is expected, enhancing the environmental issues related to proper management and disposal. Moreover, undernutrition, overweight, obesity, diet-related chronic diseases, and micronutrient deficiency coexist in the Mediterranean region, impacting the national capability to satisfy food demand (Gaaloul, Eslamian and Katlance, 2020).
In this scenario, the European Union (EU) established an action plan to reduce, reuse, and recycle materials and energy in order to raise the value of goods, materials, and extend their economic usable life. Agri-food by-products, which are generated during the various phases of agricultural and food processing operations, contain numerous nutritional, functional, and bioactive components, which can be used to develop innovative food products, reduce waste, and improve overall sustainability of our food systems (Comunian, Silva and Souza, 2021)
Within agrifood prediction scenario, there has been a great deal of interest in efficient agri-food by-product utilization in recent years because it is capable of enhancing sustainability, minimizing waste disposal, and creating value-added products within the food industry.
Many studies highlighted in the past few years that agri-food by-products have immense potential to be used in diverse food applications, such as the extraction of bioactive compounds, developing alternative feed sources for livestock, and utilizing them in innovative food formulations (Ahmad Khorairi *et al.*, 2023).
Different approaches have been developed to extract valuable compounds from waste.
Modern techniques include the green extraction of valuable compounds, the chemical and fermentative conversion of waste into basic chemicals. In particular, solid-state fermentation (SSF) with fungal is attracting extensive attention for application in waste conversion because of its low energy requirements and lack of toxicity.
Using fungal technology prevents the use of chemicals and inhibits the production of compounds, and fungal pretreatment has high lignin, cellulose and hemicellulose degradation selectivity (Chai *et al.*, 2022).
The hydrolysis of cellulose, hemicellulose, and lignin is a step that restricts the efficiency of biomass conversion. Large amounts of cellulose and hemicellulose are encapsulated by lignin, and the crystallinity and degree of polymerization of cellulose also inhibit its biodegradability. Thus, fungal pretreatment aims to remove the complex structure composed of hemicellulose, cellulose, and lignin in the raw biomass materials and release cellulose and hemicellulose under the protection of lignin for further conversion. As a biological pretreatment technology, fungal technology has many advantages, including the ability to decompose lignin, further saccharification, fermentation, accumulate lipid, and improve the pyrolysis of the treated biomass.
After fungal pretreatment, subsequent fermentation can occur to release bioactive compounds from waste. Indeed, the solid-state pretreatment will support the subsequent fermentation by enhancing the breakdown of complex polymers, which can lead to the release of bioactive compounds.
Bioactive compounds are secondary metabolites present ubiquitously in the plant kingdom and are considered non-nutritional but vital ingredients for the maintenance of human health.

Flavonoids, curcumin, carotenoids, and ascorbic acid are thoroughly investigated for their human health-maintaining properties at different stages and have reached human clinical studies, which confirm the potency of these bioactive compounds.
In recent years, several bioactive compounds have been subjected to investigation on their potential antioxidant, anticancer, and anti-inflammatory activities using different in vitro and in vivo models as well as clinical trials.
Thus, this PhD project aims to identify the different process steps (like pretreatment, volume reduction, phase change, solid removal, purification, and formulation) required to recover high-value products from agri-food residues by developing smart and effective biotechnological solutions through two steps: solid-state fermentation (SSF) and liquid- state fermentation (LSF).
The conversion of agri-food side stream coming from the Marche region crops into high-added-value and low-cost food ingredients is a challenge to formulate healthy and innovative foods and beverages.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 2:

A1) **Collection of agro-food side streams and spawn production (fungal pre-culture),** selection of by-products which are mainly available and produced in the Marche region (A1.1) and selection of the best substrates for spawn production. (A2.1).

A2) **Solid state fermentation (SSF) set up using edible mushroom *Pleurotus ostreatus*,** study of the best combination and the best growth condition for the mushroom (A3.1) and recovery of fermented substrate post fermentation for further fermentation (A3.2)

A3) **Liquid state fermentation (LSF) set up and extraction of bioactive compounds,** with probiotic yeasts like *Debaryomyces hansenii, Lachancea thermotolerans, Starmerella* spp 18, and *Saccharomyces boulardii* and extraction of bioactive compounds from fermented substrate.

A4) **Innovative food and beverage formulations** exploiting different fermented repurposed by-products.

A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 2 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Collection of by-products and spawn production***																								
	1) Collection of agrifoods by products																								
	2) spawn production																								
A2)	***Solid state fermentation***																								
	1) study of the best growth condition																								
	2) recovery of fermented substrate																								
A3)	***Liquid state fermentation and extraction of bioactive compounds***																								
	1) liquid state fermentation																								
	2) extraction of bioactive compounds																								
A4)	***Food and beverage formulations***																								
	1) formulation of innovative food and beverage																								
A5)	***Thesis and Paper Preparation***																								

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Evaluation of emerging risks in food system, and development of strategies to guarantee the safety and the quality of traditional and innovative foods (P-I-88)

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(DM 630/2024 I. 3.3. funding by PNRR)

The aim of the research activity is to track the health of traditional and innovative foods through the qualification, quantification and monitoring of emerging and non-emerging contaminants, and/or anti-nutrients in the agri-food system and to search for useful ways to avoid or mitigate their formation during technological transformation processes. The availability of analytical methods, useful for the identification and characterization of hazards in the agri-food sector, helps risk managers to predict the risks themselves and to take effective and timely preventive measures during production processes in order to protect consumers, animals, plants and the environment.

Valutazione dei rischi emergenti nel sistema alimentare, e sviluppo di strategie per garantire la salubrità e qualità di alimenti tradizionali ed innovativi.

Nell'ambito dell'attività di ricerca si intende perseguire la salubrità di alimenti tradizionali ed innovativi andando a qualificare, quantificare e monitorare contaminanti, emergenti e no, e/o anti-nutrienti nel sistema agro-alimentare e cercare vie utili per evitare o mitigare la formazione degli stessi durante i processi tecnologici di trasformazione. La possibilità di disporre di metodologie analitiche utili all'individuazione e caratterizzazione di elementi di pericolosità nel settore agro-alimentare aiuta i responsabili della gestione del rischio a prevedere i rischi stessi e a adottare misure di prevenzione efficaci e tempestive durante i processi produttivi per proteggere i consumatori, gli animali, le piante e l'ambiente.

1. State-of-the-Art

Identifying and monitoring emerging risks in food processing is a crucial aspect of ensuring the healthiness of traditional and innovative foods (Medana, 2020). One approach to risk assessment can be the identification of process markers that allow the monitoring of chemical and physical changes during processing and ensure the safety and quality of final products. In addition, it is necessary to understand the conditions that could influence the presence of any potential markers in a food system. Any food can be considered as a system that is constantly exposed to influences from the external and internal environment. Indeed, food production systems are constantly influenced by a variety of factors that, together with environmental influences, induce biological responses, that depend on the susceptibility of the system. To date, the use of markers is useful to obtain a commodity classification of the product, detect fraud and adulteration, ensure food safety, improve the product, check production and storage conditions, and evaluate the nutritional quality of the product. The product/process markers can be categorized into two types (Pellegrino at al.,1995): i) markers that derive from the degradation, denaturation and inactivation of thermolabile compounds; ii) markers derived from the formation of new molecules that are not present, or only present in trace amounts in the unprocessed product. This research project is being conducted in collaboration with the company Bonassisa Lab S.p.A. and aims to identify, quantify and reduce risks associated with emerging or not food contaminants such as acrylamide, hydroxymethylfurfural, carboxymethyl lysine, MOSH, MOAH and anti-nutritional molecules such as alkaloids, saponins, and lectins naturally present in food (Sdrigotti, 2021; Chen and Lin, 2020; Wenzl at al., 2003).

2. PhD Thesis Objectives and Milestones

Within the general objective mentioned above, this PhD thesis project can be divided into the following research activities, according to the Gantt diagram given in Table 1:

A1) **Bibliographic research -** Collection and literature analysis.
A2) **Assessment of chromatographic and spectrophotometric techniques for the analysis of contaminants and/or anti-nutrients and related risks in food matrices** to identify Risk assessment for contaminants and/or antinutrients for humans (2a) (first year) and check of the available chromatographic and spectrophotometric techniques for the analysis of contaminants and/or anti- nutritive compounds in foods (2b) (first year). Development and standardization of suitable analytical techniques for analysis of contaminants and antinutritive compounds (2c).
A3) **Implementation of strategies for the prevention and/or mitigation of the presence of contaminants or anti-nutritive compounds in cereal-based food.** Study of the influence of raw materials, processes and

storage conditions on the presence of contaminants and antinutritive compounds in foods (3a). Qualitative/quantitative analysis of the presence of process contaminants and/or anti-nutritive compounds in foods (3b)

A4) **Production of cereal based foods with minimized presence of contaminants and/ or antinutritive compounds.** Selection of suitable raw materials and modifications of food recipes (4a); Standardization of suitable formulations/recipes on pilot plants, adopting appropriate operating conditions and assessment of chemical composition, sensory quality and texture analysis of cereal based food produced (4b); Evaluation of contaminants/antinutrients on the finished products (4c); Scale up from pilot plant to industrial plant (4d).

A5) **Thesis and paper preparation -** Writing and Editing of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Bibliographic research***																								
A2)	***Assessment of chromatographic and spectrophotometric techniques for the analysis of* contaminants *and/or anti-nutrients and related risks in food* matrices**																								
	2a) Risk assessment for contaminant and/or antinutrients for humans																								
	2b) Check of the available chromatographic and spectrophotometric techniques and check of the available chromatographic and spectrophotometric techniques for the analysis of contaminants and/or anti- nutritive compounds in foods																								
	2c) Development and standardization of suitable analytical techniques for analysis of contaminants and antinutritive compounds																								
A3)	***Implementation of strategies for the prevention and/or mitigation of the presence of contaminants or anti-nutritive compounds in cereal-based food***																								
	3a) Study of the influence of raw materials, processes and storage conditions on the presence of contaminants and antinutritive compounds in foods																								
	3b) Qualitative/quantitative analysis of the presence of process contaminants and/or antinutritive compounds in foods																								
A4)	***Production of cereal based food with minimized presence of contaminants and/or antinutritive compounds***																								
	4a) Selection of suitable raw materials and modifications of food recipes																								
	4b) Standardization of suitable formulations/recipes on pilot plants, adopting appropriate operating conditions and assessment of chemical composition, sensory quality and texture analysis of cereal based food produced																								
	4c) Evaluation of contaminants/antinutrients on the finished products																								
	4d) Scale up from pilot plant to industrial plant																								
A5)	***Thesis and Paper Preparation***																								

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Characterization of mineral oil contamination in vegetable oils and fats and study of innovative and sustainable approaches for their mitigation, with a focus on the most toxic aromatic components (P-I-89)

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This PhD project aims to assess the contamination of mineral oil hydrocarbons (MOH) in vegetable oils and fats, focusing on the identification and quantification of the most toxic aromatic hydrocarbons (MOAH), those with more than three benzene rings and a low degree of alkylation, using advanced analytical techniques. Given their low concentration and analytical challenges, targeted pre-separation steps will be explored. Furthermore, the project will explore innovative and sustainable strategies to reduce MOAH contamination, focusing on environmentally friendly and scalable methods for industrial application.

Caratterizzazione della contaminazione da oli minerali in oli e grassi vegetali e studio di approcci innovativi e sostenibili per la loro mitigazione, con particolare attenzione ai componenti aromatici più tossici

Questo progetto di dottorato è finalizzato a valutare la contaminazione da idrocarburi da oli minerali (MOH) in oli e grassi vegetali, concentrandosi sull'identificazione e quantificazione degli idrocarburi aromatici (MOAH) più tossici, ovvero quelli con tre o più anelli benzenici e un basso grado di alchilazione, utilizzando tecniche analitiche avanzate. Date le loro basse concentrazioni e le sfide analitiche, verranno esplorate fasi di pre-separazione mirate. Inoltre, il progetto esplorerà strategie innovative e sostenibili per ridurre la contaminazione da MOAH, concentrandosi su metodi ecocompatibili e scalabili per applicazioni industriali.

1. State-of-the-Art

Mineral oil hydrocarbons (MOH) are complex mixtures of saturated (MOSH) and aromatic hydrocarbons (MOAH) of petrogenic origin that can enter the food supply chain through various sources, such as lubricants used in food processing machinery and migration from packaging materials. In recent years, concerns about MOH contamination in food, particularly in vegetable oils and fats have increased, due to their lipophilic nature (Purcaro et al., 2016). MOSH has been proven to accumulate in the human body, increasing the weight of specific organs and tissues. Although current dietary exposure is not considered a direct health risk, the limited safety margin and the potential long-term effects of accumulation require further study. MOAH mostly include alkylated aromatic hydrocarbons (>98%) with 1-7 benzene rings along with small amounts of non-alkylated polycyclic aromatic hydrocarbons (PAH). Among these, MOAH with 3 or more benzene rings, particularly those with a low degree of alkylation are of major concern due to their genotoxic and carcinogenic properties (EFSA, 2023). Despite growing concerns about the presence of MOH in food, the European Union (EU) has not yet established harmonized legal limits. However, a draft amendment to Regulation (EU) 2023/915 circulated by DG SANTE in late 2023 proposed a maximum limit of 2 mg/kg of MOAH in most vegetable oils, starting from January 1, 2026. The official method for mineral oil determination in vegetable oils (ISO 20122:2024), firstly introduced by Biedermann et al. (2009), involves the use of online HPLC-GC-FID, preceded by saponification to remove triglycerides and epoxidation for olefin removal. A similar approach, using microwave assisted saponification (MAS) was validated by Menegoz Ursol et al. (2022). The potential of comprehensive GC, coupled to MS for a more detailed MOH characterization has been reviewed by several authors (Biedermann & Grob, 2015; Bauwens et al., 2023). However, a reliable analytical technique capable of quantifying the most toxic MOAH sub-fraction is still lacking. Analysis of the MOAH subfraction composed of compounds with three or more aromatic rings and low alkylation is of great interest, but their determination remains highly challenging. This is mainly because they are present at significantly lower concentrations compared to the less toxic mono- and diaromatic MOAH, which dominate the total MOAH fraction. As a result, when the entire MOAH fraction is analyzed, the sensitivity toward these highly toxic components is insufficient. Moreover, analytical selectivity is further hindered by the presence of co-eluting olefinic compounds such as squalene, which can interfere with accurate quantification. Therefore, a pre-separation step is essential to selectively isolate the more hazardous subcomponents. Epoxidation is commonly used to remove olefin interference after saponification and before on-line HPLC-GC-FID, which heavily affects recoveries of MOAH, especially heavy PAHs.

Despite the several attempts made in this direction, the goal has not yet been fully achieved. Therefore, this research will focus on the development of a robust analytical protocol capable of isolating aromatic hydrocarbons with three and more benzene rings and a low degree of alkylation from vegetable oils. To this purpose, different procedures will be tested for the selective enrichment and purification of the components of interest (saponification, epoxidation, and alternative protocols) and their fractionation (SPE or HPLC on different

stationary phases), prior to the final on-line LC-GC-FID and/or GC×GC/FID-MS determination.
Mitigating MOH contamination in vegetable oils, particularly tropical oils, remains a critical issue. Contamination is often unavoidable during primary production (harvesting) and transportation. Therefore, refining process became crucial. While it is documented that deodorization helps reduce more volatile MOH, the bleaching stage requires further study. The effectiveness of conventional adsorbents like activated carbon under optimized conditions in reducing carcinogenic compounds has been demonstrated in solvent (Moret et al., 2025), and the obtained results need to be investigated in the actual oil matrix, along with the development of alternative adsorbent materials with improved decontamination potential. Parameters influencing decontamination efficiency (e.g., contaminant and adsorbent concentrations, treatment time, and temperature) will also be investigated. Therefore, there is a growing need for innovative and sustainable mitigation strategies that can be implemented on an industrial scale without compromising the quality of the final product.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Method improvement aimed at removing or reducing analytical interferences** of olefins, comparing results obtained with the current method (epoxidation) with other methods (fractionation on HPLC column, dispersive Solid-Phase Extraction – SPE - and Liquid-Liquid Extraction – LLE) to improve the profiling of the most hazardous MOAH components and to enable more accurate quantification.

A2) **Develop and compare different protocols for isolating the most toxic MOAH** (≥3 aromatic rings and low degree of alkylation) from mono- and diaromatic compounds, in order to achieve higher analytical sensitivity and selectivity towards this sub-fraction (HPLC fractionation, LLE partitioning).

A3) **Testing decontamination** properties of different authorized adsorbents (activated carbons, ACs) and non-authorized adsorbents (e.g., mesoporous silica nanoparticles, cellulosic aerogels) and modify, if necessary, their structure/surface properties in an attempt to improve their decontamination effectiveness.

A4) **Period abroad,** detailed characterization of MOSH and MOAH fractions of selected samples by HPLC-GC×GC-TOF MS/FID.

A5) **Pilot scale experiments**, evaluating and optimizing different refining stages for vegetable oils and fats, especially evaluation of different ACs during the bleaching stage to assess their impact on MOAH contamination levels and potential for decontamination.

A6) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 2 *Gantt diagram for this PhD thesis project*.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Reduce analytical interferences***																								
	1) Fractionation on HPLC column																								
	2) Solid-Phase Extraction (SPE)																								
	3) Liquid-Liquid Extraction (LLE)																								
A2)	***Isolating methods***																								
	1) Fractionation on HPLC																								
	2) LLE partitioning																								
A3)	***Decontamination trials***																								
	1) Activated carbons (ACs)																								
	2) Mesoporous silica nanoparticles, cellulosic aerogels																								
A4)	***Period abroad***																								
A5)	***Pilot-scale testing***																								
A6)	***Thesis and Paper Preparation***																								

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Design and quality assessment of improved nutritional value food products using by-products and minor crops as ingredients (P-I-90)

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Tutor: Prof. Giacomo Squeo Co-Tutor: Dr. Giusy Rita Caponio

This PhD thesis research project aims to design food products by using underutilized raw materials such as sea fennel (*Crithmum maritimum* L.) as a minor crop, and grading by-products from walnut (*Juglans regia* L.), almond (*Prunus dulcis* Mill.), and cashew (*Anacardium occidentale* L.). The study focuses on the characterization of raw materials and the optimization of their usage, based on their technological and nutritional characteristics, into innovative food items.

Progettazione e valutazione della qualità di prodotti alimentari a valore nutrizionale migliorato utilizzando sottoprodotti e colture minori come ingredienti

Questo progetto di ricerca di tesi di dottorato mira a progettare prodotti alimentari attraverso l'utilizzo di materie prime poco sfruttate come il finocchietto marino (*Crithmum maritimum* L.) quale coltura minore, e sottoprodotti da cernita di noce (*Juglans regia* L.), mandorla (*Prunus dulcis* Mill.) e anacardio (*Anacardium occidentale* L.) Lo studio si concentra sulla caratterizzazione delle materie prime e sulla ottimizzazione del loro utilizzo, sulla base delle loro caratteristiche tecnologiche e nutrizionali, in prodotti alimentari innovativi.

1. State-of-the-Art

According to annual FAO and WHO reports, two major challenges significantly impact food systems and jeopardize the ability to sustainably and equitably feed a growing global population. The first is supply chain upcycling through byproducts. If not valorized, these materials could contribute to food waste generation, which can range from 5% to 50% depending on the specific supply chain and geographic context. The second is the loss of agrobiodiversity, estimated to cost the global economy around 10 trillion dollars per year due to ecosystem degradation and the narrowing of the agricultural base. Currently, 5% of European fields are dedicated to minor crops, which could help preserve agrobiodiversity, while the remaining part hosts only 12 main species (Mattas *et al.,* 2024; Socas-Rodríguez *et al.,* 2021). From a consumer perspective, biodiversity loss and the depletion of natural resources such as food and water ranked third and fourth among long term perceived global risks in 2024 (World Economic Forum, 2025).

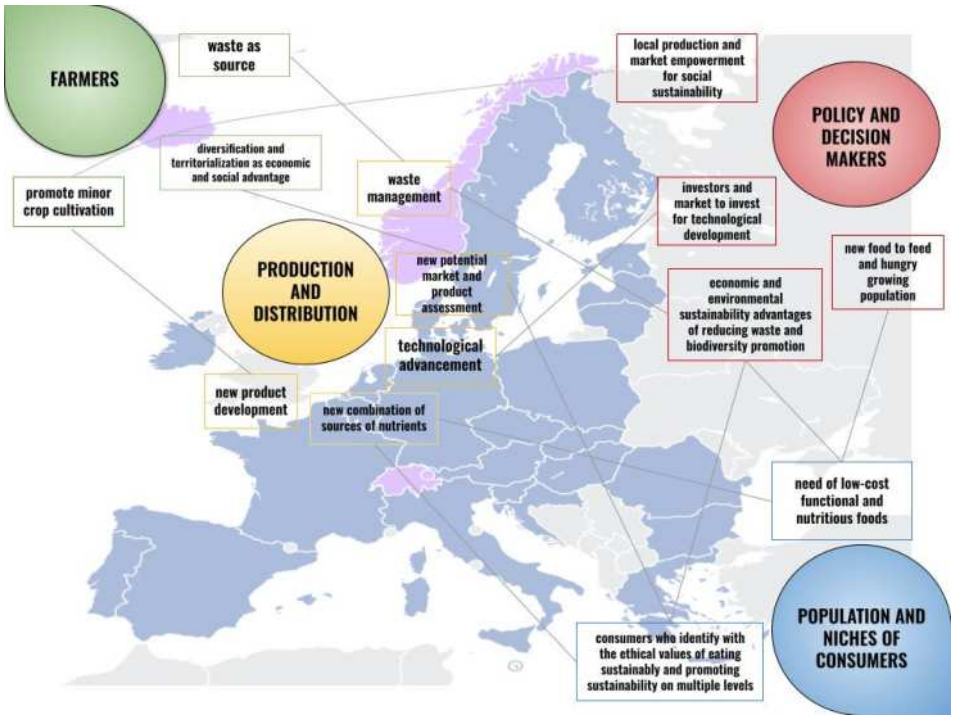


Figure 1*. Systemic perspective of product innovation using minor crops and food by-products as functional ingredients. The diagram illustrates the interconnected roles (nodes) of stakeholders within a European context.*

Food by-products are increasingly being re-evaluated as valuable ingredients, rich in nutrients and functional compounds, suitable for reintegration into new food products (Lau *et al.,* 2021). According to the Waste Framework Directive (2008/98/EC, Article 5), materials obtained from sorting processes, such as walnut, almond, and cashew off-grading, can be considered by-products. Compared to whole nuts, these fragments could be more

susceptible to oxidation. Nonetheless, they retain a similar nutritional profile and can represent a valuable raw material for the development of functional ingredients. Furthermore, combining these by-products could help balance the nutritional contribution of fatty acids and other compounds (e.g. minerals) within a single product (Tonetto *et al.,* 2025). On the other hand, minor crops, defined as traditional or underutilized plant species adapted to local conditions, offer an opportunity to diversify food sources, enhance nutritional quality, and strengthen the resilience of agrifood systems (Mattas *et al.,* 2024). Among these, sea fennel has gained interest as an aromatic ingredient, also notable for its fiber and mineral content, low fat levels, and richness in antioxidants. Its distinctive flavoring and coloring properties also make it a promising candidate as an innovative functional ingredient. However, it is well documented that its micronutrients and bioactive components are easily degraded during conventional cooking and processing (Kraouia *et al.,* 2023). Although minor crops and by-products hold promise as ingredients, developing new food involves challenges: their processing and inclusion in formulations may lead to adverse or collateral effects from both nutritional and technological standpoints. For these reasons, industry, manufacturers, and policy makers should promote consumer-oriented processing strategies, while remaining aware of the drawbacks and closely monitoring the challenges associated with processing underutilized raw materials (Knorr, 2024). A general perspective of the introduction of minor crops and by-products in a food system is shown in Figure 1. Based on these considerations, processing food items using minor crops and by-products could represent a valuable opportunity to introduce new sustainable ingredients into food systems, supporting both environmental goals and dietary improvement and diversification.

2. PhD Thesis Objectives and Milestones

This PhD thesis project is subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Bibliographic research** is fundamental to deepening the topic, understanding the state of the art and how innovative techniques could be applied to achieve results, identify unexplored facets, and valorize the new products.

A2) **Minor crop valorization** will be carried out by i) collecting and characterizing sea fennel, also according to different drying methods (A.2.1); ii) producing and assessing the chemical, technological and nutritional characteristics of two innovative food products developed by including sea fennel into the formulation, also considering the impact of sea fennel processing on technological and nutritional variables (A.2.2).

A3) **By-product valorization** will be carried out by i) collecting and characterizing walnut, almond, and cashew off-grading from a nutritional, physicochemical perspective (A.3.1); ii) designing and producing innovative food products with enhanced nutritional features using different combinations of the selected by-products (A.3.2).

A4) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Bibliographic research***																								
A2)	***Minor crop valorisation***																								
	1) Collection and characterization																								
	2) Production and characterization of innovative foods																								
A3)	***By-product valorisation***																								
	1) Collection and characterization																								
	2) Production and characterization of innovative foods																								
A4)	***Thesis and Paper Preparation***																								

3. Selected References

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Application of vacuum prior to centrifugal separation of oils from olives, avocados, and other agri-food processes (P-I-91)

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This research project aims to evaluate the use of vacuum technology in industrial processes for the extraction of olive oil, avocado oil, and in the production of fruit juices. The main objectives are to improve the quality and yield of olive oil from different cultivars, optimize avocado oil extraction, and reduce oxidation in juices. The tests will be conducted on a laboratory and industrial scale, analyzing the process parameters. The expected results aim to increase production efficiency and product quality, contributing to innovation in the agri-food sector with sustainable solutions and reduced processing times.

Applicazione del vuoto previa alla separazione centrifuga di oli da olive, avocado, e da altri processi agroalimentari

Questo progetto di ricerca intende valutare l'impiego della tecnologia del vuoto nei processi industriali di estrazione dell'olio di oliva, dell'olio di avocado e nella produzione di succhi di frutta. Gli obiettivi principali sono migliorare qualità e resa dell'olio di oliva su diverse cultivar, ottimizzare l'estrazione dell'olio di avocado e ridurre l'ossidazione nei succhi. Le prove verranno condotte su scala laboratoriale e industriale, analizzando i parametri di processo. I risultati attesi mirano a incrementare l'efficienza produttiva e la qualità dei prodotti, contribuendo all'innovazione nel settore agroalimentare con soluzioni sostenibili e tempi di lavorazione ridotti.

1. State-of-the-Art

A vacuum is a condition in which the gas pressure is lower than atmospheric pressure. In laboratories and industry, partial vacuums are created inside closed chambers using vacuum pumps. These machines suck gases out of a sealed environment, creating a pressure that is lower than atmospheric pressure. However, a perfect vacuum has never been achieved in a laboratory or observed in nature. Vacuum technology is now widely used in the food industry, where it is applied in numerous processes: pre-treatment, production, stabilization and preservation of food of plant, animal and dairy origin. The use of vacuum allows processes to be carried out that are not possible at atmospheric pressure, improving the overall quality of the products. In combination with other technologies, vacuum systems preserve or enhance the health, sensory and nutritional properties of food, reducing degradation processes and extending shelf life (Ravichandran e Upadhyay, 2019). The elimination of oxygen and thermal control limit oxidation and regulate enzymatic activity, minimizing browning and physical-chemical changes. Vacuum also affects the structural characteristics of food, modifying its consistency and porosity, and improves mass transfer and the mobility of intra- and extracellular liquids (Fleyfel et al., 2022)). In recent years, this technology has been studied as a potential innovation in the mechanical extraction process of extra virgin olive oil. Laboratory-scale experiments conducted on olive pastes from the Peranzana, Coratina and Moraiolo cultivars using a vacuum kneader have shown that high vacuum promotes an increase in phenolic compounds and the removal of molecules with a negative sensory impact (Taticchi et al., 2021). Subsequent studies in industrial plants, using cultivars such as Arbequina, Arbosana, Koroneiki and Picual, have confirmed an increase in oil extractability and an improvement in quality, thanks to a higher content of hydrophilic phenols (Veneziani et al., 2022). On the contrary, there are no studies in literature on the application of vacuum in the avocado oil extraction process. Similarly, the use of vacuum has not yet been explored in the centrifugal extraction phase of other agri-food products, although positive experiences have been reported in the field of drying, as demonstrated by the study by Li et al. (2020) on hawthorn juices. In light of the current evidence, the use of vacuum in the phase prior to centrifugal extraction appears to be a promising area for future research, particularly for olive oil, avocado oil and other agri-food processes.

2. PhD Thesis Objectives and Milestones

Within the overall objective described above, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram provided in Table 1:

A1) Application of vacuum technology in virgin olive oil extraction on an industrial scale.

- **A1.1:** Experimental trials with vacuum-assisted malaxation using selected Apulian cultivars (Coratina, Ogliarola, Peranzana, Leccino) at various ripeness stages.

- **A1.2:** Comparative testing of traditional and vacuum-based processes, focusing on phenolic and volatile compound profiles, extractability and oil quality.
- **A1.3:** Optimization of process parameters: vacuum pressure levels, retention time, evaporation rate and paste mixing speed in the vacuum malaxer.
- **A1.4:** Data analysis correlates vacuum intensity with oil yield and quality, defining optimal operational settings.

A2) Exploratory laboratory-scale application of vacuum in avocado oil extraction.

- **A2.1:** Experimental use of a mini vacuum malaxer on avocado pastes to evaluate vacuum's effect on yield, volatile and phenolic content.
- **A2.2:** Investigation of vacuum's potential to reduce off-flavours caused by overripe fruits.
- **A2.3:** Conditional scale-up to industrial trials if lab results confirm process improvement, including protocol development for operational parameters ensuring high oil quality and process efficiency.

A3) Laboratory evaluation of vacuum application in fruit juice and protein extraction processes.

- **A3.1:** Testing of vacuum conditions applied between crushing and centrifugation to assess effects on yield, color, and nutritional/organoleptic profiles.
- **A3.2:** Identification of processing conditions that minimize oxygen exposure and temperature increases to preserve quality attributes.
- **A3.3:** Definition of parameters for optimal integration of vacuum in centrifugal extraction of juices and proteins.

A4) Dissemination and writing of results.

- **A4.1:** Drafting of the PhD thesis.
- **A4.2:** Preparation of scientific papers and participation in academic conferences with oral/poster presentations.

Table 1 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Application of vacuum in the olive oil extraction process***																								
	1) Industrial-scale experimental tests																								
	2) Data analysis and interpretation																								
A2)	***Application of vacuum in the avocado oil extraction process***																								
	1) Laboratory-scale experimental tests																								
	2) Industrial-scale experimental tests																								
	3) Data analysis and interpretation																								
A3)	***Application of vacuum in the juice extraction process***																								
	1) Laboratory-scale experimental tests																								
	2) Industrial-scale experimental tests																								
A4)	***Thesis and Paper Preparation***																								

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Supercritical CO_2 as a Green Technology for the Extraction and Characterization of Bioactives from Alpine Botanicals: Application in Oleolites (P-I-92)

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This PhD project aims to develop a green extraction approach using supercritical carbon dioxide (SC-CO_2) to extract and characterize oleolites enriched with bioactives from alpine botanicals. An experimental design approach using response surface methodology will be utilized to optimize key extraction parameters and the extracted oleolites will be extensively characterized for their antioxidant activity, oxidative stability, and physicochemical properties. Further profiling will be carried out using Supercritical Fluid Chromatography (SFC) to identify and quantify key compounds. The efficacy of the enriched oleolite will be evaluated for potential use in functional food and nutraceutical applications aimed at enhancing nutrition and promoting well-being.

Anidride carbonica supercritica cone tecnologia sostenibile per l'estrazione e la caratterizzazione di composti bioattivi da botaniche: applicazione per la produzione di oleoliti

Questo progetto di dottorato mira a sviluppare un approccio di estrazione sostenibile utilizzando anidride carbonica supercritica (SC-CO_2) per estrarre e caratterizzare oleoliti arricchiti con bioattivi da botaniche alpine. Verrà utilizzato un approccio di progettazione sperimentale che utilizza la metodologia della superficie di risposta per ottimizzare i parametri chiave di estrazione. Gli oleoliti estratti saranno ampiamente caratterizzati per la loro attività antiossidante, stabilità ossidativa e proprietà fisico-chimiche. Il profilo dei composti presenti negli oleoliti sarà effettuato utilizzando la cromatografia con fluidi supercritici (SFC) per identificare e quantificare i composti chiave. L'efficacia dell'oleolita arricchito sarà valutata per il potenziale utilizzo in alimenti funzionali e applicazioni nutraceutiche volte a migliorare la nutrizione e promuovere il benessere.

1. State-of-the-Art

Advances in Oleolite extraction using Supercritical fluid technology

The rising global interest in sustainable and health-driven food systems has increased the demand for functional foods and nutraceuticals enriched with bioactive compounds from natural sources. Alpine botanicals, in particular, are valued for their rich repertoire of polyphenols, flavonoids, and other phytochemicals with potential health benefits. However, utilizing these compounds in a way that is both efficient and eco-friendly remains a significant challenge.

Conventional extraction techniques rely heavily on petroleum-based organic solvents such as hexane, chloroform, and tetrahydrofuran, which pose environmental and health concerns. Supercritical Fluid Extraction (SFE) uses supercritical carbon dioxide (SC-CO_2) to selectively extract thermolabile and lipophilic bioactives under mild conditions, without leaving behind harmful solvent residues. Supercritical Fluid Chromatography (SFC), a less-explored but powerful analytical tool, allows for the rapid, high-resolution separation and quantification of these bioactives in complex lipid-rich matrices, offering a greener alternative to traditional HPLC. However, SC-CO_2 alone exhibits limited solubility for certain bioactives, necessitating the use of co-solvents to enhance extraction efficiency.

Among potential co-solvents, vegetable oils have gained considerable attention for their ability to improve the solubility and stability of extracted compounds while offering a direct food-compatible medium. Oils such as rice bran oil (RBO), virgin coconut oil (VCO), and sunflower oil have been successfully employed to extract and stabilize lipophilic bioactives like lycopene, xanthones, and flavonoids (Dhakane-Lad et al., 2023; Kok et al., 2021; Pattiram et al., 2022). Moreover, edible oils have been traditionally used to infuse or macerate herbs, spices, and medicinal plants, providing a historical foundation for their modern application in bioactive extraction (Yara-Varón et al., 2017).

While the use of edible oils as co-solvents in SFE has shown promise, their integration with supercritical chromatographic methods for full-spectrum profiling remains underexplored. Moreover, limited studies have explored the use of vegetable oils as co-solvents in SFE specifically for developing functional oleolites, especially from high-value alpine botanicals.

This study addresses these gaps by investigating the use of sunflower oil as a food-compatible co-solvent in SFE to extract bioactives from selected alpine botanicals. The project also focuses on developing and validating a

supercritical fluid chromatography method for profiling the enriched oil, aiming to quantify and assess the stability of key bioactives. The resulting oleolites are envisioned as natural, ready-to-use functional food ingredients or nutraceutical products, offering a sustainable and consumer-friendly alternative in the health and wellness sector.

Experimental methodology to obtain and characterize oleolite from alpine botanicals

The production of oleolite will be performed using a supercritical fluid extractor with sunflower oil as co-solvent. The key parameters (pressure, temperature, co-solvent ratio and time) will be optimized using response surface methodology. Colorimetric assays, density, viscosity along with DPPH and Total Phenolic Content assays will be performed to study the overall antioxidant activity and physicochemical properties of the oleolite. Oxidative stability studies will be studied using isothermal calorimetry, peroxide value, anisidine value and TBARS assay. Furthermore, extensive profiling of the oleolites will be done using supercritical chromatography to provide insights into the components present in the extracted oleolite. This project aims to address these gaps by optimizing the use of sunflower oil as a co-solvent in SFE for the extraction of bioactives from alpine botanicals. By systematically studying the extraction process and developing a validated SFC method for bioactive characterization, this study seeks to advance sustainable extraction techniques and expand the potential applications of oleolites (bioactive-enriched oils) within the food and nutraceutical industries.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

M1- Literature review
M2- Studies on Physicochemical properties: Optimization of extraction conditions and characterization of the oleolite using supercritical fluid technology.
M3- Studies on oxidative stability: advanced techniques to analyze the oxidative stability of oleolite enriched with bioactives
M4- Food application evaluation: Assess the efficacy of the oleolites as in real food matrixes
M5- Period abroad
M6- Writing and Editing of PhD thesis, papers and/or poster communication.

Table 1 Gantt diagram for this PhD thesis project.

Milestones	**1st year**				**2nd year**				**3rd year**			
Terms	I	II	III	IV	I	II	III	IV	I	II	III	IV
M1 Literature review												
M2 Physicochemical properties												
M3 Oxidative stability												
M4 Food application												
M5 Period abroad												
M6 Writing												

3. Selected References

Dhakane-Lad, J., Kar, A., & Patel, A. S. (2023). SC-CO 2 extraction of lycopene from red papaya using rice bran oil as a co-solvent lessens its degradation during storage. *Separation Science and Technology*, *58*(13), 2357–2368. https://doi.org/10.1080/01496395.2023.2255738

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Home sensory dietary patterns and the acceptability of school meals: the role of parental and child individual variables (P-I-93)

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This project aims to investigate how home sensory dietary patterns influence the eating behavior of school-age children with regard to school meals, in terms of acceptability and actual consumption of the dishes offered. Questionnaires specifically designed for children and parents will assess exposure to and preference for foods with specific sensory profiles and will be correlated with individual variables. Using a *Sensory Epidemiology* approach, the study aims to deepen the factors that influence the adoption of a varied and balanced diet, with the goal of supporting school food innovation and developing gamified tools aimed at engaging young participants.

I pattern sensoriali della dieta domestica e l'accettabilità dei piatti del menu scolastico: il ruolo delle variabili individuali dei genitori e dei bambini

Questo progetto si propone di indagare come i pattern sensoriali della dieta domestica influenzino il comportamento alimentare di bambini in età scolare rispetto all'offerta scolastica, in termini di accettabilità e consumo effettivo dei piatti proposti. Questionari appositamente sviluppati per bambini e genitori valuteranno l'esposizione e la preferenza per alimenti con specifici profili sensoriali e saranno messe in relazione alle variabili individuali. Attraverso un approccio di *Sensory Epidemiology*, lo studio mira a comprendere i fattori che incidono sull'adozione di una dieta varia ed equilibrata, con l'obiettivo di supportare l'innovazione alimentare scolastica e sviluppare strumenti gamificati mirati ad ingaggiare i giovani partecipanti.

1. State-of-the-Art

Food preferences in childhood can strongly influence eating behavior in adulthood and future food consumption. Sensory stimulation plays a pivotal role in eating decisions and is a primary driver of food choices among children (Schwartz et al., 2011). Sensory properties, particularly taste, function as "nutrient-sensors" that provide a signal to the brain and body about the arrival of specific macronutrients, thereby fulfilling a functional role linked to nutritional and metabolic outcomes (De Graaf & Kok, 2010). However, protective sensory mechanisms often act as barriers to healthy eating in children due to the rejection of unpleasant sensory characteristics, such as bitter taste, which also elicit negative emotional responses. Beyond sensory aspects, several factors manifest significant variability among children, including differences in taste responsiveness, attitudinal and behavioral characteristics, such as eating rate, and psychological traits, like food neophobia, defined as reluctance to try new or unfamiliar foods. At different developmental stages, factors will have different weights in shaping children's preferences and diets (Varela et al., 2024). The home environment will largely influence dietary patterns through household food availability and parental feeding practices. It has been demonstrated that the types of foods consumed and provided by parents predict children's eating habits, contributing to the formation of specific sensory dietary patterns. For instance, greater availability of fruits and vegetables at home led to increased intention to eat them, more enjoyment of eating them, and higher consumption of these foods (Lwin et al., 2020). Both adult and peer models have been found to influence children's acceptance of novel foods or bitter vegetables, indicating that social facilitation impacts children's intake patterns. Correspondingly, external food environments, particularly the school food environment, become increasingly important as children grow. The strategic and educational value of school canteens must not be underestimated; they serve as widely utilized platforms to reach children and adolescents across diverse socioeconomic backgrounds, reduce food insecurity, promote exposure to novel foods, and increase familiarity, thereby implicitly reinforcing social norms around healthy eating. Despite the recognized importance of these factors, large-scale studies investigating the impact of individual variables in a unitary frame and the influence of home dietary patterns on actual school meal consumption contexts remain scarce. This research aims to elucidate how and to what extent such sensory dietary patterns affect the acceptability and consumption of school food offerings. Furthermore, it explores how these patterns vary according to individual characteristics, including demographic and anthropometric features, attitudes, and psychological traits. To address these issues, we propose a *Sensory Epidemiology* approach, a promising method to explore dietary patterns through the perceptual properties of the foods habitually chosen and consumed (Forde & de Graaf, 2022). Since these properties guide food choices by influencing intake quantity and frequency, they are essential variables in constructing healthy dietary styles and controlling weight gain. Additionally, when conducting sensory testing with children, it is important to implement procedures that are appropriate for their age, as well as their psychosocial and cognitive abilities. This involves not only adapting and simplifying existing methods but also developing ad hoc questionnaires specifically designed to meet children's needs. Incorporating gamification elements into testing procedures can enhance task enjoyment and effectively engage and motivate children. We hypothesize that the sum of individual factors characterizing a group, including habitual exposure to a nutritionally and sensory diverse food repertoire, promotes greater dietary openness and higher acceptability of novel or

different food offerings, such as those presented in school canteens. Therefore, this study aims to deepen understanding of the factors facilitating or hindering the adoption of varied and high-quality diets, with potential positive implications for both public health and environmental sustainability. The ultimate objectives are twofold: first, to support innovation in primary school food offerings by aligning them more closely with the dietary sensory patterns of target groups; second, to contribute to the development of targeted tools to actively engage young participants while facilitating dialogue among research, schools, and families.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities, according to the Gantt chart presented in Table 2:

A1) **Data collection using a multidisciplinary approach** on children and their parents from five primary schools (approximately 700 participants). A structured questionnaire will be administered to the children to collect individual variables, including demographic characteristics, levels of food neophobia, eating rate, anthropometric measurements, as well as liking and emotional profiles for target foods. A corresponding questionnaire will be administered to the parents to gather demographic information, food neophobia levels, eating rate, attitudes toward food choice, and frequency of consumption of specific food categories, also described in sensory terms, to define familiar sensory dietary patterns (A1.1). During the field study, in the school canteens, acceptability and actual consumption of specific target dishes served in the school menus will be recorded (A1.2). During the first year of the PhD, the development, validation, and optimization of the questionnaires were conducted through a pilot study involving 200 children and their parents from a primary school in Florence. Additionally, the protocol for acquiring data of interest in the school canteens context was tested.

A2) **Statistical data analysis**. Subject segmentation according to dietary sensory patterns through data distribution analysis and study of differences and similarities between clusters for different variables using ANOVA models and multivariate methods (Principal Component Analysis). Multivariate predictive models of the adoption of specific dietary patterns (Logistic regression) to test the effect of variables on the acceptability of dishes consumed in the canteen.

A3) **Contribution to the development of targeted tools** to actively engage young participants through gamification during educational interventions.

A4) **Period abroad** aimed at collaborating to optimize knowledge and interventions to improve the adoption of healthy eating habits among children.

A5) **Writing and editing** of the PhD thesis, scientific papers, and oral and/or poster communications.

Table 2 Gantt diagram for this PhD thesis project (2nd and 3rd year).

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Data Collection (Multidisciplinary approach)***																								
	1) Children's and parents' questionnaires																								
	2) Field study in the school canteens																								
A2)	***Statistical Data Analyses***																								
A3)	***Contribution to the development of targeted tools***																								
A4)	***Period abroad***																								
A5)	***Thesis and Paper Preparation***																								

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Innovative strategies for oxidation protection in pistachio products (P-I-94)

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The PhD project, in collaboration with BMC Srl, aims to identify the quality marker in pistachios responsible for the occurrence of an abnormal flavor that can be described as spicy, pungent, or bitter. The project proposes studying lipid oxidation and hydroxymethylfurfural formation in pistachio raw material and paste and using sustainable plant extracts to protect the product from degradation.

Strategie innovative per il rallentamento dell'ossidazione dei prodotti a base di pistacchio

Il progetto di dottorato, condotto in collaborazione con BMC Srl, mira a identificare il marcatore di qualità nei pistacchi responsabile della comparsa di un sapore anomalo che può essere descritto come piccante, pungente o amaro. Il progetto si propone di studiare l'ossidazione lipidica e la formazione di idrossimetilfurfurale nella materia prima e nella pasta di pistacchio e di utilizzare estratti vegetali sostenibili per proteggere il prodotto dalla degradazione.

1. State-of-the-Art

The genus *Pistacia* contains 11 species, with the cultivated species of pistachios belonging to the species *Pistacia vera* L. Pistachio occupies the most relevant economic position due to its use as a food product (salted and roasted) and as a food ingredient in various culinary preparations (Bayram and Bayram, 2022). World pistachio production shows the USA as the largest producer with 61% of world production, equivalent to 675,853 metric tons in 2023/2024, followed by Turkey, Iran, Syria and the European Union with 16,15, 5 and 3% of world production respectively (USDA, 2024). Most of the imported pistachios in Italy are consumed within the country, most of its pistachios from the United States (48%) and Germany (which acted as a transit country) (15%) (The European Market Potential for Pistachios, 2024). Pistachio nuts are a rich source of various biologically active compounds, including tocopherols, phytosterols, and phenolic compounds. Distinguished by their unique nutritional composition, pistachios are particularly abundant in cardioprotective constituents such as oleic acid, phytosterols, phenolics, and tocopherols. These bioactive components contribute to their potential as a functional food with notable antioxidants and anti-inflammatory properties. By scavenging and neutralizing free radicals or inducing the gene expression of endogenous antioxidants, these compounds may support antioxidant defense systems and reduce oxidative damage and stress (Salvador et al., 2020). The most significant challenge confronting the pistachio industry is the reduction in shelf life resulting from fungal contamination, aflatoxin B1, hull browning, and lipid oxidation during the postharvest period. The potential for contamination by fungal agents arises during the process of ripening in the orchard, the harvesting stage, and the storage phase. Consequently, factors such as elevated temperatures and humidity, protracted drought, and substantial insect activity (Taghipour et al., 2023). The oxidation of fat and the formation of hydroperoxides in unsaturated fatty acids, particularly polyunsaturated fatty acids, during the storage of pistachio powder directly impact its quality level (Tavakolipour et al., 2016). The lack of literature on the identification of quality characteristics attributable to the bitter and/or spicy flavor of Californian pistachio or pistachio-derived products makes it difficult to identify a compound to be monitored in terms of concentration prior to product processing. Certainly, the sensory characteristics of the product are highly dependent on the pre- and post-harvest treatments and the process the raw material undergoes along all processing stages from refining to heat treatment, whereby the chemical composition of the ingredients influences the degree of oxidation and the formation of hydroperoxides, along with the volatile component due to the rate of decomposition of fatty acids. Based on literature and cross-checking among the chemical compounds responsible for bitter flavors, evidence of flavors attributable to the spicy/bitter/pungent attribute has never been found in pistachios. According to Noguera-Artiaga et al. (2019), sensory evaluation of different pistachio cultivars revealed that the Kerman cultivar did not exhibit a predominance of bitterness or astringency, with average scores of 1.0 and 0.7, respectively, on a scale of 0 to 10. The microclimate and the degree of fruit ripeness influences acid composition and moisture. The effect due to country of origin includes differences that occur according to several secondary variables, mainly cultivar, environment, and soil management (Polari et al., 2019). Several compounds may be responsible for bitterness. However, according to Luo et al. (2025), none of these compounds have been identified in the Kerman cultivar, nor has their presence been correlated with the perception of bitterness and/or pungency in the fresh fruit. However, as Lafitte et al. (2016) reported, linoleic acid may be responsible for bitter flavors in poppy seeds and the Kerman cultivar has the highest reported linoleic content (Rabadán et al., 2018). Considering that most nuts are roasted at high temperatures, roasting can induce complex chain reactions, including the Maillard reaction, caramelization, and lipid oxidation. According to Mohammadi Moghaddam et al. (2015), increasing the roasting temperature causes an increase in fruit bitterness. At high temperatures, carbohydrates and proteins change, fats oxidize, and seeds acquire a bitter taste. In this context, 5-HMF, a furfural aldehyde formed by the Maillard reaction and caramelization, is associated with bitter flavors in roasted foods, according to Luo et

al. (2025). The threshold for this compound is 30 µmol/L. Liu et al. (2022) evaluated the concentration of 5-HMF in pistachio samples subjected to different heat treatments. One treatment resulted in the development of 4.77 ppm of 5-HMF, which corresponds to 37.82 µmol/L—a value slightly above the identified threshold. It has been shown that 5-HMF can easily form through enolization and dehydration of sugars during the Maillard reaction and caramelization under acidic, low-humidity conditions. Some studies have shown that lipid peroxidation can be mitigated and delayed through the use of plant extracts and polysaccharides (i.e. rosemary and green tea) (Singh et al., 2025; Ahmadi et al., 2024).

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project (in collaboration with with BMC srl) can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Identification of quality marker in pistachio**, with a study on the oxidation of lipids, and Maillard reaction to identify bitter/astringent/spicy compounds.

A2) **Identification of quality marker of pistachio paste**, with a study on the oxidation of lipids, and Maillard reaction following the oxidation kinetics through the evaluation of peroxide value, p-anisidine value and free organic acidity and specific HPLC determination to identify the bitter/astringent/spicy compounds during the shelf life.

A3) **Application of sustainable natural extracts to prevent oxidation** to create sustainable pastes with the use of by-products of food industry, to give stability and to reduce the oxidation and abnormal flavor during the shelf life.

A4) **Paper preparation**, writing and editing scientific papers.

A5) **Participation in conferences and workshops**, preparation of oral and/or poster communications.

A6) **Training abroad** at Université de Bourgogne, Dijon, France.

A7) **Thesis Preparation**.

Table 1 Gantt diagram for this PhD thesis project.

		Second year												**Third year**										
Activity	Months	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
A1)	***Identification of quality marker in pistachio***																								
	1) Study on the oxidation of lipids																								
	2) Study of Maillard reaction																								
A2)	***Identification of quality marker of pistachio paste***																								
	1) Study on the oxidation of lipids																								
	2) Study of Maillard reaction																								
A3)	***Application of sustainable natural extracts to prevent oxidation***																								
	1) Rosemary bioactives																								
	2) Green tea bioactives																								
A4)	***Paper preparation***																								
A5)	***Participation to conferences and workshop***																								
A6)	***Training abroad***																								
A7)	***Thesis Preparation***																								

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Transition from gas to electrical ovens at industrial level: optimization of the cooking profile and product formulation (P-I-95)

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The aim of this industrial PhD project is to achieve product equivalence between biscuits baked in traditional gas ovens and those baked in electric ovens by understanding and controlling the impact of different heat transfer mechanisms on biscuit quality. Through comprehensive evaluation of thermal behavior, ingredient interactions, and dough rheology, the project seeks to identify the critical factors that influence texture, spread ratio, color, flavor, and structure. This knowledge will support formulation adjustments to replicate the sensory and structural attributes of gas-baked biscuits using electric baking systems, enabling an industrial ecological transition without compromising the product quality characteristics.

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Transizione da forni a gas a forni elettrici a livello industriale: ottimizzazione del profilo di cottura e della formulazione dei prodotti

Obiettivo di questo progetto di dottorato industriale è raggiungere l'equivalenza di prodotto tra biscotti cotti in forni industriali tradizionali a gas e forni elettrici, comprendendo e controllando l'impatto dei diversi meccanismi di trasferimento del calore sulla qualità dei prodotti. Tramite la valutazione del comportamento termico, delle interazioni tra gli ingredienti e della reologia degli impasti, il progetto mira a identificare i fattori critici che influenzano la consistenza, lo spread ratio, il colore, il sapore e la struttura. In al modo si potrà modificare opportunamente la formulazione per ottenere biscotti che, cotti in forni elettrici, abbiano caratteristiche sensoriali e qualitative simili a quelle di biscotti cotti in forni a gas, consentendo così una transizione ecologica a livello industriale senza compromettere la qualità dei prodotti.

1. State-of-the-Art

Since the early development of industrial baking technologies, the transition from traditional gas-fired ovens to electric ones has posed significant challenges in maintaining consistent biscuits quality. The baking process is influenced by complex interactions between ingredient composition, dough rheology, and the heat transfer mechanisms within the oven. Depending on the oven type, heat transfer occurs predominantly through radiation or convection, which affects the thermal profile of the dough and consequently the timing and extent of key ingredient transitions such as starch gelatinization, fat melting, leavening gas release, and Maillard browning (Mesias et al., 2021).

Schouten et al., (2022) conducted a comprehensive study on the impact of heat transfer modes on acrylamide formation and key quality parameters in biscuits. Their research compared ventilated and static baking at 175 °C, demonstrating that convective baking leads to a faster temperature rise, more uniform heat distribution, and higher acrylamide levels at equivalent baking times. The study also found a strong negative correlation between biscuit moisture content and acrylamide concentration, emphasizing the crucial role of moisture migration and heat transfer in biochemical changes and quality outcomes.

Furthermore, Jovicic et al., (2020) investigated the role of individual heat transfer mechanisms within model baking ovens heated by porous volumetric ceramic burners. Their study demonstrated that radiant heat significantly contributes to crust formation and surface browning, while convection enhances moisture removal. The work highlighted the potential of advanced radiant heating technologies to optimize baking efficiency and product quality by modulating the balance between heat transfer modes. Acquaticci et al., (2024) compared lab-made biscuits baked under static and ventilated conditions, demonstrating that baking mode significantly influences both baking time and Volatile Organic Compound (VOC) concentrations. Specifically, ventilated baking led to faster baking and slightly reduced levels of key VOCs such as furfural, furfuryl acetate, 5-methylfurfural, furfuryl alcohol, 3-methylbutanal, and hexanal compared to static baking. These findings underscore the combined impact of baking conditions and recipe composition on biscuit moisture, water activity, color, texture, and chemical safety markers, providing important insights for optimizing industrial baking processes.

This research will contribute to advancing the understanding of how different heat transfer mechanisms in gas and electric ovens influence ingredient thermal transitions and final biscuits quality. The objective is to identify critical points where ingredient reactions differ between oven types and to propose formulation or process adjustments to optimize biscuits quality in electric ovens.

2. PhD Thesis Objectives and Milestones

Within the overall objective of achieving comparable quality for biscuits baked using electric or gas ovens, this

PhD project is subdivided into the following research activities, aligned with the Gantt chart presented in Table 1:

A1) Bibliographic Review and Theoretical Framework

- Conduct an extensive literature review on biscuit baking processes, ingredient thermal transitions, and heat transfer mechanisms in gas and electric ovens, highlighting studies on ingredient interactions, dough rheology, and baking quality parameters to establish a strong theoretical foundation for experimental work.

A2) Test samples production and characterization

- Industrial production of different types of products (e.g. Maria biscuits, soda cracker, high fat, high sugar, and high fiber biscuits)
- Physico-chemical and sensory characterization of test samples, assessing key quality attributes such as texture, spread ratio, dimensions, color, moisture content, water activity, acrylamide content and product acceptance.
- Assessment and comparison of the cooking oven thermal profiles.

A3) Correlation between Thermal Profile and Product Quality

- Integrate DSC data with oven temperature profiles to identify critical baking stages and ingredient transformations.
- Analysis of the impact of radiant *vs.* convection heat transfer on ingredient reactions and final biscuit quality.

A4) Ingredient interaction

- Perform lab-scale formulation trials by varying one ingredient factor at a time to study ingredient sensitivity to oven types.

A5) Formulation Optimization and Pilot Plant Validation

- Develop modified formulations to replicate gas oven biscuit quality in electric ovens.
- Validate optimized formulations through pilot plant trials and sensory/instrumental evaluation.

A6) Thesis writing and papers preparation.

Table 1 Gantt diagram for this PhD thesis project.

Activity	*Months*	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***	***15***	***16***	***17***	***18***	***19***	***20***	***21***	***22***	***23***	***24***
A1)	***Bibliographic Review***																								
A2)	***Test samples production and characterization***																								
	1) Industrial Production																								
	2) Physico-chemical Analysis																								
	3) Comparison of cooking thermal profile																								
A3)	***Correlation between Thermal Profile and Product Quality***																								
	1) Integrate DSC data with oven temperature																								
	2) Impact of radiant *vs.* convection heat transfer																								
A4)	***Ingredient Interaction***																								
	Lab-scale formulation trials																								
A5)	***Formulation Optimization and Pilot Plant Validation***																								
	1) Modified formulations																								
	2) Validate formulations through pilot plant productions																								
A6)	***Thesis writing and papers preparation***																								

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Athlete hydration and development of a validated questionnaire for monitoring hydration status in different sports disciplines (P-I-96)

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This PhD thesis research project is aimed at designing, testing the reliability and validity of a questionnaire to assess the hydration status of competitive athletes, to use this tool to conduct large-scale studies and collect national data in different sports disciplines, an area currently lacking systematic information. The secondary objective is to verify whether there is a correlation between the different tools used to evaluate hydration status, to see if these methods can be used individually and interchangeably with each other. This new tool for assessing hydration status in the sports population could prove to be of great help to these subjects, ensuring optimal health and optimal performance; it could also facilitate population-based studies by speeding up data collection time and minimizing running costs, thus helping to speed up research in this area.

Idratazione dell'atleta e sviluppo di un questionario validato per il monitoraggio dello stato di idratazione in diverse discipline sportive

Questo progetto di ricerca per la tesi di dottorato ha lo scopo di progettare e testare l'affidabilità e la validità di un questionario per valutare lo stato di idratazione degli atleti agonisti, con l'obiettivo di utilizzare tale strumento per condurre studi su larga scala e raccogliere dati a livello nazionale in diverse discipline sportive, un ambito attualmente privo di informazioni sistematiche. L'obiettivo secondario è verificare se esiste una correlazione tra i diversi metodi di valutazione dello stato di idratazione, per valutare se possano essere utilizzati singolarmente e in modo intercambiabile. Questo nuovo strumento per la valutazione dello stato di idratazione nella popolazione sportiva potrebbe rivelarsi di grande utilità per questi soggetti, garantendo una salute ottimale e migliori prestazioni; potrebbe inoltre facilitare gli studi epidemiologici, accelerando i tempi di raccolta dei dati e riducendo al minimo i costi di gestione, contribuendo così a promuovere la ricerca in questo settore.

1. State-of-the-Art

With the growing number of people taking up sport, both competitive and amateur, it is essential to understand the diet of this section of the population, which requires specific nutrients, carefully distributed across the different phases of sports preparation: training, pre-race, intra-race, post-race, and recovery or rest periods. A crucial aspect of sports nutrition is hydration, which plays an even more significant role. Maintaining an adequate hydration status is essential for mental and physical performance and overall health (Laja García et al., 2019).

Deviation from the correct state of hydration, known as euhydration, can be detrimental to health. Excess hydration (overhydration) can lead to exercise-associated hyponatremia (EAH), a potentially life-threatening condition preventable with appropriate fluid intake (Leggett et al., 2018). Conversely, insufficient hydration (hypohydration), due to excessive sweating or inadequate water intake, can impair exercise performance with as little as a 2% loss of body weight (McDermott et al., 2017). Losses of 4% or more can cause heat illnesses such as heat exhaustion, heat stroke, and even death (Maxwell et al., 2009; Kerksick et al., 2018).

In the current sports context, awareness of hypohydration and its consequences is growing, both among athletes and sports professionals. Athletes must adopt effective hydration strategies to maintain optimal water balance during training and competitions to enhance performance and safeguard their health.

Various methods for assessing hydration status have been tested in recent years, but no universally accepted "gold standard" currently exists (Armstrong, 2007). Further research is needed to develop new tools, including specific questionnaires tailored to the sports population, which are still lacking.

Incorrect hydration, whether by excess or deficiency, can severely impact athletes. Mild dehydration can cause premature fatigue and reduced performance, while severe dehydration can lead to muscle cramps, heat stroke, and kidney damage. Conversely, overhydration may result in hyponatremia, a dangerous condition caused by abnormally low blood sodium levels.

Currently, in Italy, there is a lack of specific national data regarding the hydration status of the population, making it difficult to assess the prevalence of these issues in both sports and the general population. This gap likely stems from the absence of large-scale assessment tools and the lack of a universally recognized "gold standard". This project therefore aims to address this need by developing and validating a new questionnaire designed for use in the Italian athletic population

2. PhD Thesis Objectives and Milestones

Within the overall objective described above, this PhD thesis project can be subdivided into the following

activities, according to the Gantt diagram reported in Table 1:

A1) **Literature review and critical analysis of current hydration assessment methods**, with particular attention to athletes' needs and characteristics of the sports population, through critical review of existing tools and methodologies (A1.1), and identification of methodological gaps and requirements for sport-specific, scalable tools (A1.2).

A2) **Design and development of the initial version of the questionnaire** through definition of content domains and generation of the item pool with expert consultation (A2.1), and preparation of the draft questionnaire in electronic format (A2.2).

A3) **Pilot testing and statistical validation of the questionnaire** to assess its reliability (A3.1) and validity through comparison with hydration biomarkers (A3.2).

A4) **Large-scale data collection** on the hydration status of competitive athletes through recruitment (A4.1) and administration of the validated questionnaire with supplementary data collection (A4.2).

A5) **Data analysis and interpretation** to identify hydration patterns and risk factors (A5.1), and to evaluate correlations between questionnaire outcomes and objective hydration measures (A5.2).

A6) **Writing and editing of the PhD thesis**, and preparation of scientific papers, oral and/or poster presentations (A6).

Table 1 Gantt diagram for this PhD thesis project.

		2nd Year												3rd Year											
Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Literature review & analysis***																								
	1) Critical analysis of tools																								
	2) Identification of gaps																								
A2)	***Design of questionnaires and tools***																								
	1) Requirements & construct mapping																								
	2) Prototyping & pre-validation																								
A3)	***Pilot testing & validation***																								
	1) Reliability testing																								
	2) Validity testing																								
A4)	***Large-scale data collection***																								
	1) Athlete recruitment																								
	2) Administration of questionnaire and tools & data collection																								
A5)	***Data analysis & interpretation***																								
	1) Hydration knowledge & status of athletes																								
	2) Correlation evaluation																								
A6)	***Thesis writing & dissemination***																								

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Nutritional needs of lung cancer patients: assessment of nutritional status for the development of new personalized nutrition interventions (P-I-97)

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Tutor: Prof. Alice Rosi

This PhD thesis research project is aimed at evaluating the nutritional and metabolic status of patients with lung cancer by assessing body composition, nutrient-related biomarkers and multi-omics data, and their association with treatment outcomes. This will allow to provide predictive models for precision nutrition strategies to improve patient's outcome.

Esigenze nutrizionali del paziente con tumore del polmone: valutazione dello stato nutrizionale per la definizione di nuovi interventi di nutrizione personalizzata

Questo progetto di tesi di dottorato mira a valutare lo stato nutrizionale e metabolico dei pazienti con tumore del polmone attraverso l'analisi della composizione corporea, dei biomarcatori correlati ai nutrienti e dei dati multo-omici, e dalla loro associazione con gli esiti del trattamento. Questo permetterà di sviluppare modelli predittivi per strategie di nutrizione di precisione volte a migliorare l'esito clinico dei pazienti.

1. State-of-the-Art

Lung cancer is a globally significant malignancy, affecting both men and women (WHO, 2023). In 2020, over 2.2 million people were diagnosed with lung cancer, confirming it as the second most prevalent type of cancer worldwide (Malvezzi *et al.,* 2023). Among the primary causes leading to this malignancy, there is the long-term exposure to tobacco smoke (Malhotra *et al.,*2016). Other risk factors could be identified in environmental pollutants, genetic predisposition, and unhealthy lifestyles and diets (Malhotra *et al. ,*2016).
Despite a general decline in global cancer mortality, lung cancer remains one of the major clinical challenges, imposing a substantial burden on society through significant economic consequences and pressure on health care resources (Looijmans *et al.,* 2018). Patients with lung cancer encounter a range of difficulties, lower Quality of Life (QoL), and higher levels of psychological distress compared with other cancer populations (Maguire *et al.,* 2019). They may also experience dissatisfaction or distrust in the medical system and frequently present comorbidities that negatively affect physical performance and overall well-being (Maguire *et al.,* 2019). Furthermore, rapid nutritional decline due to disease progression often leads to clinically significant malnutrition (Polański *et al.,* 2021). Thus, the challenges faced by lung cancer patients extend well beyond their initial diagnosis, profoundly influencing various aspects of their well-being and QoL.
Although cancer has been considered a proliferation disorder, emerging evidence suggests it should also be considered a metabolic disease, given that the metabolic status of patients plays a crucial role in shaping clinical outcomes (Coller, 2014). In this context, the implementation of precision nutrition is needed to address malnutrition and metabolic consequences in a dynamic and individualized way. Despite this, it is noteworthy that lung cancer patients undergoing clinical treatments are rarely provided with individualized dietary interventions. Substantial evidence supports the connection between nutrition and metabolic regulation in cancer (McIntyre *et al.,* 2023). Cancer cells exhibit unique metabolic requirements differing from healthy cells, thereby influencing cell survival and growth (McIntyre *et al.,* 2023). Both cell-intrinsic factors like genetics and cell-extrinsic factors such as diet and drug therapy influence nutrient utilization in cancer cells (Cuyàs, 2020). Moreover, the impact of metabolism on the immune system function, the microbiome profile and the glycome-associated signaling pathways will dictate the response and outcome of cancer patients. Understanding these interactions opens new therapeutic possibilities and highlights the role of nutrition in cancer treatment. Recent studies shown that diet significantly influences tumor metabolism by modulating nutrient availability within the tumor microenvironment, thereby affecting cancer progression (Wang et al., 2025).
A well-balanced diet is fundamental to good health, as it provides essential nutrients critical for therapeutic success in lung cancer patients, helps manage the adverse effects of treatments, accommodates other chronic conditions, and aims to prevent malnutrition and cancer-associated cachexia (Polański et al., 2023). Conversely, dietary imbalances have been shown to disrupt systemic metabolic health, adversely impacting treatment efficacy (McIntyre et al., 2023). Moreover (poly)phenols and carotenoids have demonstrated significant potential in cancer prevention and management by enhancing antioxidants, reducing oxidative stress, modulating inflammation, and inhibiting cancer cell proliferation through various studies (Briguglio et al., 2020). Nonetheless, precision nutrition studies addressing the nutritional status of lung cancer patients are lacking.

2. PhD Thesis Objectives and Milestones

This PhD thesis project aims to evaluate the nutritional and metabolic status of patients with lung cancer and to develop predictive models that will guide precision nutrition strategies to improve patient outcomes. Within this overall objective, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Systematic literature review,** to evaluate the current state-of-the-art.
A2) **Prospective study,** to perform sample and data collection activities for further analyses and model development while ensuring compliance with ethical and regulatory guidelines.
A3) **Data integration and modelling,** to establish a model for precision nutrition recommendations able to characterize the nutritional and metabolic status of lung cancer patients.
A4) **Writing and editing** of the PhD thesis.

Table 1 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Systematic review***																								
A2)	***Prospective study for sample and data collection***																								
	1) Prospective study preliminary planning																								
	2) Enrolment, sample and data collection																								
	3) Sample preparation and processing																								
A3)	***Data integration and modelling***																								
A4)	***Thesis and Paper Preparation***																								

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Food systems transformation for healthy and sustainable diets (P-I-98)

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This PhD thesis research project aims to encourage a transition in the food system towards healthier and more sustainable diets. This objective will be achieved by developing policy interventions that encourage consumers to improve their dietary behaviors using the tools available to consumers, such as food-based dietary guidelines.

Trasformazione dei sistemi alimentari per diete sane e sostenibili

Questo progetto di tesi di dottorato ha l'obiettivo ti promuovere la trasformazione dei sistemi alimentari verso diete più sane e più sostenibili. Questo obiettivo sarà raggiunto sviluppando politiche e strategie di intervento mirate ad incoraggiare il consumatore a migliorare le proprie abitudini alimentari, partendo da un miglioramento degli strumenti a disposizione del consumatore stesso, ossia le linee guida per una sana alimentazione.

1. State-of-the-Art

Food systems largely contribute to environmental damage, with food production responsible for 30% of global greenhouse gas emissions (1). At the same time, the current food production system is unable to provide equitable access to healthy diets, given that various forms of malnutrition, such as micronutrient deficiencies, undernutrition and overnutrition, are widely spread. Therefore, urgent changes to food systems are needed to encourage a shift towards healthy and environmentally sustainable diets.

Food-based dietary guidelines are one approach that can be used to achieve this goal, as they are an educational and awareness-raising tool that informs and supports consumers to be compliant with the nutritional recommendations. However, limitations in the use of this tool can be identified. Firstly, despite the scientific evidence supporting the guidelines and the relevant messages included, it is well known that adherence to the dietary recommendations is still low (2). This is mainly due to difficulties in communicating the messages clearly and easily to end users, as well as a lack of appropriate dissemination of dietary guideline documents (3, 4). Additionally, it should be noted that most current food-based dietary guidelines focus more on nutritional aspects rather than on a holistic view that considers all dimensions of diet, such as environmental impact and social considerations. While environmental sustainability has achieved some relevance through the inclusion of general advice on this topic (5), a methodology for incorporating sustainability considerations into food-based dietary guidelines has not developed yet. It is clear that an approach that considers different dimensions of sustainability while supporting consumers' dietary choices would improve the effectiveness of food-based dietary guidelines by focusing on the health of the planet and that of people.

For this reason, the main objective of the PhD project is to develop an innovative methodology for producing food-based dietary guidelines that could serve as a model to be shared among European countries, in the context of the Horizon Europe PLAN'EAT project. Once validated, this framework will be applied to the Italian context.

2. PhD Thesis Objectives and Milestones

Among the PhD thesis objectives, different activities will be developed, in consideration of the timing outlined in the Gantt chart (Table 1).

A1) **Define and test the FBDG framework**, including the True Cost Accounting (TCA) methodology, and identify an efficient communication strategy. The TCA methodology aims to evaluate the true cost of food in terms of its environmental, social, and health impacts. The results of this approach can consequently help to identify which consumption patterns should be recommended, taking these three aspects into consideration.

A2) **Conduct qualitative tests** (e.g., focus group or Delphi methodology) with policymakers to verify the applicability of the framework at the development stage, and with consumers to assess their understanding of it.

A3) **Finalize the FBDG framework** based on the results of the test to make it more applicable and reliable.

A4) **Apply the developed and tested framework** to the Italian context to produce a concrete example of food-based dietary guidelines derived from its application.

A5) **Establish food-based dietary guidelines based on TCA**, with the aim of recommending eating habits that have the lowest environmental, social and health impacts, while remaining close to current food habits and culinary traditions.

A6) **Produce** scientific papers and posters for congresses, reports on activities, and PhD thesis.

Table 1. *Gantt diagram for this PhD thesis project.*

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Define the framework***																								
	Identify how to apply the TCA methodology																								
	Identify the efficient communication strategy																								
A2)	***Qualitatitve test at European level***																								
	Test with stakeholders																								
	Test with consumers																								
A3)	***Finalize the framework***																								
	Adjust the framework based on the test feedback																								
A4)	***Apply the framework to the Italian context***																								
	TCA method to the Italian dietary pattern																								
	Prepare the food-based dietary guidelines																								
A5)	***Develop dietary guidelines***																								
	Identify which food consumption should be promoted based on the lowest environmental, social and health impact																								
	Adapt the recommendations to the usual and traditional habits																								
A6)	***Writing and editing***																								
	Scientific papers, posters, PhD thesis																								

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Datasets integration for the development of dietary strategies aimed at promoting cardiometabolic health through plant-based diets (P-I-99)

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This PhD project aims to apply an integrated multi-omics approach (genomics, epigenomics, metagenomics, metabolomics and proteomics) to understand how dietary (poly)phenols influence cardiometabolic health in humans, contributing to the development of personalized strategies within the framework of precision nutrition.

Integrazione di dataset per lo sviluppo di strategie dietetiche indirizzate alla promozione della salute cardiometabolica attraverso diete a base vegetale

Questo progetto di dottorato ha l'obiettivo di applicare un approccio multi-omico (integrando genomica, epigenomica, metagenomica, metabolomica e proteomica) per comprendere come il consumo di (poli)fenoli influenzi la salute cardiometabolica negli esseri umani, contribuendo allo sviluppo di strategie personalizzate nell'ambito della nutrizione di precisione.

1. State-of-the-Art

The growing interest in (poly)phenols (PPs) within the field of precision nutrition (PN) is particularly remarkable, due to presence of PPs in plant-based foods and their potential beneficial effects, that might allow the design of diets targeting chronic disease prevention (Del Rio et al., 2013). At the same time, researchers face significant challenges due to the marked heterogeneity in individual responses to dietary PP consumption, representing a considerable obstacle in analyzing and fully understanding their potential for preventing non-communicable diseases (Favari et al., 2024). The complexity of individual responses to PPs is influenced by numerous factors, including age, sex, ethnicity, lifestyle, dietary preferences, genetic profile, gut microbiome composition, enzymatic profiles, overall physiological state, and even medications being taken (Milenkovic et al., 2017). The interplay between various factors creates unique individual responses that require careful consideration in nutritional interventions. PN addresses this individual complexity by analyzing multiple factors for more targeted outcomes. This requires the integration of multiple layers of understanding, beginning with basic biological, social, and environmental factors, and extending to more complex facets, including genetic variants, proteins, metabolites, gut microbial species, and their enzymes (Palmnäs et al., 2020). The progress of this field has been greatly improved by innovative tools including multi-omics techniques, machine learning models, and artificial intelligence. Looking to the future, emerging technologies in both single and integrative multiple omics fields hold promise to reveal detailed subject-specific multi-layered profiles. PN might benefit from the integration of big and omics data and could enable unprecedented precision in identifying disease risk factors and developing optimal dietary strategies through holistic approaches.

2. PhD Thesis Objectives and Milestones

This PhD thesis project will be directed to select multi-omics application to nutritional science, investigating the different biological layers determining individual response to PPs. The final aim will be advancing our ability to provide truly personalized dietary recommendations and interventions, mainly aimed at preventing cardiometabolic disease through PP-rich diets. Within the overarching aim outlined, the project will be structured into the following work packages:

A1) **Sample set refinement**: evaluation and curation of available omics datasets to select high-quality and biologically relevant samples for downstream multi-omics analyses.

A2) **Single-omics analyses:** bioinformatic analysis of data from individual omics platforms:

- A2.1) Genomics: investigation of the genetic variants associated with specific phenotypes and individual metabolic capacities.
- A2.2) Epigenomics: examination of epigenetic modifications that regulate gene expression in response to dietary PPs and influence cardiometabolic health outcomes.
- A2.3) Metagenomics: analysis of the composition and functional potential of the gut microbiota.

- A2.4) Metabolomics: identification of PP-derived metabolites and metabolic signals associated with cardiometabolic function.
- A2.5) Proteomics: exploration of proteins involved in the bioactive mechanisms of dietary PPs.

A3) **Multi-omics integration:** Bioinformatic integration of multi-omics datasets to identify associations and predictive patterns of individual response to PP intake.

A4) **Writing and editing**: preparation of the PhD thesis, scientific publications, and participation in conferences.

Table 2 Gantt diagram for this PhD thesis project.

Activity	**Months**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
A1)	***Sample Set Refinement***																								
A2)	***Single omics analysis***																								
	A2.1) Genomics																								
	A2.2) Epigenomics																								
	A2.3) Metagenomics																								
	A2.4) Metabolomics																								
	A2.5) Proteomics																								
A3)	***Multi-omics analysis***																								
A4)	***Thesis and Paper Preparation***																								

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Investigating the citrus fruit polyphenols and Their Metabolites on Immunoaging, Neurodegeneration, and Skeletal Muscle (P-I-100)

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This PhD thesis research project aims to explore how polyphenols found in citrus fruits, along with the compounds they break down within the body, impact the aging process in three key areas. Specifically, it will investigate their effects on the decline of the immune system (immunoaging), the progression of neurodegenerative diseases, and the age-related deterioration of skeletal muscle. The overarching goal is to understand if and how these citrus-derived compounds can mitigate the detrimental effects of aging on these vital physiological systems.

Studio dei polifenoli degli agrumi e dei loro metaboliti su immunosenescenza, neurodegenerazione e muscolo scheletrico

Questo progetto di ricerca di tesi di dottorato mira a esplorare come i polifenoli presenti negli agrumi, insieme ai composti in cui si scompongono all'interno dell'organismo, influenzino il processo di invecchiamento in tre aree chiave. Nello specifico, studierà i loro effetti sul declino del sistema immunitario (immunoinvecchiamento), sulla progressione delle malattie neurodegenerative e sul deterioramento del muscolo scheletrico correlato all'età. L'obiettivo principale è comprendere se e in che modo questi composti derivati dagli agrumi possano mitigare gli effetti dannosi dell'invecchiamento su questi sistemi fisiologici vitali.

1. State of Art:

Citrus fruits provide bioactive polyphenols such as hesperidin, naringin, eriocitrin, and nobiletin, which exhibit antioxidant, anti-inflammatory, and cytoprotective effects. In HepG2 cells, flavonoids undergo metabolism that alters activity, yet many studies use parent compounds at unrealistic doses. In SH-SY5Y neurons, nobiletin and hesperidin protect against oxidative stress and support synaptic plasticity. In CD56+ NK cells, citrus metabolites modulate inflammation by suppressing NF-κB and MAPK signaling. In C2C12 myoblasts, naringin and hesperidin enhance mitochondrial function and differentiation. However, reliance on young cells, neglect of metabolites, and limited complex culture models reduce translational relevance for aging-related dysfunctions.

2. PhD thesis objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 2:

A1) Elucidate the Metabolic Transformation of Citrus Fruits Polyphenols Using HepG2 Liver Cells.

A2) Investigate the Neuroprotective Potential of Citrus Polyphenols and Their Metabolites in SH-SY5Y Neuronal Cells Under Oxidative and Amyloid Stress

A3) Characterize the Anti-Inflammatory and Immunomodulatory Effects of Citrus Polyphenols and Their Metabolites in CD56+ Natural Killer (NK) Cells

A4) Assess the Myo protective Effects of Citrus Polyphenols and Their Metabolites in C2C12 Skeletal Muscle Cells

A5) Writing and Editing of the PhD thesis, scientific papers and oral and/or poster communications.

Table 2 Gantt diagram for this PhD thesis project.

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	**Metabolic Transformation and Bioavailability of Citrus Polyphenols Using HepG2 Liver Cells**																								
A2)	**Neuroprotective Potential of Citrus Polyphenols and Their Metabolites in SH-SY5Y Neuronal Cells Under Oxidative and Amyloid Stress**																								
A3)	**Anti-Inflammatory and Immunomodulatory Effects of Citrus Polyphenols and Their Metabolites in CD56+ Natural Killer (NK) Cells**																								
A4)	**Myo protective Effects of Citrus Polyphenols and Their Metabolites in C2C12 Skeletal Muscle Cells**																								
A5)	**Thesis and Paper Preparation**																								

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Nutritional and functional sustainability for human and planetary health: the study of the dietary habits and metabolic profile of centenarians from Abruzzo (P-I-101)

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This PhD thesis research project aims to conduct a comprehensive analysis of the metabolic, genomic, functional, and nutritional profiles of centenarians and nonagenarians from the Abruzzo region. The study intends to explore how lifestyles and eating habits affect longevity and quality of life and collect important information to create nutritional and functional guidelines that will help to improve overall health.

1. State-of-the-Art

Human health and well-being are influenced by epigenetic, environmental, and lifestyle factors, including dietary habits. This is particularly important for European societies, where the aging population is growing, along with the incidence of age-related diseases such as cardiovascular, metabolic, and neurodegenerative disorders (Pes et al., 2021).

In this context, many studies have shown a connection between healthy dietary patterns, lifestyle choices, and longevity. In fact, some areas of the world, known as 'Blue Zones'—characterized by high longevity—such as the island of Okinawa in Japan, the island of Ikaria in Greece, the mountain area of the island of Sardinia in Italy, and the peninsula of Nicoya in Costa Rica, share common features such as the consumption of homegrown foods, diets rich in antioxidants, moderate intake of pork, and low consumption of fish, likely influenced by the local availability of food resources (Pes et al., 2022). A diet rich in vegetables, legumes, and whole grains has been linked to a lower risk of age-related diseases, thanks to the presence of vitamins, minerals, fibre and bioactive compounds with antioxidant and anti-inflammatory properties (Leitão et al., 2021). In addition, Blue Zone populations tend to maintain high levels of physical activity as part of their daily lives, often through regular walking and low-intensity movements, which positively influence health outcomes (Ungvari et al., 2023). Epigenetic mechanisms are also crucial in understanding how dieting influences the aging process. Indeed, the environment and lifestyle can disrupt the system of epigenetic modifications that promote healthy aging. This disruption leads to changes in cellular function, providing a 'biological reading' of the aging process (Puca et al., 2018).

The region of Abruzzo, located in central Italy, is among the leading areas in the country for its high number of nonagenarians and centenarians (ISTAT, 2022). The region's food biodiversity suggests a clear example of a diet that promotes longevity. Local products—such as “Santo Stefano di Sessanio” lentils and wild greens like orapi (Chenopodium bonus-henricus)—are used in traditional recipes with nutritional and functional properties, involved in health promotion. Moreover, intermittent fasting, known for its potential benefits on longevity, has interesting similarities with traditional dietary habits observed in the Abruzzo region, such as “sdijuno” — an extended overnight fast followed by a substantial morning meal. This practice may have tuned the body’s innate response to daily nutrition, potentially optimizing metabolic and immune responses to dietary stressors (Angelino et al., 2022).

Based on the collected data, specific nutritional recommendations will be developed to promote successful aging and longevity in the general population, derived from the principles of the Mediterranean diet and traditional Abruzzo eating habits. The project will further contribute to the preservation and promotion of Abruzzo’s culinary heritage, supporting regional biodiversity and environmental sustainability.

2. PhD Thesis Objectives and Milestones

A1) Estimate the number of nonagenarians and centenarians, as well as the frequency of longevity in the Abruzzo population, based on the most recently updated municipal and provincial data from ISTAT (Italian National Institute of Statistics), and compare them with Italian so-called "Blue Zones."

A2) Identify the cohort of centenarians and nonagenarians in the region.

A3) Evaluate the antioxidant potential of extracts from selected products and recipes from Abruzzo, using an in vitro intestinal model of oxidative stress, and identify the bioactive compounds responsible.

A4) Examine the dietary habits and lifestyles of Abruzzo’s centenarians.

A5) Determine the pro- or anti-inflammatory potential of traditional dishes consumed by centenarians grown and living in Abruzzo by measuring specific inflammatory markers.

A6) Identify specific foods abandoned by the contemporary Western diet that may correlate with the rise in age-related pathological conditions.
A7) Establish a biobank (plasma, saliva, urine, DNA, and RNA) of Abruzzo's centenarians for research purposes.
A8) Develop and propose a trademark or logo certifying the quality and authenticity of Abruzzo's traditional products and recipes, linking them to longevity.
A9) Create an editorial project exploring the connections between diet, environment, and longevity in Abruzzo, including descriptions of key traditional recipes identified by centenarians across different provinces.
A10) Present the project's findings at national and international conferences and publish them in scientific journals.
A11) Organize public events to disseminate the project's results and future developments.

Table 2 Gantt diagram for this PhD thesis project.

1st Year	1nd Year	2nd Year	2nd Year	3rd Year	3rd Year
Semester I	**Semester II**	**Semester I**	**Semester II**	**Semester I**	**Semester II**
A1					
	A2				
	A3	A4			
		A5			
		A6	A7	A8	
				A9	
					A10
					A11

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Sustainable Diets for Health and Equity: Advancing Food Policies in Urban Environments (P-I-102)

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This PhD thesis research project aims to investigate the real-world implementation of nutritional guidelines within the population, with particular emphasis on the co-benefits for public health and the reduction of greenhouse gas emissions associated with a shift towards such diets. The primary objective is to establish a robust data foundation to inform food and health policies, promote public awareness, and facilitate the transition towards more sustainable and evidence-based dietary patterns that address both health and environmental sustainability challenges.

Diete sostenibili per la salute e l'equità: promuovere politiche alimentari negli ambienti urbani

Il progetto si propone di studiare l'applicazione effettiva nella popolazione delle linee guida nutrizionali, ponendo particolare attenzione ai co-benefici per la salute pubblica e alla riduzione delle emissioni di gas serra che la transizione verso queste diete può favorire. L'obiettivo principale è creare una base di dati solida che possa supportare le politiche alimentari e sanitarie, promuovendo una maggiore consapevolezza e facilitando la transizione verso diete più sostenibili e consapevoli, capaci di rispondere alle sfide della salute e della sostenibilità ambientale.

1. State-of-the-Art

Climate change impacts human health both directly and indirectly and shifting dietary habits represents one of the key strategies to mitigate these effects, while simultaneously delivering health benefits. With the global food production system accounting for approximately 30% of greenhouse gas emissions, is crucial to identify dietary patterns that are both health-promoting and environmentally sustainable (Willett et al., 2019).

Recent studies have shown that adopting sustainable diets can significantly reduce mortality and cancer incidence while also contributing to lower greenhouse gas emissions (Laine et al., 2021). Various models for healthy and sustainable eating have been proposed globally, reflecting geographical differences. Notable examples include MyPlate in the United States (Schwartz & Vernarelli, 2019) and the Mediterranean diet (Guasch-Ferré & Willett, 2021), which share many similarities. Both approaches emphasize a reduced intake of highly processed foods, limit the consumption of red meat, sugar and salt, and encourage the consumption of locally sourced, seasonal, and plant-based foods. Additionally, both models highlight the importance of physical activity as a pivotal component of a healthy lifestyle.

The project aims to provide a robust scientific foundation to guide public health policies toward dietary recommendations that are both healthy and sustainable, while ensuring social equity. By analyzing dietary patterns and socio-cultural barriers that hinder the transition to sustainable diets, the research develops practical, locally tailored recommendations to reduce social inequalities and promote evidence-based policymaking. The project seeks to accelerate progress toward achieving international sustainable development goals, and climate change mitigation and adaptation focusing on Italian cities. It adopts an integrated approach that emphasizes health and equity in the planning of local policies and interventions, prioritizing evidence-based measures that reduce social and gender inequalities while delivering significant direct and indirect health benefits.

Through the analysis of dietary patterns, greenhouse gas emissions, and socio-cultural barriers, the research develops actionable recommendations for local implementation, focusing on sustainability and social justice. The primary focus lies on internationally and nationally recognized dietary models, particularly European frameworks such as the Mediterranean diet. The study examines the health and environmental benefits of these models, especially regarding greenhouse gas emissions. It also evaluates the accessibility of these dietary patterns for specific population groups, considering gender, socio-economic, and cultural differences, along with factors such as the consumption of local products and their seasonality.

A central element of the research involves assessing Metabolic Food Waste. This analysis examines the excessive consumption of food in obese and overweight individuals compared to those with normal weight, investigating its impact on individual well-being and the environment. This evaluation highlights how imbalanced diets affect both health and environmental resources (Serafini & Toti, 2016).

The ultimate goal of the research is to establish a solid scientific basis for public health policies that promote both human health and environmental sustainability, with a strong focus on equity. Particular attention goes to the acceptability and accessibility of healthy and sustainable diets across the population, addressing the socio-economic, cultural, and geographical differences that influence these aspects. Social disadvantage remains a critical determinant of health even in developed countries, significantly impacting morbidity, premature mortality,

and the growing demand for healthcare services (Guasch-Ferré & Willett, 2021).
The only effective response to these risks, which include environmental, clinical, and social factors, lies in the "Health in All Policies" approach. This framework calls for collaboration among stakeholders at various decision-making levels and a redefined public policy governance guided by scientific evidence and tailored to local contexts. In this framework, the research focuses on the role of nutrition as a tool for climate change mitigation, with particular attention to the health benefits associated with promoting these healthy and sustainable lifestyles.

2. PhD Thesis Objectives and Milestones

This project aims to investigate the real-world implementation of nutritional guidelines within the population, with particular emphasis on the co-benefits for public health and the reduction of greenhouse gas emissions that may result from a shift towards such dietary patterns. The primary objective is to establish a robust data foundation to inform food and health policies, enhance public awareness, and support the transition towards more sustainable and conscious eating habits—capable of addressing both health-related and environmental sustainability challenges.
Key focus areas will include:

1) **Analysis of Adherence to Nutritional Guidelines**. This section examines existing data to assess the population's adherence to sustainable dietary recommendations. The analysis will identify dietary patterns that demonstrate the highest potential for reducing greenhouse gas emissions while simultaneously contributing to significant public health benefits, such as a reduced incidence of diet-related chronic diseases (e.g., type 2 diabetes, cardiovascular disease).

2) **Study of Socio-Economic and Cultural Disparities**. This analysis aims to identify population groups that face significant barriers to accessing healthy and sustainable diets. Key determinants such as gender, socio-economic status, and cultural background will be examined to better understand disparities in dietary practices. The findings will inform the development of empirically grounded recommendations to improve the accessibility and acceptability of sustainable diets across diverse demographics. Particular attention will be given to urban settings, where targeted food policies may produce more immediate and measurable outcomes. In addition, the analysis will assess the potential of sustainable dietary patterns to reduce socio-economic and gender-based inequalities in access to nutritious food by examining data across different geographical areas and social groups.

3) **Assessment of Metabolic Food Waste**. The study evaluates Metabolic Food Waste, defined as food consumption exceeding nutritional needs, through an observational analysis comparing overweight and obese individuals to those with normal weight. This research highlights the negative implications of metabolic food waste for both public health and environmental sustainability.

4) **Development of Concrete Recommendations.** Based on the conducted analyses, this section formulates recommendations to promote sustainable diets, particularly in urban areas of Italy. The approach focuses on inclusivity and aims to reduce social inequalities. Proposed solutions enhance the accessibility and acceptability of healthier lifestyles while minimizing environmental impact.

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Study of flavan-3-ols metabolised and non-metabolised at colonic level through the use of several chromatographic techniques (P-I-103)

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This PhD research project aims to evaluate both metabolized and non-metabolized high molecular weight (poly)phenols, namely proanthocyanidins (PACs), in human faeces. Limited literature exists regarding the quantification of unabsorbed PACs in faeces. This could be due to their propensity to form complexes with proteins during gastrointestinal transit, which complicates their detection using chromatographic techniques.

Studio di flavan-3-oli metabolizzati e non metabolizzati a livello colonico attraverso l'utilizzo di molteplici tecniche cromatografiche

Questo progetto di tesi di dottorato ha l'obiettivo di valutare i (poli)fenoli ad alto peso molecolare, metabolizzati e non metabolizzati a livello colonico, con particolare attenzione alle proantocianidine (PACs). Ad oggi, la letteratura disponibile sulla quantificazione delle PACs non assorbite a livello gastrointestinale in feci umane è ancora limitata. Uno dei fattori potrebbe essere la loro tendenza a formare complessi con le proteine durante il transito gastrointestinale, rendendo difficile la loro rilevazione.

1. State-of-the-Art

Several studies have focused on the determination and structural characterization of proanthocyanidins (PACs) in food matrices, applying various analytical protocols. One of the most widely used techniques is phloroglucinolysis followed by reversed-phase high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) (Kennedy & Jones, 2001). Phloroglucinolysis is an acid-catalysed reaction that causes the cleavage of the interflavan bonds of PACs under acidic conditions, yielding a C4-phloroglucinol adduct from the extension units. This method provides detailed information regarding the mean degree of polymerisation, monomeric flavan-3-ol subunit composition, and degree of galloylation of PACs.

In addition to reversed-phase HPLC-MS, other analytical techniques have been developed to assess PACs, including:

- Hydrophilic interaction liquid chromatography (HILIC) coupled with fluorescence detection (FLD), which is particularly useful to separate and detect PACs according to their degree of polymerisation. A study led by Bussy et al. (2021) validated a method for cocoa PACs determination using a cocoa-based commercially available reference material (Bussy et al., 2021).
- Gel permeation chromatography (GPC), which is employed for molecular size distribution analysis. Bindon et al. (2010) applied GPC to characterise size distribution of PACs isolates (Bindon et al., 2010).

Despite the availability of these techniques for food matrices, there is limited literature regarding their application to human faecal samples. While some studies have investigated the presence of PACs in the faeces of animals fed with PAC-rich diets, research on human faeces remains scarce (Choy et al., 2014; Pereira-Caro et al., 2020).

This gap derives from the uncertainty surrounding the fate of PACs during gastrointestinal digestion. They may not be degraded in the early stages of digestion, reaching the colon in their native form where they are degraded by the gut microbiota, or are excreted as unabsorbed PACs. Current literature primarily focuses on identifying colonic catabolites resulting from PAC ingestion, as was recently proposed following an *in vitro* faecal fermentation study evaluating the influence of flavan-3-ol structure on the production of phenolic catabolites (Di Pede et al., 2022). This has been further confirmed recently in animals (Liu et al., 2025). Several studies have also investigated the interaction of PACs with proteins during gastrointestinal transit. For instance, a study investigated the interaction between tannins and salivary proline-rich proteins. It also examined interactions with dietary proteins and digestive enzymes in the gut (Baxter et al., 1997). Indeed, the high number of hydroxyl groups in PACs facilitates their interaction with other macromolecules, particularly proteins, mainly proline-rich ones. This can potentially complicate their extraction and quantification from complex biological matrices. The affinity of PACs for proteins is influenced by both the physicochemical properties of proteins themselves (e.g. charge, size, and conformation) as well as the structural characteristics of PACs, particularly their degree of polymerisation. Higher polymerisation is associated with stronger interactions (Hagerman & Butler, 1981). To date, there is limited information on the detection of unabsorbed PACs in human faeces and there is a lack of standardised protocols to analyse PACs in faecal samples.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities:

1. **Development of a protocol for the extraction and identification of PACs in faeces.**
   This will consist in comparing different extraction protocols to determine the most effective for PAC extraction from human faecal samples. Tests on the phloroglucinolysis reaction will also be conducted, in parallel with the development of the extraction protocol.
2. **Development and optimization of analytical techniques for quantifying PACs in faeces.**
   The main techniques will include:
   - HPLC-MS, useful for phloroglucinolysed samples.
   - HILIC-FLD, useful for separating PACs based on their degree of polymerization.
   - GPC/SEC, useful for molecular size distribution analysis.
3. **Application of the methods developed to human faecal samples.** Once the protocol is developed, it will be applied to human faecal samples from human intervention studies with dietary (poly)phenols to determine the content of unabsorbed PACs.
4. **Writing and editing of the PhD thesis, scientific papers, and oral and/or poster communications.**

Table 2 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Protocol development***																								
	1) Protocol of extraction																								
	2) Phloroglucinolysis reaction																								
A2)	***Analytical technique optimization***																								
	1) HPLC-MS																								
	2) HILIC-FLD																								
	3) GPC-SEC																								
A3)	***Application of the methods to human faecal samples***																								
	1) Samples preparation																								
	2) Samples analysis																								
	3) Data analysis																								
A5)	***Thesis and Paper Preparation***																								

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Nutritional and technological strategies to study and develop cereal-based products for different population targets (P-I-104)

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This PhD project aims to develop nutritional and technological strategies for studying and enhancing the nutritional quality of cereal-based products, which are staple foods consumed worldwide. The research activity will focus on evaluating the bioaccessibility of macronutrients and micronutrients from different starchy foods using in vitro digestion models, as well as investing their effect in vivo on post-prandial glucose metabolism. The developed products will be designed to meet the nutritional needs of different population groups, with particular attention to the growing elderly population and to meet the increasing challenges related to the sustainability of food production.

Strategie nutrizionali e tecnologiche per studiare e sviluppare prodotti a base di cereali per diversi target di popolazione

Questo progetto di tesi di dottorato mira a sviluppare strategie nutrizionali e tecnologiche per migliorare la qualità nutrizionale dei prodotti a base di cereali, alimenti fondamentali a livello globale. L'attività si concentrerà sulla valutazione della bioaccessibilità e biodisponibilità di macro e micronutrienti in vari alimenti amidacei, utilizzando protocolli di digestione in vitro e analizzando l'effetto in vivo sulla glicemia post-prandiale. I prodotti sviluppati risponderanno alle esigenze nutrizionali di diversi gruppi di popolazione, con particolare attenzione alla fascia anziana e alla sostenibilità della produzione alimentare.

1. State-of-the-Art

Nutrition is a key modifiable factor in promoting health and well-being (Liu et al., 2022). Healthy dietary habits also play a preventive role in the development of chronic diseases. Given the rising elderly population worldwide, it is increasingly important to promote healthy aging. Aging is associated with various physical, physiological, and cognitive changes, which means that dietary models considered appropriate for adults may no longer be adequate for older individuals. Physiological changes in gastrointestinal function and composition, difficulties in chewing and swallowing, and altered sensory perception can, over time, lead to deficiencies in essential nutrients, resulting in the malnutrition commonly observed in the elderly (Table 1).

Table 1 *Common deficiencies and symptoms prevalent in the elderly population (Qin et al.,2024).*

Deficiency	Symptoms
Vitamin D3 & Calcium	Bone loss - Osteoporotic - Fractures
Vitamin K	Blood coagulation - Bone health
Vitamin B6 & B12	Alzheimer's disease - Dementia
Zinc & Iron	Immune system - Infections - Anemia
Protein	Malnutrition - Fraility - Dysphagia
Carbohydrate	Loss of physical activity - Fraility - Wound healing
Fat	Cardiovascular diseases - Alzheimer's diseases

Based on these premises, it is crucial to rethink food formulations to better meet the specific needs of the elderly population, with particular attention to the food matrix (FM) effect. FM is a key factor in the development of foods for older adults, as it can significantly influence nutrient bioavailability. Bioavailable compounds refer to the fraction of nutrients that are both digested and absorbed, a process largely dependent on bioaccessibility. Therefore, FM is a central parameter in evaluating the health impact of food and represents a promising approach for meeting the nutritional needs of various population groups through the development or reformulation of food products (Aguilera et al., 2019).

Among staple foods, cereal-based products represent a significant source of energy worldwide. Nutritional recommendation suggests that 45–60% of daily energy intake should come from carbohydrates, with a particular focus on foods with a low glycaemic index (GI). Epidemiological studies suggest that diets rich in foods that elicit lower postprandial glycaemic responses can play a crucial role in reducing the risk of developing chronic diseases, such as type 2 diabetes (Livesey et al., 2013). One parameter used to evaluate carbohydrate quality is starch digestibility, particularly slowly digestible starch, for which a cause-and-effect relationship with reduced postprandial glycemia has been established (EFSA, 2011). Moreover, intervention studies will be conducted to evaluate the effect of cereal-based products on post-prandial glycaemic response. Finally, the use of alternative

ingredients from circular economy will be explored as a promising way to increase the quality of starch-based products in terms of micronutrients and bioactive compounds from a sustainable perspective.
Therefore, the PhD project aims to evaluate the nutritional quality of starchy foods commonly consumed in Italy and specifically developed for a targeted population. Starch and micronutrient bioaccessibility will be evaluated using validated in vitro digestion methods, to support reformulation strategies to improve overall dietary quality.

2. PhD Thesis Objectives and Milestones

This PhD thesis project can be divided into the following activities according to the Gantt diagram given in Table 2:

A1) **Estimation of SDS intake of the Italian population** through the selection of commonly consumed cereal-based products sold on the Italian market (A1.1), quantification of carbohydrate digestibility with in vitro digestion method (A1.2) and evaluation of SDS intake of different target of population according to recent consumption data (A1.3).

A2) **Evaluation of FM effect on cereal-based products,** through the study of macro and micronutrient accessibility of two types of matrices (A2.1) and reformulation of these products to enhance their nutrient content and bioaccessibility in differenttargetst of population (A2.2).

A3) **Evaluation of in vivo effect of cereal based-products,** after consumption of different reformulations (A3.1).

A4) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 2 *Gantt diagram for this PhD project.*

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
A1)	***Estimate the SDS intake of Italian population***																																				
	1) Selection of prepacked food products																																				
	2) Analysis of carbohydrate digestibility																																				
	3) Selection of recent consumption data from FoodEx2 database and analysis of SDS intake based on the developed database																																				
A2)	***Evaluation of FM effect on cereal-based products***																																				
	1) analysis of FM in macro and micronutrient accessibility of two different food products																																				
	2) Reformulating the analyzed foods to enhance their nutrient content and bioaccessibility in different targets of population																																				
A3)	***Evaluation of in vivo effect of cereal-based products***																																				
	1) In vivo effect on post-prandial glycaemic response of different cerea-based products																																				
A4)	***Thesis and Paper Preparation***																																				

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Set up of a probiotic and germinated brown rice (GBR) formulation for health benefits (P-I-105)

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The aim of this PhD project is to develop a symbiotic formulation for human health by combining germinated brown rice (GBR) and probiotics belonging to the genera *Lactobacillus* and *Bifidobacterium*. Germinated brown rice (GBR) presents a high nutritional profile and its cultivation is environmentally sustainable. Among the bioactive compounds, γ-aminobutyric acid (GABA) and γ-oryzanol are valuable for general health. Due to its insoluble dietary fibers and minerals, GBR stands out as a valuable prebiotic feature to promote beneficial intestinal microbiota. The synergistic combination of GBR and probiotics could represent a promising strategy for maintaining good overall health, paving the way for new personalized nutritional strategies.

Messa a punto di una formulazione a base di riso integrale germogliato (GBR) e probiotici per la salute umana

L'obiettivo di questo progetto di dottorato è formulare un simbiotico per la salute umana combinando riso integrale germogliato (GBR) e probiotici appartenenti ai generi *Lactobacillus* e *Bifidobacterium*. A differenza del riso bianco, che sfrutta notevoli risorse di energia diminuendo anche il contenuto nutrizionale, la coltivazione del riso integrale è ecosostenibile e mantiene un elevato profilo chimico-nutrizionale. Inoltre, la germinazione dei chicchi in acqua aumenta la produzione di molecole bioattive con comprovati effetti benefici. Tra queste, l'acido γ-amminobutirrico (GABA) e il γ-orizanolo sono particolarmente preziosi per la salute generale. Grazie alle sue fibre alimentari insolubili e ai minerali, il GBR si distingue come un prezioso prebiotico in grado di promuovere la crescita dei batteri lattici (LAB), noti per i loro effetti benefici sul microbiota intestinale. La combinazione sinergica di GBR e probiotici potrebbe rappresentare una strategia vincente per il mantenimento di una buona salute generale, aprendo la strada a nuove strategie nutrizionali personalizzate nel rispetto della sostenibilità ambientale.

1. State-of-the-Art

The recent heightened demand for integrative and natural approaches to health maintenance has brought nutraceuticals to the forefront of research. Consumers are increasingly seeking alternative ways to support their well-being through targeted dietary strategies which involve the use of minimally processed and/or whole foods rich in bioactive compounds capable of providing physiological benefits. Among these, brown rice (BR) has proven to be a particularly promising cereal-derived prebiotic due to its valuable nutritional profile. Unlike white rice (WR), which undergoes intensive processing which removes most of its beneficial ingredients, BR retains its bran and germ. Therefore, its content of insoluble dietary fiber, essential minerals, vitamins, and antioxidants is significantly higher than WR. Among the bioactive compounds of interest, ferulic acid, phytic acid, tocotrienols, γ-aminobutyric acid (GABA) and γ-oryzanol are associated with a variety of health benefits (Pino *et al.*, 2022). Indeed, regular consumption of BR has been linked to the prevention of chronic diseases (e.g., obesity, osteoporosis, certain types of cancer, type 2 diabetes, and neurodegenerative disorders), due to its ability to modulate glucose metabolism, to improve insulin sensitivity, and to regulate lipid profiles (Bhoyar *et al.*, 2025). From an environmental perspective, BR represents a more sustainable alternative to WR. Its processing requires significantly less water and energy. Furthermore, the generation of agro-industrial by-products is considerably fewer and avoids the intensive refining steps that contribute to pollution. For this PhD project, BR will be kindly provided by the Italian company Mister Biofood s.r.l. (Crespadoro, Italy), known for its organic farming methods and commitment to environmental stewardship.

The germination of BR grains in water improves nutritional value. In addition, GBR provides a high source of fiber that would not be digested in the intestine (Hutkins *et al.*, 2025) promoting the selective growth of beneficial microorganisms and inhibit the harmful.

LABs, especially the strains belonging to the genera *Lactobacillus* and *Bifidobacterium*, play a pivotal role in gut microbiota. These beneficial bacteria contribute to the maintenance of the intestinal barrier, stimulate immune responses, and possess anti-inflammatory and antimicrobial properties. During the fermentation process, LABs metabolize the indigestible fibers and phenolic compounds of GBR. This enzymatic activity results in the breakdown of complex substrates into smaller and bioavailable molecules, which lead to the production of short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate. SCFAs have been widely recognized for their positive effects on gut health, on metabolic regulation, and on mood and cognitive function via the “gut-brain axis” (Meerschaert & Chiu, 2025).

The aim of this PhD research proposal is to develop a symbiotic formulation based on GBR and selected probiotic strains for human well-being. The project involves the set-up of the symbiotic formulation GBR and LABs to enhance the bioavailability of nutrients, and to impart targeted health advantages. In addition, this research study

satisfies key sustainability objectives through the encouragement of the consumption of cereal-derived foods. Using a robust and multidisciplinary approach, this PhD project aims to develop a novel functional nutraceutical to improve gut health, lowering environmental footprint and creating a more sustainable agri-food system.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 2:

A1) **Literature review:** This phase will focus on studying and exploring the cultivation methods of various cereals, particularly brown rice, as well as its nutritional profile and intrinsic properties. The main methods for increasing the content of bioactive compounds will be studied. In addition, the link among nutrition, gut microbiota, and its interaction with overall well-being will be explored in depth.

A2) **Screening and selection of promising lactic acid bacteria strains with probiotic features:** strains belonging to the *Lactobacillus* and *Bifidobacterium* genera will be screened for their probiotic characteristics, as well as for their antimicrobial activity against the main gastrointestinal pathogens. In addition, their survival in a simulated gastrointestinal tract (GIT) will be *in vitro* evaluated, and their synergy with GBR will be assessed;

A3) **Setting up of a probiotic blend and study of interactions among the selected bacterial strains:** once the most promising lactic bacteria have been selected, several combinations will be tested in order to obtain the most promising blend. Various antagonism tests will be carried out among the different strains;

A4) **Development of the symbiotic formulation:** the GBR will be lyophilized and supplemented with the selected probiotic blend. Various encapsulation methods will be screened. Some of the methods may include spray drying method, freeze drying, extrusion, emulsion encapsulation, spray-freeze drying and nano-encapsulation. The stability of the experimental supplement will be evaluated during the shelf-life. Different types of coatings will be screened to choose the best one (e. g. plant cellulose, alginates, pectin, starch-based, chitosan etc);

A5) ***In vitro* evaluation of the functional biological properties of the formulated supplement through the SHIME system:** the formulated symbiotic will be *in vitro* tested using the SHIME system, by reproducing the human gastrointestinal tract and evaluating the survival of the probiotics in the supplement formulation**;**

A6) **Data management and dissemination, thesis and paper preparation**: writing of the PhD thesis, scientific papers and oral and/or poster communications.

Table 2 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Literature review***																								
A2)	***Screening and selection of LABs***																								
	1) Safety tests																								
	2) Biofilm formation																								
	3) Antimicrobial activity																								
	4) Survival during the simulated GIT																								
A3)	***Set up of a probiotic blend***																								
A4)	***Development of the symbiotic formulation***																								
	1) Chemical characterization of BR																								
	2) Germination of BR grains																								
	3) Evaluation of synergy between LABS and GBR																								
	4) Evaluation of encapsulation methods																								
A5)	***In vitro experiment with SHIME system***																								
A6)	***Data management and dissemination, thesis and paper preparation***																								

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Exploiting the microbiome of traditional and sustainable plant-based fermented foods with pro- and post-biotic properties (P-I-106)

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This PhD research project aims to characterize the microbiome of traditional plant-based fermented foods and exploit its diversity for the design of novel pro- and postbiotic sustainable fermented foods.

Caratterizzazione del microbioma di alimenti fermentati tradizionali e vegetali con effetto pro- e post-biotico

Questo progetto di dottorato ha l'obiettivo di caratterizzare il microbioma di alimenti fermentati tradizionali vegetali e di disegnare nuovi prodotti fermentati sostenibili con attività pro- e postbiotica.

1. State-of-the-Art

Fermentation is among the earliest and most cost-effective techniques for food production and preservation. Beyond its economic and historical significance, fermentation naturally contributes to reducing the bulk of materials for easier transportation, eliminating harmful substances, improving the nutritional quality and visual appeal of foods, decreasing cooking energy demands, and enhancing overall food safety [1]. Due to these characteristics, fermented foods (FFs) have always been an interesting field to be explored.
Despite they have been studied for decades, all the potential of these foods, and the communities that live within them, is still unlocked. FFs may be an underexplored source of microbes able to produce bioactive compounds, such as neurotransmitters and vitamins, which may influence health. Moreover, nowadays there is an urgent need to develop valid alternatives to animal origin products, to improve the sustainability of the food systems, as well as to counteract the spreading of chronic diseases. It also important to underline the new growing interest of the consumer in alternative foods, related to higher consciousness about the rapid environmental deterioration over the past few decades, which is boosting the industries to develop more sustainable products.
As people become increasingly aware of the health benefits associated with plant-based foods, there has been a notable shift in eating habits. This change not only promotes individual well-being but also contributes to improved food sustainability and reduced environmental impact. As a result of these trends, both the market and academic interest in this area are evolving rapidly [2]. However, the consumption of plant-based products (PBP) is still limited, due to the poor nutritional content (e.g., lack of certain vitamins and amino acids) and sensorial traits. Fermentation of plant-based materials (legumes, cereals) may help enhance nutritional value and masking off-flavours.
The fermentation process typically involves a diverse range of microorganisms—including yeasts, bacteria, and fungi—functioning individually or in mixed cultures, either simultaneously or sequentially, with shifts in the dominant microbial populations over time. Among the most interesting taxa for cereal fermentation, there are *Leuconostoc*, *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Bifidobacterium* and *Bacillus*.
According to different studies fermentation, beyond enhancing sensory qualities with the release of various volatile compounds that are perceived by consumers as aromas, has been shown to markedly improve the nutritional value of PBPs. Indeed, it enriches the content of essential amino acids, vitamins, and bioavailable minerals (reducing and degrading antinutrients, such as phytates, that reduce minerals absorption), thereby increasing both the digestibility and nutritional appeal of the foods. An additional enzymatic benefit of fermentation is the reduction of oligosaccharides—compounds commonly found in beans and vegetables—that are known to cause digestive discomfort, such as flatulence.
Cereals and legumes generally provide a favorable environment for microbial growth and have even been proposed as prebiotic substrates. This is largely due to their high content of indigestible components such as dietary fiber, specific peptides, proteins, and lipids that promote the proliferation of beneficial bacteria (FAO/WHO, 2001). Among these, inulin and various oligosaccharides—naturally found in fruits, vegetables, and cereals—have received considerable scientific attention for their demonstrated ability to support microbial growth [2].
Traditional fermented foods consumed in the Mediterranean area, such as table olives, are a reservoir of potentially beneficial microbes, that can be used for the design of synthetic consortia useful for driving targeted fermentations and developing innovative, nutrient and sensorially accepted PBPs. Therefore, the aim of this PhD project is to characterize the microbiome of traditional FFs from the Mediterranean area, to identify microbial consortia and their metabolic pathways involved in the production of beneficial metabolites (e.g., vitamins, particularly B12, niacin and folate). Based on these results, ad-hoc synthetic communities will be designed to develop novel PBPs.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Literature review and meta-analysis of fermented food microbiome.** Traditional fermented foods from the Mediterranean area (e.g. table olives) will be characterized by shotgun metagenomics, to identify microbiome consortia involved in the production of beneficial metabolites (e.g., vitamins) and with relevant genomic traits (e.g., phytase activity, flavour and exopolysaccharides production).

A2) **Targeted strain isolation, characterization and synthetic communities' development.** Based on A1 results, Lactic Acid Bacteria strains will be isolated, phenotypically characterized to confirm the interesting activities, and mixed in ad hoc designed consortia.

A3) **Development of plant-based fermented foods with improved nutritional and sensorial properties. Legumes (**chickpea, peas) and cereals (rice, sorghum) will be mixed in different formulations, and fermentation will be carried out using microbial consortia designed to develop novel PBPs (e.g., yoghurt-like). PBPs will be analysed for vitamin content, nutrient composition and sensorial profiles.

A4) **SHIME trial and gut metagenome analysis.** The most promising PBPs will be tested in a mSHIME model to evaluate the impact on the gut microbiome and metabolome.

A5) **Data analysis.**

A6) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	**Literature review and meta-analysis**																								
A2)	***Strain isolation and characterization***																								
A3)	***Formulation of plant-based fermented foods***																								
A4)	***SHIME trial and gut metagenome analysis***																								
A5)	***Data analysis***																								
A6)	***Thesis and Paper Preparation***																								

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Study of sourdoughs microbial communities to develop novel biotechnological strategies for the food industry (P-I-107)

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This PhD thesis research project aims to investigate the complex microbial ecosystem of sourdough using advanced omics technologies to isolate and identify microorganisms with nutritional and functional properties and design synthetical microbial consortia to evaluate their effectiveness in industrial baking applications.

Studio delle comunità microbiche del lievito naturale per lo sviluppo di nuovi protocolli biotecnologici applicabili all'industria alimentare

Questo progetto di tesi di dottorato mira ad investigare il complesso ecosistema microbico presente nel lievito naturale, utilizzando le nuove tecniche -omiche per isolare e identificare microrgansimi aventi proprietà nutrizionali e funzionali per poi assemblarli in comunità microbiche sintetiche e testarne la loro applicabilità a livello industriale.

1. State-of-the-Art

Sourdough fermentation is a traditional method used in breadmaking to enhance flavor, texture, shelf life, and several nutritional attributes of leavened baked goods (Gobbetti *et al*., 2019). Sourdough, one of the oldest examples of natural starters, is defined as a mixture of flour and water, spontaneously fermented by lactic acid bacteria (LAB) and yeast, having acidification and leavening capacities (Arora *et al*., 2021). Sourdough can be classified in several types based on the inoculum used to initiate the fermentation, as well as its process technology. The latter divides sourdoughs into four types: 0, I, II and III (De Vuyst *et al*., 2023). Type 0 sourdough, often called biga, is a flour-water mixture fermented for a limited amount of time with baker's yeasts, nevertheless, during the process LAB species, which are naturally present in the flour and as contaminants of the baker's yeast can grow (Reale *et al*. 2013). Type I is a traditional sourdough, spontaneously fermented at 20-30°C by multiple backsloppings, which lead to a stable and active community of microorganisms, whereas in type II sourdough, commonly used at industry level, starter cultures are added to the mixture and used for a single step fermentation under controlled conditions. Type III is a dehydrated type II-sourdough, yet this process compromises the viability of the microbiota due to the high temperatures employed (De Vuyst *et al*., 2023).

Sourdough microbiota is mainly constituted by LAB and yeasts. The establishment of a mature and stable sourdough primary relies on sourdough propagation, a procedure that involves the addition of sourdough to fresh flour and water thus providing an ecological environment for the persistence of sourdough starters (De Vuyst *et al*., 2023. Indeed, the frequent backsloppings and the acidic environment generate a complex microbiome of interacting players (dominants, subdominants and satellites), and the structure and metabolic network of this microbiome are constantly influenced by consistent biotic pressure and spatiotemporal changes (Calabrese *et al*., 2022). As a result, in most cases, sourdough is prone to instability such that the metabolic networks are constantly threatened. Metacommunity interplay is, therefore, critical in maintaining stability as a precondition for ensuring sourdough functionality.

Nowadays, industrials sourdoughs are extremely simplified (composed of one or two strains) and although this procedure ensures standardized production, it also reduces sourdough biodiversity thus possibly limiting its full potential. Recent studies (Calabrese *et al*., 2022) have shown the great complexity of the sourdough microbial community, where subdominant players cooperate synergistically with dominant species by expressing accessory genes that are critical for key metabolic pathways and, although not encoding key functions, satellite members may be metabolically active and equipped with unique genes and enzymes.

A similar approach will be used in this study, but with few implementations which will allow to move from the current state of the art to practical industrial applications. Given the many modern applications of sourdough biotechnology, the characterization of dominant and subdominant strains for their main metabolic traits, prior to the assembly of the synthetic community, will help defining the specific contribution of individual strains and thus the selection of the best combination of microorganisms ideal for the considered protocol.

Finally, the assembly of a synthetic microbial community and its application on a pilot-scale (30L fermenter) is a concept unique in its kind. Indeed, while so far few research studies have studied the possibility to reconstruct a sourdough community based on its original microbial consortium, none of them optimized the process for key nutritional, functional and technological advantages, nor included the possibility of upscaling the process in a semi-liquid environment, which would allow complete industrial automation.

2. PhD Thesis Objectives and Milestones

Based on the above considerations, this research project aims at:

1. Studying the sourdough microbial community using new -omic techniques, with a focus on dominant and sub-dominant populations.

2. Selecting microorganisms with metabolic aptitudes affecting *i*) nutritional (proteolysis and protein digestibility, reduction of the glycemic index, degradation of anti-nutritional factors), *ii*) functional (synthesis of functional compounds), and *iii*) technological (*e.g.*, exopolysaccharides synthesis) parameters.

3. Assembling synthetic microbial communities, based on specific traits and studying their performances under bakery industry applications.

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

1. **Sampling** of 5 type II-sourdoughs (from the 5 different establishments of the company Grande Impero (Glamour Food S.p.A.).
2. **Study of the sourdoughs microbial community using culture-dependent** (e.g., isolation and identification of lactic acid bacteria and yeasts, with particular attention to including dominant, sub-dominant, and satellite species) flours used in the production facility will also be analyzed, both in their native form and after enrichment, to better isolate microorganisms occurring at low cell densities that may nonetheless contribute to the microbial and biochemical dynamics of the sourdough ecosystem, **and independent approaches** (total DNA extraction and high-throughput sequencing of 16S and 26S rRNA genes to comprehensively characterize the microbiota).
3. **Characterization of the isolates** by evaluating their main metabolic traits (acidification kinetics fermentative profile, proteolytic, phytase and beta-glucosidase activities, antimicrobial activity, EPS synthesis, etc.).
4. **Assembling synthetic communities**, to produce new generation type II-sourdoughs. The assembly will involve the use of dominant, sub-dominant, and satellite species, taking into account their original abundance in the isolation matrix as well as their specific metabolic and technological traits. The robustness of the synthetic community will be assessed during propagation procedures. and the metabolome of natural sourdoughs will be analyzed using chromatographic techniques and NMR spectroscopy, in order to obtain a comprehensive overview of the community's contribution to the sourdough system, by comparing it with the original sourdough.
5. **Scaling-up**, first on a pilot scale and then on an industrial level, of the biotechnological protocols for the production of improved baked goods.
6. **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1. Gantt diagram for this PhD thesis project.

Activity/month	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
Activity 1																		
Activity 2																		
Activity 3																		
Activity 4																		
Activity 5																		
Activity 6																		

3. References

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Pomegranate (*Punica granatum* L.) by-products extracts with high antioxidant and antimicrobial action as food additives in a circular economy and environmental sustainability perspective – "From waste to wealth" objective (P-I-108)

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This PhD project investigates pomegranate (*Punica granatum* L.) peels as a sustainable source of antioxidant and antimicrobial compounds. Leveraging their abundance of phenolics, these bioactives are extracted using green methods: Enzyme- and Ultrasound-Assisted Extractions. Extracts are characterized and tested against spoilage and pathogenic bacteria, with a particular focus on improving their antimicrobial action. Application as edible-coating and safety, including interaction with gut microbiota and urolithin formation, are assessed. The final goal is to develop a natural food additive that improves shelf life, in line with environment sustainability and growing demand for health-oriented products.

Estratti di sottoprodotti di melograno (*Punica granatum* L.) ad alta azione antiossidante e antimicrobica come additivi alimentari in un'ottica di economia circolare e sostenibilità ambientale - Obiettivo "Da rifiuto a ricchezza"

Il progetto di dottorato mira a studiare le bucce di melograno (*Punica granatum* L.) come fonte sostenibile di antiossidanti e antimicrobici. Tali composti bioattivi vengono estratti utilizzando metodi ecologici con enzimi e/o ultrasuoni. Gli estratti sono caratterizzati e testati contro batteri alteranti e patogeni, con l'obiettivo di migliorarne l'efficacia antimicrobica. Vengono valutate l'applicazione come rivestimento edibile e la sicurezza, l'interazione con il microbiota intestinale e la produzione di urolitine. L'obiettivo finale è sviluppare un additivo alimentare naturale che migliori la conservabilità, in linea con i requisiti di sostenibilità ambientale e la crescente domanda di prodotti orientati alla salute.

1. State-of-the-Art

Pomegranate (*Punica granatum* L.) is cultivated globally with over 500 cultivars and a production of around 1.5 million tons per year. Pomegranate peel, which makes up about 50% of the fruit's weight, contains high levels of phenolic compounds, particularly hydrolysable tannins like punicalagin, ellagic acid, and anthocyanins (Siddiqui et al., 2024). The concentration of these compounds varies depending on cultivar, growth conditions, and ripeness. Punicalagin is the most abundant compound in the peel (28–104 mg/g) and is known for its antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (Kharchoufi et al., 2018). Other bioactives include punicalin, catechin, gallic acid, epigallocatechin, and kaempferol derivatives. These compounds have been found in significant amounts in pomegranate by-products, especially peels (Man et al., 2022). Green extraction methods like Ultrasound-Assisted Extraction (UAE) and Enzyme-Assisted Extraction (EAE), utilized to extract these bioactive compounds from different matrixes, are replacing traditional techniques due to their lower environmental impact, shorter processing times, and higher yields using food-grade solvents such as ethanol and water. EAE, using enzymes like pectinase, protease, or cellulase, facilitates the release of compounds by degrading plant cell walls, often serving as a pre-treatment to UAE (Cano-Lamadrid et al., 2022). UAE uses ultrasound waves to disrupt cell structures and enhance mass transfer, improving polyphenol extraction (Faulisi et al., 2024). Pomegranate peel extracts (PPEs) have shown strong antimicrobial effects especially against Gram-positive bacteria, while Gram-negative bacteria are more resistant due to their outer membrane structure. PPEs also demonstrate antifungal activity against different mycotoxin-produce strains (Chen et al., 2020). The effectiveness depends on the solvent, with aqueous extracts often outperforming ethanolic ones (Sweidan et al., 2023). Edible coatings in active packaging are emerging as sustainable food preservation tools. Made from different biopolymers, such as chitosan, they can carry bioactives for gradual release during storage, exploiting their antimicrobial and antioxidant activities (Martins et al., 2024). In recent years, growing attention has been given to the interaction between polyphenols and the gut microbiota. Non-extractable polyphenols, such as ellagitannins, are metabolized by gut microbiota into low-molecular-weight phenolics, such as ellagic acid, promoting the growth of beneficial bacteria. Gut microbiota, in some cases, facilitates the hydrolysis and metabolism of ellagic acid into urolithins. Urolithins have shown promise *in vitro* with potential antioxidant, estrogenic, anti-inflammatory, and anticarcinogenic activities (García-Villalba et al., 2022). The metabolic transformation of ellagic acid varies among individuals; three distinct urolithin metabotypes have been identified and may serve as biomarkers of microbiota health (Tomás-Barberán et al., 2014; García-Villalba et al., 2017).

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) The **bibliography research** will begin and follow all stages of the project to ensure that the best extraction methods for bioactive compounds are used, the best edible coating is formulated, and the most effective *in vitro* digestion method is applied to study how the microbial population varies following the ingestion of the extract.
A2) **Optimization of the extracts (A2.1)** obtained from pomegranate peels (PP) using the two extraction methods (EAE and UAE) followed by **chemical-physical characterisation (A2.2)** of the extratcs obtained.
A3) **Evaluation of antimicrobial activity and spectrum**.
A4) **Comparison between the extracts** to determine which one exhibits better antioxidant and antimicrobial activity.
A5) **Development of an edible coating enriched with the PPE extracts (A5.1)** at different concentrations and **application of coating on a selected food item (A5.2)** based on the subsequent target microorganisms to be tested.
A6) **Study of the shelf life, including microbiological evaluation.**
A7) **Optimization of *in vitro* digestion** to evaluate the effect that PPE extracts have on the composition of the intestinal microbiota.
A8) **Writing of the final thesis, scientific articles, oral presentations and project posters** which will take place during the analysis period.

Table 1. Gantt diagram for this PhD thesis project.

Activity		**First year**											**Second year**												**Third year**											
n.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A1	***Bibliographic research***																																				
A2	***A2.1) Recovery of bioactive compounds from PP A2.2) Chemical-physical characterisation of extracts***																																				
A3	***Antimicrobial assays***																																				
A4	***Comparison between extracts***																																				
A5	***A5.1) Formulation of an edible coating added with PPE A5.2) Application of edible coating in food formulation***																																				
A6	***Shelf life and microbiological evaluation***																																				
A7	***In vitro digestion***																																				
A8	***Thesis and scientific papers preparation***																																				

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Biotechnological valorization of legumes for the development of high-value added ingredients to be used in plant-based food formulations (P-I-109)

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Tutor: Prof. Francesca Patrignani

This PhD research project is aimed at developing and optimizing biotechnological strategies—such as microbial fermentation and non-thermal processing techniques—for the valorization of legumes as functional ingredients in plant-based food systems. Experimental procedures will be conducted at both laboratory and pilot scales to identify optimal formulations and processing conditions for the production of high-protein, nutritionally enhanced, and organoleptically acceptable legume-based dairy analogues, providing a foundation for their industrial-scale application.

Valorizzazione biotecnologica dei legumi per lo sviluppo di ingredienti ad alto valore aggiunto da utilizzare in formulazioni alimentari a base vegetale

Questo progetto di dottorato di ricerca è finalizzato allo sviluppo e all'ottimizzazione di strategie biotecnologiche, come la fermentazione microbica e le tecniche di lavorazione non termica, per la valorizzazione dei legumi come ingredienti funzionali nei sistemi alimentari a base vegetale. Saranno condotte procedure sperimentali sia su scala di laboratorio che pilota per identificare formulazioni e condizioni di lavorazione ottimali per la produzione di analoghi lattiero-caseari a base di legumi ad alto contenuto proteico, nutrizionalmente migliorati e organoletticamente accettabili, fornendo le basi per la loro applicazione su scala industriale.

1. State-of-the-Art

In recent years, increasing consumer awareness regarding environmental sustainability, nutrition, and health has driven a significant shift toward plant-based diets. This includes the growing popularity of vegan, vegetarian, and flexitarian dietary patterns (Cramer et al., 2017; Sabaté & Soret, 2014; Sottile, 2023). In parallel, global population growth and the need to adopt more sustainable diets will significantly increase the demand for alternative protein sources by 2030 (FAO, 2021). The food industry is therefore challenged to provide sustainable, nutritious, and palatable alternatives to animal-based products. Legumes represent a promising solution due to their high protein content, but they are also associated with several drawbacks, including the presence of undesirable compounds (e.g., aldehydes responsible for grassy notes), fermentable oligosaccharides (like raffinose), and antinutritional factors (such as phytates, which impair mineral absorption) (Shi et al., 2021; Coda et al., 2015). To overcome these issues, biotechnological approaches involving safe microbial strains (GRAS status) have gained attention for their ability to enhance both nutritional and sensory properties of legume-based matrices. For instance, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Bacillus subtilis* have been shown to increase antioxidant levels in red beans (Jhan et al., 2015), while *Lactiplantibacillus plantarum* has been used to mitigate off-flavors and reduce antinutritional compounds such as trypsin inhibitors and tannins (Coda et al., 2015; Shi et al., 2021).Additionally, non-thermal technologies like High-Pressure Processing (HPP) are being explored for their potential to improve microbiological safety, structural properties, and functional stability of plant-based ingredients (Fasolin et al., 2019; Tabilo-Munizaga et al., 2019). When applied to legume proteins, HPP can also promote enzymatic hydrolysis and the release of bioactive peptides while maintaining food safety (Seyed et al., 2021). These technological and microbiological strategies form the foundation of my PhD research, which aims to develop legume-based “cheese analogues” by combining fermented protein matrices with different lipid sources and processing methods (e.g., pressing, drainage). The goal is to create innovative plant-based products that are nutritionally valuable, organoleptically acceptable, and technologically scalable. Building on this background, the aim of this PhD research project is to develop innovative, high-protein plant-based dairy alternatives through the biotechnological enhancement of legumes. The research will focus on the formulation of legume-based matrices fermented with selected microbial strains, combined with different types of plant fats, and subjected to various draining and pressing techniques. The ultimate goal is to obtain a nutritionally valid and sensorially acceptable alternative food product, using both fermentation and innovative processing technologies to valorize legumes. The research activities are structured into several phases: a comprehensive review of legume substrates and microbial strains already explored in the literature; the selection and testing of different processing methods to optimize texture and sensory quality; the characterization of fermented products through microbiological, nutritional, and rheological analyses; shelf-life studies and pilot-scale applications to evaluate industrial feasibility; and finally, the dissemination of results through thesis writing, scientific publications, and conference presentations. These phases are distributed across the three years of the doctoral program, following a structured timeline to ensure continuous progress and validation of results.

2. PhD Thesis Objectives and Milestones

This research project aims to evaluate and develop high-protein plant-based alternatives to dairy products, using

legumes as the raw material in combination with biotechnological processes and non-thermal technologies. The PhD thesis project can be divided into the following activities, summarized in the Gantt chart shown in Table 1:
A1**) Literature review** on legume matrices and microbial strains used in other studies on these substrates.
A2) **Evaluation of different sample preparation methods** in order to identify the procedure that allows the creation of varied textures and products by modifying formulations and growth conditions.
A3) **Analysis of the samples using various analytical techniques** and statistical processing of the obtained data. Data analysis will enable the observation of changes induced by fermentation on the matrix, both in compositional terms (quantitative and qualitative) and structural aspects.
A4) **Shelf life studies and industrial-scale applications**; this includes conducting a time-based study of the produced items and evaluating the scale-up of the process.
A5) **Writing and publication of the PhD thesis, posters, scientific articles, and oral presentations.**

Table 1 Gantt diagram for this PhD thesis project.

Attività	Mese	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Bibliographic research***																		
	1) Study of legume matrices and related research																		
	2) Evaluation of microbial strains applied to similar products																		
A2)	***Evaluation of different sample preparation systems***																		
	1) Trials on various matrices and different microbial strains																		
	2)Combinations of vegetable fats and different draining methods																		
A3)	***Sample analysis using various analytical techniques and data processing***																		
	1) Microbiological analyses																		
	2)Other analyses (proteins, antioxidants, antinutritional compounds...)																		
	3) Rheological analyses																		
A4)	***Shelf-life study and industrial-level application***																		
	1)Study and preparation of samples																		
	2) Monitoring and data collection																		
	3) Scale-up trials																		
A5)	***Writing and dissemination of the PhD thesis, posters, scientific articles, and oral presentations***																		

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Development of Functional Dairy Products made from Buffalo Milk: The role of Probiotics, Prebiotics and Production Technology (P-I-110)

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This PhD project aims to develop functional dairy products from Buffalo milk. The main objective is to formulate two probiotic and possibly prebiotic milk-based products: a Buffalo milk ice cream and a Buffalo milk spreadable fresh cheese, both enriched with green extracts from vegetable by-products. These functional products will be characterized by their nutritional value, microbial profile and sensory properties. Special attention will be given to the survival of probiotic strains throughout processing and storage, as well as to the stability of added functional compounds to ensure their long-term effectiveness.

Sviluppo di prodotti lattiero-caseari funzionali a base di latte di bufala: il ruolo di probiotici, prebiotici e tecnologia di produzione

Questo progetto di tesi di dottorato mira a sviluppare prodotti lattiero-caseari funzionali a base di latte di Bufala. L'obiettivo principale è quello di formulare due prodotti probiotici ed eventualmente prebiotici: un gelato e un formaggio fresco spalmabile di Bufala, entrambi arricchiti con estratti *green* derivanti da sottoprodotti di origine vegetale. Questi prodotti funzionali saranno caratterizzati in termini di profilo nutrizionale, composizione microbica e proprietà sensoriali. Particolare attenzione sarà data alla valutazione della sopravvivenza dei ceppi probiotici durante il processo di produzione e durante la conservazione del prodotto, oltre che alla stabilità dei composti funzionali aggiunti, in modo da garantirne l'efficacia a lungo termine.

1. State-of-the-Art

Milk and dairy products have been essential in human diets for centuries due to their high nutritional value. Among the various types of milk, buffalo milk stands out for its superior composition. Compared to cow's milk, it contains higher levels of protein and fat, more minerals such as calcium, phosphorus, and magnesium, and lower cholesterol, making it a valuable base for producing functional foods (Khan et al., 2022). In recent years, there has been growing interest from both the scientific community and the food industry in functional dairy products, foods that offer health benefits beyond basic nutrition. The dairy matrix is considered ideal for this purpose due to its stability and frequent consumption. Fortification strategies commonly involve adding vitamins (D, B12, folic acid), minerals (calcium, iron, zinc), omega-3 fatty acids, and natural antioxidants to enhance health properties (Aydemir and Altun, 2024; Savatinova and Ivanova, 2024). Additionally, there is a focus on producing low-fat and low-sugar formulations to combat diet-related diseases such as obesity and diabetes. Probiotics and prebiotics, respectively beneficial live microorganisms and non-digestible fibers that support gut health, are widely used to improve the functional value of dairy foods (Rashidinejad et al., 2022). At the same time, sustainability has become a key priority. As a result, new strategies are focusing on the use of agri-food by-products rich in bioactive compounds like polyphenols, fibers, vitamins, and antioxidants. This not only enhances the nutritional profile of foods but also supports food waste reduction and the circular economy (Eliopoulos et al., 2022). In light of these considerations, the proposed PhD project aligns with current trends in sustainable innovation within the food sector. While most functional dairy products currently on the market are fermented foods like yogurt, kefir, and cheeses made from cow's milk, this study aims to expand the range by using buffalo milk. The project focuses on creating two innovative products: functional Buffalo milk ice cream and spreadable fresh cheese, enriched with green extracts from food by-products and functionalized with probiotics and prebiotics. Different strains and extract types will be tested. A chemometric approach, using experimental design and multivariate statistical analysis, will be applied to optimize the formulation and processing. The final products will be assessed for their nutritional, microbiological, and sensory properties, with particular attention to probiotic viability, and the stability of their metabolites as well as added bioactive compounds during processing and storage. Shelf-life studies will complete the assessment. **Figure 1** shows the experimental plan of this PhD thesis project.



Figure 1: Experimental plan of this PhD thesis project.

2. PhD Thesis Objectives and Milestones

To achieve the objectives of the above-mentioned PhD project, the following activities will be carried out, according to the Gantt chart shown in **Table 1**:

A1) **Selection and characterization of raw materials and functional element,** through basic analysis of Buffalo milk (A1.1), extensive screening of probiotic strains (A.1.2), prebiotic fibers such as FOS, inulin and fruit fibers (A.1.3) and green extracts (A1.4) from different agri-food by-products (fennel waste, spent coffee grounds, citrus peels and tomato waste).

A2) **Preliminary formulation** will be carried out to develop a standard base recipe of Buffalo milk ice cream (A2.1) and spreadable fresh cheese (A2.2), which will serve as the control in the experimental design.

A3) **Use of an experimental design combined with a chemometric approach** will be employed to develop a mathematical model aimed at minimizing the number of trials required to optimize both the composition of the functional products (A3.1) (ice cream and spreadable cheese) and the processing parameters (A3.2).

A4) **Evaluation of the developed products** will include analysis of their nutritional profile (A4.1), probiotic viability (A4.2), prebiotic activity, and the stability of the incorporated bioactive compounds. The formulations will also be assessed for the potential formation of bioactive peptides, conjugated linoleic acid (CLA), antimicrobial compounds, and other health-promoting components. Functionality will be investigated through *in vitro* assays and, where appropriate, *in vivo* models (A4.3). Finally, a shelf-life study will be carried out, involving nutritional, microbiological, functional, and sensory characterisation over time (A4.4).

A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Selection of row matherials***																								
	1) Characterisation of Buffalo milk																								
	2) Screening of probiotic strains																								
	3) Choice of prebiotic fibers																								
	4) Selection of green extracts																								
A2)	***Product formulation***																								
	1) Buffalo Ice cream																								
	2) Buffalo spreadable fresh cheese																								
A3)	***Process and products optimization***																								
	1) Optimization of product composition																								
	2) Optimization of processing variables																								
A4)	***Nutritional and functional evaluation***																								
	1) Chemical and nutritional characterization																								
	2) Probiotics viability evaluation																								
	3) Functionality evaluation																								
	4) Study of nutritional, functional, microbiological and sensory properties during shelf-life																								
A5)	***Thesis and Paper Preparation***																								

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Baked goods with a high nutritional profile: from their design using alternative ingredients to the health-related properties (P-I-111)

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The project aims to develop innovative methods for bakery products with high nutritional quality, using flours from winemaking and fruit and vegetable by-products to promote circular economy. Prototypes will be created with partial replacement of conventional flour, enriched with fiber and bioactive compounds. The study focuses on interactions between wheat proteins and phenolic compounds, which are crucial for optimizing formulation. The goal is to obtain sustainable and nutritionally enhanced products, while exploring new opportunities for the integration of functional ingredients in the food sector.

Prodotti da forno con un alto profilo nutrizionale: dal loro design con ingredienti alternativi alle loro proprietà salutari

Il progetto sviluppa metodi innovativi per prodotti da forno di alta qualità nutrizionale, usando farine da sottoprodotti enologici e ortofrutticoli per promuovere l'economia circolare. Verranno creati dei prototipi con farina convenzionale parzialmente sostituita, ricco di fibre e composti bioattivi. Lo studio si focalizza sulle interazioni tra proteine del frumento e composti fenolici, cruciali per ottimizzare la formulazione. L'obiettivo è ottenere prodotti sostenibile e nutrizionalmente superiore, valorizzando ingredienti funzionali nel settore alimentare.

1. State-of-the-Art

In recent years, ready-to-eat food products have gained widespread popularity due to their convenience. However, they are often characterized by high levels of fat and sugar. Simultaneously, there is a growing demand for foods with enhanced nutritional value and health-promoting properties (Patel et al., 2017). Baked goods, widely consumed, represent ideal carriers for functional ingredients aimed at improving their nutritional profile (Rocchetti et al., 2021). Globally, approximately 1.3 billion tons of food are wasted annually, prompting the 2030 Agenda to promote sustainable practices, including the recovery and valorization of food by-products still rich in bioactive compounds (Melini et al., 2020).
Wheat-based bakery products are particularly suitable for functional enrichment and may contribute to the prevention of chronic diseases (Eswaran et al., 2013). Among functional compounds, polyphenols play a key role in protective effects against cardiovascular, metabolic, cancer-related, and neurodegenerative diseases (FAO, 2019). The incorporation of functional ingredients derived from plant-based waste into baked goods is therefore a promising strategy. Agro-industrial by-products represent valuable sources of fiber, vitamins, and polyphenols with potent antioxidant properties (Helkar et al., 2016). In particular, winemaking process involving *Vitis vinifera* L. generates substantial quantities of by-product (approximately 30% of grape weight), including pomace, stalks, and lees (Barba et al., 2016). Italy, as a world leader in wine production, generates large volumes of these residues, and their valorization aligns with the principles of circular economy (Provenzano et al., 2024). These by-products are rich in phenolic compounds such as resveratrol, catechin, and quercetin, which exert antioxidant and anti-inflammatory effects (Teixeira et al., 2014). Dietary fiber also plays a key role in metabolic health; however, its intake remains insufficient in traditional diets, suggesting the need for alternative sources (Tolve et al., 2020). In addition to their health benefits, polyphenols may interact with wheat proteins when incorporated into bakery products. These interactions, which occur through covalent and non-covalent bonds, can modify protein structure, potentially affecting dough properties, texture, and protein digestibility (Jia et al., 2016).

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in *Table 1*:

1. Bibliographic research: study of the literature concerning the use of wine lees and fruit and vegetable by-products for the production of functional bakery products.

2. Obtaining ingredients from by-products:

2.1 Physico-chemical and techno-functional characterization of the ingredients: study of proximate composition, antioxidant activity, total phenol content, polyphenol characterization by UHPLC-ESI-MS/MS analysis; study of the physical and chemical interactions of ingredients from by-products with wheat flours and evaluation of the main technological properties.

3. Formulation of products enriched with the alternative ingredients from by-products: the alternative

ingredients will be incorporated in the formulation of baked goods and then optimized the final products.

3.1 Physico-chemical and nutritional characterization of formulated products: study of proximate composition (moisture, carbohydrates, proteins, fats, fibers and ash) antioxidant activity (ABTS and DPPH assays), total phenol content (Folin-Ciocalteu assay), characterization of polyphenols by UHPLC-DAD analysis; bioaccessibility of polyphenols; determination of hardness and friability; consistency, viscosity; rheology assessments; determination of the predicted glycemic index; determination of minerals.

3.2 Study of shelf life

3.3 Consumer test

3.4 Biological tests for the evaluation of health properties: biological evaluation of the finished products through *in vitro* gastrointestinal digestion models on human colon cells

4. Selection of prototypes to be produced on a semi-industrial scale

5. **Writing of scientific articles and final thesis**

Table 1: *Gantt diagram for this PhD thesis project.*

Activity / Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1.	***Bibliographic research***																								
2.	***Obtaining ingredients from by-products***																								
	2.1 Physico-chemical and techno-functional characterization of ingredients																								
3.	***Formulation of products enriched with alternative ingredients from by-products***																								
	3.1 Physico-chemical and nutritional characterization of formulated products																								
	3.2 Study of shelf life																								
	3.3 Consumer test																								
	3.4 Biological tests for the evaluation of health properties																								
4.	***Selection of prototypes to be produced on a semi-industrial scale***																								
5.	***Writing scientific articles and final thesis***																								

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Study for the formulation of innovative durum wheat bakery products and extension of their shelf-life (P-I-112)

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This PhD research project aims to develop innovative bakery products based on durum wheat, with a dual focus on extending shelf-life and improving nutritional profiles. By incorporating alternative ingredients derived from nuts waste and other plant-based sources, and by optimizing strategies to extend shelf-life, experimental trials will be conducted at both laboratory and industrial scale to assess product quality, safety, and nutritional value over time. The objective is to define effective formulation and preservation strategies suitable for industrial application.

Studio per la formulazione di prodotti da forno innovativi da semola di grano duro ed incremento della loro shelf-life

Questo progetto di ricerca di dottorato ha l'obiettivo di sviluppare prodotti da forno innovativi a base di grano duro, con un duplice focus sul prolungamento della shelf-life e sul miglioramento del profilo nutrizionale. Attraverso l'inclusione di ingredienti alternativi provenienti da scarti di frutta secca e altre matrici vegetali e l'ottimizzazione di strategie per l'incremento della shelf-life, verranno condotte prove sperimentali su scala di laboratorio e industriale per valutare la qualità, la sicurezza e il valore nutrizionale dei prodotti nel tempo. Il fine è definire strategie di formulazione e conservazione efficaci e trasferibili a livello industriale.

1. State-of-the-Art

The bakery sector plays an important role both economically and in terms of nutritional impact. Bakery products are widely consumed across cultures and play an essential role in daily human diets. However, like most processed foods, they are subject to various forms of spoilage—physical, chemical, and microbiological—that affect their shelf-life (Smit et al., 2010). For these reasons, ingredients selection and the use of appropriate packaging becomes crucial for developing bakery products that are both nutritionally rich and shelf-life stable. To improve shelf-life and nutritional quality, recent research has focused on the strategic selection of ingredients and on the development of preservation strategies aimed at extending product stability. In this context, flours derived from plant-based foods could represent a promising option, especially in response to growing consumer demand for healthier and more sustainable food choices. Among these, flours derived from nuts waste are considered promising ingredients; these flours are naturally rich in bioactive compounds, including polyphenols, tocopherols, and essential fatty acids, contributing to antioxidant potential and improved nutritional profiles of bakery products (Pycia & Ivanišová, 2020; Burbano & Correa, 2021). Moreover, nuts processing industries produce large volumes of waste, often discarded due to aesthetic defects, which can be repurposed as functional flours, supporting circular economy goals and reducing food waste. Despite their nutritional benefits, these flours are also susceptible to rapid oxidation due to high levels of unsaturated fats. To counteract this and in response to the various shelf-life challenges of bakery products, strategies like adding natural antioxidants can be employed to achieve products that, in addition to having an interesting nutritional profile, also possess an extended shelf life. (Leyva-Porras et al., 2021). Additionally, advances in food packaging technologies—such as modified atmosphere packaging (MAP) and active packaging systems—can significantly extend shelf-life by controlling oxygen exposure and releasing antioxidant agents (Ahmed et al., 2017; Amin et al., 2022). This PhD research advances these recent developments by investigating innovative formulation and preservation strategies that combine alternative plant-based ingredients and advanced packaging solutions. The objective is to create bakery products that align with modern nutritional needs and offer enhanced shelf-life, supporting health, quality, and sustainability goals.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1. Bibliographic research

A2. Improvement of the nutritional profile of bakery products through the inclusion of selected ingredients

A2.1 Selection and characterization of ingredients from nuts waste.

This activity includes a comprehensive review of literature and the design of experimental plans, while also exploring the selection of alternative ingredients derived from nuts processing waste not meeting commercial standards, which represent a valuable opportunity for upcycling in food formulations. Subsequently these

alternative ingredients will be subjected to physicochemical and nutritional characterization to assess their potential for use in bakery products with particular attention to their technological performance, nutritional profile and sensory impact.

A2.2 Study and application of the selected ingredients in the formulation of bakery products.

This activity focuses on studying ingredient formulations using Design of Experiments (DoE) to achieve at least two nutritional claims on the label, such as "high in fiber" and "high in protein" and with particular attention to the lipidic profile. It then focuses on optimizing process parameters for obtaining bakery products, including mixing, leavening, and baking. The next step is the rheological characterization of doughs formulated with alternative ingredients. Finally, it includes the chemical, structural, and sensory characterization of the final products.

A3. Shelf-Life extension of bakery products through optimization strategies

A3.1 Study of impact of raw materials on product quality and shelf-life.

This activity involves studying the impact of raw material quality on oxidative stability and sensory properties. It then focuses on identifying and applying natural antioxidants to reduce lipid oxidation and improve product shelf-life.

A3.2 Implementation of packaging strategies to extend shelf-life.

This activity involves evaluating the effectiveness of modified atmosphere packaging (MAP) in slowing product deterioration. It then focuses on studying the interaction between packaging and the product to minimize the effects of moisture, oxidation, and sensory changes over time.

A4. Data analysis and scientific dissemination

A4.1 Data processing.

A4.2 Scientific publication writing.

Table 1*. Gantt diagram for this PhD thesis project.*

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1	***Bibliographic research***																								
A2	***Improvement of the nutritional profile***																								
	A2.1) Selection of alternative ingredients																								
	A2.2) Inclusion of selected alternative ingredients in the bakery product																								
A3	***Shelf-life extension of bakery products***																								
	A3.1) Study of impact of raw materials on product quality																								
	A3.2) Implementation of packaging strategies for shelf-life extension																								
A4	***Data analysis and scientific dissemination***																								
	A4.1) Data processing																								
	A4.2) Scientific publication writing																								

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Chemical characterization, photochemical behaviour and environmental fate of agricultural technological products (P-I-113)

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This PhD project aims to completely characterize novel commercial agricultural technological products (adjuvants, fertilizers and pesticides) produced using clean processes and based on active substances extracted from plants. After determining their chemical composition, their fate in food and environment will be fully unravelled by modelling their distribution and assessing their degradation paths and by-products.

Caratterizzazione chimica, dell'attività fotochimica e del destino ambientale di prodotti agricoli tecnologici

Questo progetto di dottorato mira a caratterizzare in maniera esaustiva nuovi prodotti agricoli tecnologici commerciali (adiuvanti, fertilizzanti e pesticidi) realizzati con processi puliti e basati su sostanze attive estratte dalle piante. Dopo averne determinato la composizione chimica, verrà studiato nel dettaglio il loro destino negli alimenti e nell'ambiente, modellandone la distribuzione e valutandone i meccanismi e i sottoprodotti di degradazione.

1. State-of-the-Art

Pesticides and fertilizers are nowadays extensively used to boost agricultural production in response to growing global food demand. They may therefore persist in the environment and eventually accumulate in the food chain, leading to potential toxic effects on non-target organisms, including humans (Rad *et al.*, 2022). To address these concerns, WHO and FAO (Food and Agriculture Organization of the United Nations) have set protocols such as the International Code of Conduct on Pesticide Management, to assess the risks derived from pesticides exposure to human health and set protection measures (World Health Organization, 2014). The increasing synthesis and use of these chemicals highlight the need for more sustainable production practices and a deeper understanding of their emissions and environmental impacts. Indeed, the effects of pesticides and fertilizers on the environment, as well as on food safety, have become an issue of great concern in the last decades.
After their application, these substances can be subjected to different transformation, transportation and accumulation pathways that strictly depend on chemical properties, bioavailability and on the environmental compartment. Degradation usually occurs following multiple routes that involve photolytic decomposition by solar light, hydrolysis, redox reactions and metabolic reactions, and which could lead either to less or more toxic by-products (Raffa and Chiampo, 2021). These substances and their degradation products may accumulate in soil, leach into groundwater, be transported by runoff to rivers or lakes, volatilize and be transported and deposited on other sites. On the other hand, after their application on growing crops they could enter the plant system through penetration into fruits and leaves, depending on physicochemical properties of these active substances, permeability of the cuticle and environmental conditions (Wang and Liu, 2006). To mitigate the negative impacts of synthetic agrochemicals, biodegradable and biocompatible materials have been recently introduced to produce safer and more environmentally friendly substances. This approach is based on the principles of sustainable agriculture, which aim to maximize crop growth while minimizing the environmental impacts on cultivations. The reduction of usual chemical products can be obtained through different approaches, one above all the use of active substances obtained from plants (e.g. essential oils, vitamins, antioxidants). These novel products can for example act as biopesticides, exploiting microorganisms that could prey to infesting species or the natural chemicals with pest-repelling properties synthesized by plants themselves (Abdollahdokht *et al.,* 2022). Another common application of these technologies is as biofertilizers, using different active components for improving the nutrients uptake by specific crops. These next-generation products are designed to be low in toxicity, biodegradable, and more selective in their action. Additionally, because they are naturally recognized by plant systems, they are often more readily absorbed and effective.

2. PhD Thesis Objectives and Milestones

Considering the current state of art and the purposes of this project, it is necessary to underline that the chemical characterization and the assessment of environmental impact of agricultural technological products require different levels of action and the exploitation of a comprehensive panel of analytical techniques. The work plan established to achieve the foreseen objectives is briefly described below supported by a Gantt diagram (Table 1):

1. **Complete assessment of products' chemical composition** using a combination of different analytical techniques, that include Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) for elemental analysis,

High Resolution-Liquid Chromatography Mass Spectrometry (HR-LCMS) for identifying the main organic constituents and Gas Chromatography-Mass Spectrometry (GC-MS) for assessing the composition of the volatile fraction.

2. **Assessment of the photochemical behaviour** by exposing the products to irradiation in order to simulate field conditions and have an insight into their stability and transformation under sunlight exposure.
3. **Application trials under controlled conditions** (e.g. in a climatic chamber where temperature, humidity, and light exposure are regulated) on edible crops such as rice and lettuce. These trials will help assess the influence of environmental factors on the distribution and fate of the products in food and the environment.
4. **Field application trials** in order compare results obtained under controlled conditions with those observed in real-world scenarios.
5. **Study of degradation pathways and by-products with ecotoxicological tests** using high resolution mass spectrometry coupled with MS-MS experiments to elucidate the molecular structure of such degradation products. Regarding the evaluation of toxicity, laboratory tests will be coupled with *in silico* tests to assess the impact of these substances on ecosystems and human health.
6. **Data analysis and elaboration** as well as writing scientific papers and oral and/or poster communications at conferences.

Table 1 Gantt diagram with all the planned activities of the PhD project.

Activities	**Semester 1**	**Semester 2**	**Semester 3**	**Semester 4**	**Semester 5**	**Semester 6**
1-Complete assessment of products' chemical composition						
2-Assessment of the photochemical behaviour						
3-Application trials under controlled conditions						
4-Application trials in field						
5-Study of degradation pathways and by-products with ecotoxicological tests						
6-Data analysis and elaboration						

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Animal-derived proteins in the confectionery industry: Knowledge, Applications, and Alternatives (P-I-114)

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This project aims to investigate milk proteins and their chemical, physical, and bio-functional properties within the confectionery industry. The study addresses a current gap in literature concerning the structure-function relationship between milk proteins and their application in confectionery products. Following the chemical-physical characterization of representative samples of commercial milk proteins, model systems will be developed to study the structure-function relationship in a controlled and rational manner. Based on the insights gained, various formulations will be tested and optimized to enhance both sensory and functional properties. Additionally, the development of new formulations with improved digestibility and bioactivity will be pursued.

Proteine di origine animale nell'industria dolciaria: conoscenze, applicazioni e alternative

Lo scopo di questo progetto di dottorato è quello di studiare le proteine del latte e le loro proprietà chimiche, fisiche e bio-funzionali per l'applicazione di queste nell'industria dolciaria. L'obiettivo principale è identificare la proteina o la combinazione di proteine più adatta per il loro impiego in specifici prodotti dolciari. Dopo la caratterizzazione dei campioni, tramite l'utilizzo di sistemi modello, verranno studiate in modo controllato e razionale la relazione tra struttura e funzione. Le conoscenze acquisite saranno poi applicate alla formulazione e all'ottimizzazione di prodotti dolciari, con l'obiettivo di migliorarne sia le proprietà sensoriali che quelle funzionali.

1. State-of-the-Art

According to the third annual 'State of Snacking' report released in 2021 (Mondelez International Public, 2022), consumers seek snacks that support well-being throughout the day. Snack choices are driven by health and nutritional needs. Common claims that can be found in snacks are "Rich in Vitamins", "Low in Sugar, Carbs, or Fat", "Boost Immunity", and "High in Protein". Among the proteins used in the food industry, dairy proteins are the most widely utilized due to their high nutritional-biological value, the extended range of functional characteristics that they offer, and their sensory profile, which tends to be neutral and pleasant compared to their plant alternatives. (Balivo et al., 2024).

The dairy industry produces a vast variety of milk protein ingredients specifically designed for specific applications. These include whey powders, caseins and caseinates, whey protein concentrates and isolates, milk protein concentrates and isolates, and hydrolyzed milk protein. The specific processes used in the manufacture of these products can modify the native structures of proteins, which can impact protein functionality (Singh, 2009). Milk proteins serve various key functions in food products, including emulsifying, thickening, gelling, and foaming capacity (Andiç and Boran, 2015). As an example, as reported by Kumar (2015), whey protein concentrate-based formulations significantly enhanced the texture and stability of spreadable creams, demonstrating that WPC can act as an effective structuring agent in such applications. Moreover, since different fractions can be obtained, they may exhibit distinct nutritional values and functional properties. Each protein has unique characteristics that depend on its features (structure and amino acid composition), leading to high variability in the chemical, physical, and bio-functional properties of food. Furthermore, they are known to possess numerous biological properties, such as the release of bioactive peptides. In particular, the bioactivity of these latter can be inactive within the intact sequence but can be released by enzymatic proteolysis and/or during gastro-intestinal digestion (Balivo et al., 2024). Moreover, this study becomes more challenging when considering the interactions of milk proteins with other ingredients. The composition, ingredients, and water content can affect, indeed, the ability of the proteins to hydrate and perform their intended functions. Identifying the most suitable protein classes for a specific application is a challenging task that requires in-depth knowledge of proteins, the impact of technological processes, and anticipation of changes that may occur during the products shelf life.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram provided in Table 1:

A1) **Bibliographic research**: A thorough review of the scientific literature will be conducted to gather current knowledge, identify emerging trends, and discover any gap regarding milk proteins and their use in the confectionery industry.

A2) **Milk protein characterization**: Milk protein samples will be analyzed to determine the key chemical-physical properties, which are essential for a deep understanding of their behavior and function within confectionery products.
   1) To evaluate the physical functionality of different milk protein samples, techniques such as particle size analysis, solubility, water-holding capacity, and foaming/emulsifying properties will be performed.
   2) To investigate the chemical features, proteins degree of glycation, amino acid composition, and the digestibility, to investigate the gastro-intestinal fate and potential presence of bioactive peptides, will be assessed.

A3) **Milk proteins in confectionery products**: Milk proteins will be utilized in the development of specific confectionery products to evaluate their chemical-physical and functional properties and enhance the final product formulation concerning texture, stability, nutritional value, and sensory appeal.
   1) Model systems will be developed to study, under controlled conditions, the structure-function relationships of milk proteins.
   2) To evaluate their effect on product performance, various recipes with different types of milk protein and/or concentrations will be examined.
   3) Laboratory-scale prototypes will be developed.
   4) Final products will be analyzed to assess physical (texture, rheology…) and chemical (*in vitro* digestion) characteristics.
   5) The final prototypes will undergo biofunctional evaluation to assess their potential bioactivity.

A5) **Assemble a unique protein database**: collect all the information concerning the chemical-physical characterization of animal proteins and their alternatives, their performance in specific application and bio-functionality.

A4) **Thesis and paper preparation**: Writing and editing the PhD thesis, scientific papers, and oral and/or poster communications.

Table 1 *Gantt diagram for this PhD thesis project.*

Activity \ Months		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	***Bibliographic research***																								
A2)	***Milk proteins characterization***																								
	1) Physical characterization																								
	2) Chemical characterization																								
A3)	***Milk proteins in confectionery products***																								
	1) Study of proteins in model systems																								
	2) Development of formulations on lab scale																								
	3) Trials on pilot scale																								
	4) Physical-chemical characterization of final prototypes																								
	5) Biofunctional study on final products																								
A4)	***Assemble a unique protein database***																								
A5)	***Thesis and Paper Preparation***																								

3. Selected References

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Poster presentation sessions
PhD students – 2nd year
Abstracts

Innovative methods in breadmaking, packaging and distribution able to increase nutraceutical value, extend shelf-life and reduce waste of Bakery products (P-II-1)

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This PhD project thesis aims to develop innovative techniques to increase the shelf life of bakery products, thereby extending their distribution area and subsequent consumption. The multifactorial approach includes using PDO sourdough, fortifying with food by-products sourced from the fruit and vegetable agri-food chain, and employing Modified Atmosphere Packaging (MAP) with compostable film to prolong shelf life, reduce food wastage, and minimize the use of chemicals in the agri-food chain.

Metodi innovativi nella produzione del pane, nell'imballaggio e nella distribuzione in grado di aumentare il valore nutraceutico, prolungare la durata di conservazione e ridurre gli sprechi dei prodotti da forno

Il presente lavoro di ricerca ha l'obiettivo di sviluppare tecniche innovative per aumentare la shelf-life dei prodotti da forno al fine di estendere l'area di distribuzione e di conseguenza, il consumo. L'approccio multifattoriale include l'utilizzo di lievito madre DOP, l'arricchimento con sottoprodotti alimentari provenienti dalla filiera agroalimentare di frutta e verdura e l'impiego di confezionamento in atmosfera modificata (MAP) con film compostabile per prolungare la shelf life e ridurre gli sprechi alimentari e l'utilizzo di sostanze chimiche nella filiera agroalimentare.

Key words: Sourdough fermentation, Food by-product valorization, Shelf-life extension, Modified atmosphere packaging, Compostable packaging, Food waste reduction

1. Introduction

In accordance with the PhD thesis project previously described, this poster reports the main results of the first two activities concerning:

A1) **Study and determination of rheological behaviour of biopolymer films for a new packaging system** will help to establish the correct usage of these materials or optimize them based on the final product (A1.1). Additionally, shelf-life monitoring tests will be conducted using smart sensors (A1.2).

2. Materials and Methods

Bread samples were prepared according to the official Tuscan Bread PDO protocol under the supervision of the Consortium. The sourdough underwent two refreshments (4 h at 28 °C), followed by a biga (600 g flour, 100 g sourdough, 200 mL water, 16 h at 17 °C). The final dough (1 kg flour, 550 mL water) underwent autolysis, shaping, proofing, and baking at stepwise temperatures (240–190 °C), then cooled under laminar flow for 2 h. Five packaging films were tested: PLA, PP, PET, PBS, and PBS-CHT. Packages were flushed with 100% CO_2 and monitored for water vapor (ESP8266 sensors), gas retention (CheckPoint3, EVO-P), and mass loss (precision scale). Twelve loaves were sliced and packaged in four configurations:

1. air (control);
2. PET outer + PBS inner in CO_2 with sensors;
3. air + 1% antifungal aerosol;
4. air + 1.2% antifungal aerosol.

Packages were stored at room temperature for 3 weeks. Daily non-destructive measurements (O_2, CO_2, T, RH) were performed. At intervals, destructive analyses included Aw, texture, pH, volatile acidity, lactic and acetic acid.

3. Results and Discussion

3.1 Study and determination of rheological behaviour of biopolymer films for a new packaging system

To evaluate packaging material performance under modified atmosphere (100% CO_2), samples were monitored for water vapor content and gas retention over 6 days. Water vapor concentration (PPMV, dry) was calculated using temperature, pressure, and relative humidity data, following Vaisala (2013) formulas. At time zero (t = 0 h),

readings reflected environmental conditions. After sealing (ti), PPMV increased due to water migration from bread. Most materials stabilized between 18,000–22,000 PPMV, except PLA, which showed a sharp decline after 96 h (down to ~12,000 PPMV), indicating poor water vapor barrier properties (**Figure 1**).
Gas loss (LCO_2) and mass loss (Lm) were calculated as percentage differences between initial and final values. Compostable films (PLA, PBSA, PBSA-CHT) showed high CO_2 loss (>85%) and relevant mass loss (up to 6.5%), while conventional polymers, especially PET, maintained internal conditions effectively ($LCO_2 \approx 8\%$, $Lm < 1\%$). Variability in PP and PLA was likely due to sealing defects (**Table 1**). PET demonstrated the best overall performance in preserving a modified atmosphere and product mass. Based on the results obtained regarding the affinity of the materials for packaging, a non-destructive gas analysis prototype and a novel packaging method were developed to extend the shelf life of **Pane Toscano DOP** (**Figure 2**).

Figure 1. Average water vapor content (PPMV) versus time (confidence intervals shown with an α= 0.05).

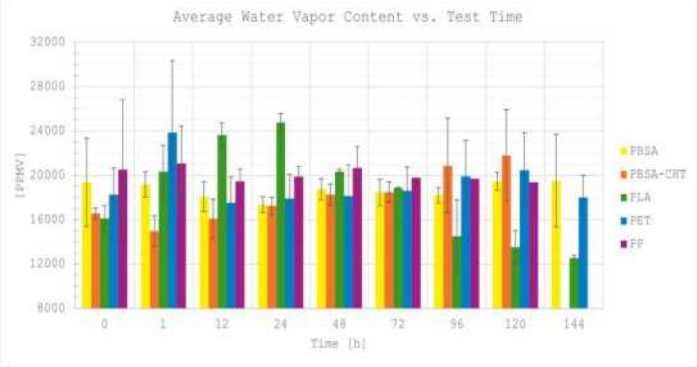
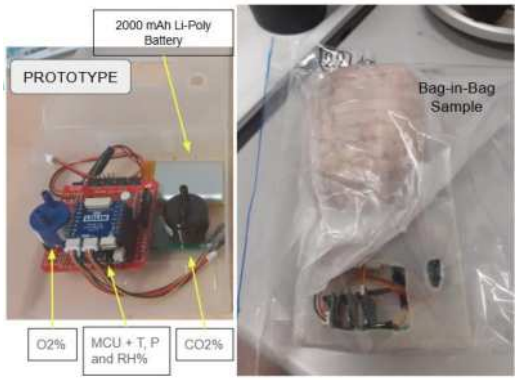


Table 1. Average temperature, gas composition, and mass reduction percentage results for different types of films (confidence intervals shown are with α = 0.05).

FilmType	Avg.T(℃)	LCO2[%]	Lm[%]
PBSA	19.79± 0.05	94.59± 4.24	4.01± 0.58
PBSA-CHT	20.98± 0.11	89.01± 3.84	4.00± 0.85
PLA	21.36± 0.08	85.76± 22.52	6.51± 0.29
PET	19.20± 0.06	8.34± 8.38	0.21± 0.05
PP	19.87± 0.12	66.11± 31.15	0.63± 0.87

Figure 2. Prototype for non-destructive gas analysis in a bag-in-bag system for Pane Toscano DOP, with sensors for O_2, CO_2, T, P, and RH%, powered by a 2000 mAh Li-Poly battery.



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Valorization of Food Waste for New Packaging Solutions (P-II-2)

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Plastic dominates food packaging for its cost, strength, and moisture resistance, but poor gas barriers often require multilayers, complicating recycling (Arrieta et al. 2024). Bioplastics from renewable sources, such as chitin and its derivative chitosan (CS), are gaining interest (Al Sagheer et al. 2009). While traditionally obtained from crustaceans, insect residues offer sustainable alternatives (Kaya et al. 2017). My doctoral research focuses on extracting CS from Hermetia illucens and Tenebrio molitor (exuviae, larvae, and adults). An optimized, uniform process improved yield and sustainability, and the resulting CS films were characterized for food packaging applications.

Valorizzazione di Scarti dell'Industria Alimentare per Applicazioni di Packaging Innovativi

La plastica domina il packaging alimentare grazie al costo, resistenza e impermeabilità all'umidità, ma le scarse proprietà barriera ai gas richiedono spesso multistrati, difficili da riciclare (Arrieta et al. 2024). Le bioplastiche da fonti rinnovabili, come la chitina ed il chitosano (CS), suscitano interesse (Al Sagheer et al. 2009). Sebbene tradizionalmente ottenuta da crostacei, i residui di insetti rappresentano un'alternativa sostenibile (Kaya et al. 2017). La mia ricerca di dottorato si concentra sull'estrazione di chitosano da Hermetia illucens e Tenebrio molitor (esuvie, larve e adulti). Un processo ottimizzato ha migliorato resa e sostenibilità, e i film ottenuti sono stati caratterizzati per applicazioni nel packaging.

Key words: Chitosan, food waste valorization, bio-based, circular economy, insects

1. Introduction

In line with the previously described PhD project, this poster presents the main results of the activities carried out:
(A1) Extraction of chitin and its conversion to chitosan from different unconventional sources.
(A2) Production of chitosan-based films followed by characterization of their optical and barrier properties.

2. Materials and Methods

Chitosan was extracted from the insect biomasses through a multi-step process aimed at removing mineral and protein fractions, followed by deacetylation to convert chitin into chitosan.
All biomasses were initially dried at 60 °C for 48 hours, ground, and sieved to a particle size of 200 µm. The demineralization step was carried out by treating the biomass with 0.25 M HCl at room temperature for 3 hours under constant agitation at 250 rpm. This was followed by deproteinization using 1 M NaOH at 70 °C for 24 hours, also under continuous agitation at 250 rpm. After each step, the samples were washed with distilled water until neutral pH was achieved and then oven-dried at 60 °C to constant weight.
The resulting chitin was subjected to deacetylation using a 50% (w/v) NaOH solution at 130 °C for 3 hours under constant stirring at 250 rpm. The chitosan obtained was again washed to neutrality and dried at 60 °C to constant weight.
Chitosan films were prepared by dissolving the polymer in a 1% (v/v) aqueous acetic acid solution at room temperature under constant stirring for 24 hours. The resulting solution was centrifuged at 12,000 rpm for 10 minutes to remove any undissolved residues. Films were obtained via solvent casting by pouring the solution into Petri dishes and allowing it to dry at room temperature under a fume hood for 24 hours. The dried films were then conditioned with silica gel at ambient conditions to remove residual moisture.
The films were subsequently characterized by their optical properties, including transparency and haze, and their barrier performance was assessed by measuring the oxygen transmission rate (OTR) at 23 °C and 0% relative humidity.

3. Results and Discussion

3.1 Chitosan extraction and characterization

Chitosan was successfully extracted from four insect-derived biomasses: exuviae and adults of *Hermetia illucens*,

and exuviae and larvae of *Tenebrio molitor*. The optimized extraction protocol, applied uniformly across all matrices, resulted in chitosan of consistent quality. For all the biomasses, the chitosan yield was consistent with the expected values based on literature data, indicating that the applied extraction method was effective in recovering chitosan from the biomass (Table 1). The intermediate chitin and final chitosan products were characterized using FT-IR spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction (XRD). FT-IR confirmed the presence of characteristic functional groups and successful deacetylation of chitin to chitosan, compared with commercial chitosan from shrimp. XRD analysis was used to evaluate the crystallinity index of chitin, which was consistent with an α-chitin morphology. SEM imaging further revealed morphological differences among the various sources: chitin derived from *Tenebrio molitor* exhibited a scale-like structure with overlapping layers forming micro- and nanopores, whereas *Hermetia illucens*-derived chitin showed a more fibrous organization, arranged in a honeycomb-like pattern.

Table 1 *Chitin and chitosan recovered percentage from all the biomasses*.

Biomass	Chitin [%]	Chitosan [%]
Hermetia illucens exuviae	10.5 ± 3.2^{b}	6.2 ± 1.8^{b}
Hermetia illucens adult	6.4 ± 1.5^{a}	3.8 ± 1.3^{a}
Tenebrio molitor exuviae	11.7 ± 2.8^{b}	7.0 ± 1.2^{b}
Tenerbio molitor larvae	4.8 ± 1.9^{a}	2.9 ± 0.9^{a}

3.2 Film formation and functional characterization

Chitosan-based films were successfully fabricated through solvent casting, resulting in uniform, flexible, and transparent materials, with an average thickness ranging between 13 and 14 µm Their optical properties were assessed based on transparency and haze. All films showed moderate transparency and weak UV-shield capabilities, positioned between those of PP and PET (Figure 1). Haze values, all below 3%, varied depending on the biomass source.

Barrier properties were assessed through oxygen transmission rate (OTR) measurements at 23 °C and 0% relative humidity. The films demonstrated promising oxygen barrier performance, with OTR values never exceeding 0.5 $cm^3m^{-2}day^{-1}$.

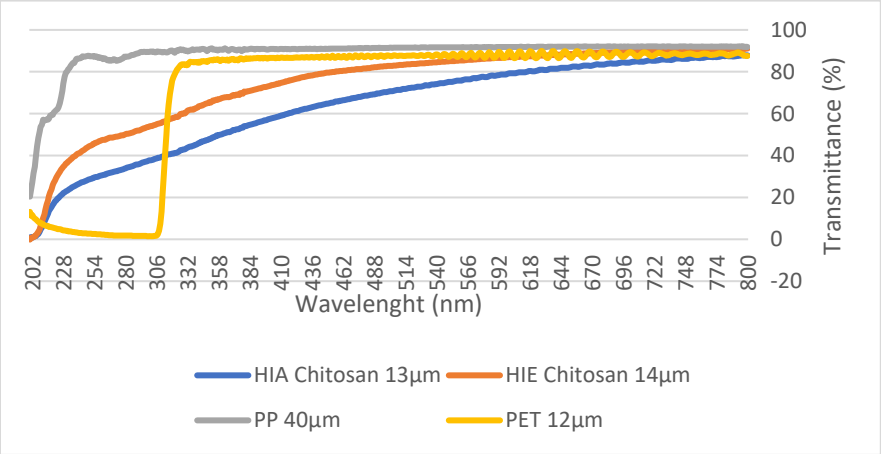


Figure 1 *UV-Vis spectra of HIA-CS, HIE-CS compared to PP and PET*

These findings confirm the feasibility of extracting chitosan from insect biomass and using it to develop biodegradable films with functional properties suitable for sustainable food packaging applications. The origin of the biomass influenced both the extraction efficiency and the final film performance, highlighting the role of raw material selection in tailoring biopolymer-based packaging solutions.

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Safety Assessment of Recyclable Coffee Packaging: Screening NIAS and Quantifying Primary Aromatic Amines in Polyolefin-Based Materials (P-II-3)

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This PhD thesis research project focuses on understanding food-packaging interactions in new polyolefin multilayer structures and validating their application for coffee powder packaging.
The following activities of the PhD project are presented. Firstly, a method for the screening of NIAS in packaging materials was implemented. Secondly, a method for the quantification of PAAs migrating from food packaging was developed. The test conditions were selected to reflect the intended final use of the packaging materials, specifically for roasted & ground coffee packaging.
Key words: Food safety, NIAS, multilayer packaging, PAAs, coffee packaging

1. Introduction

This study addresses two key safety aspects in innovative PE-based laminates intended for coffee powder packaging:

- The screening of NIAS, for which SPME was selected due to its speed and high sensitivity;
- The quantification of PAA migration using mass spectrometry, chosen for its high selectivity, which helps overcome the overestimation issues commonly associated with conventional methods

2. Materials and Method

The multilayer packaging materials selected for the study, each differing in barrier type and layer composition, were coded as follows:

- A: PE 25 µm / EVOH 5 µm / PE 90 µm
- B: PE 25 µm / EVOH 4 µm / PE 60 µm
- C: OPE 25 µm / MPE 25 µm / COPE 50 µm
- D: OPE 25 µm / COPE 75 µm

For NIAS screening 3 cm × 0.5 cm cut-outs of the laminates were analyzed by HS-SPME-CG-MS. 20 µL of a 75 µg/mL solution of 2,6-Di-tert-butylphenol were added as internal standard. Analyses were performed in duplicate using a TRACE 1300 GC (Thermo Fisher, USA) with a Robotic Pro autosampler (Gerstel, Germany). Two SPME fibers were compared on a ZB-5MSplus Zebron Capillary GC Column (Phenomenex Inc., California, USA) of 30 m × 0.25 mm × 0.25 µm: a Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber with 50 µm DVB layer and 30 µm CAR/PDMS layer, and a Polydimethylsiloxane (PDMS) fiber of 100 µm. The identification of the compounds detected was carried out by MS comparison with NIST library. Toxicity was evaluated according to Cramer rules with the software Toxtree (Ideaconsult Ltd.).
For PAAs migration testing, UNI EN 1186-3:2003 specifies a surface-to-volume ratio of 1 dm²/100 mL of food simulant. Accordingly, pouches were made from each of the selected material and filled with food simulant B (3% acetic acid, w/v). Pouches were then stored for 10 days at 60 °C, as required by Regulation (EU) No 10/2011 for migration testing of long-shelf-life products (over 6 months).
The simulant extract was filtered through a 0.45 µm glass microfiber filter (Whatman, GE Healthcare, Little Chalfont, UK). 8 µL of food simulant were injected in an Acquity UPLC separation module (Waters, Milford, MA, USA) coupled to a Q Exactive hybrid quadrupole-Orbitrap MS through a HESI-II probe for electrospray ionization (Thermo Scientific, San Jose, CA, USA). PAAs were separated using an Acquity UPLC BEH C18 column (1.7 mm, 2.1 x 50 mm).
For quantification, the 'Protocol C' from the JRC document "*Technical guidelines on testing the migration of primary aromatic amines from polyamide kitchenware and of formaldehyde from melamine kitchenware*" (Simoneau et al., 2011) was considered. This work focuses on five selected PAAs, that were used as standards: 2,4-Toluenediamine (2,4-TDA) 2,6-Toluenediamine (2,6-TDA), 4,4'-Methylenedianiline (4,4'-MDA), 3,3'-Dimethylbenzidine (3,3'-DMB) and Aniline (ANL).

3. Results and Discussion

3.1 NIAS screening

The first step involved testing two fibers: DVB/CAR/PDMS and PDMS. Both fibers identified mostly the same compounds though with different response intensities. The DVB/CAR/PDMS fiber, with its porous mixed coating, better extracted semi-volatile and volatile compounds with medium retention times (10–30 minutes). As expected,

the PDMS fiber extracted non-polar, less volatile compounds (Vera et al., 2011), showing higher peaks at retention times of 40–60 minutes.
The initial NIAS screening using HS-SPME-GC-MS revealed several identifiable peaks. A large portion consists of esters, such as ethyl acetate, triacetin, dibutyl adipate, and tributyl aconitate. Hydrocarbons, including alkanes like decane, dodecane, and tetradecane, as well as alkenes such as 1-dodecene, may result from degradation, residual monomers, or additives.
Toxicity of the identified compounds was evaluated according to Cramer's classification. This divides chemicals into three toxicity classes, with Class I representing low toxicity, Class II intermediate toxicity, and Class III the highest toxicity (Vera et al., 2010).
Only four of the detected compounds were classified as toxic according to Cramer's rules. Detection of these compounds reflects the wide variety of substances used in packaging manufacturing. Like butylated hydroxytoluene (BHT) an antioxidant preventing oxidative degradation of the material.
These findings highlight the relevance of HS-SPME as a rapid and effective screening tool for detecting potential volatile and semi-volatile contaminants in food packaging materials (Aznar et al., 2011).

3.2 PAAs determination

The aim of this preliminary step is to obtain a calibration curve to quantify the levels of PAAs migrated into the food simulant. Individual standards of these substances were prepared with a concentration of 1000 µg/mL in ethanol. A single solution containing all the selected PAAs was prepared by diluting them to 10 µg/mL with a 3% (w/v) aqueous acetic acid solution. This was further diluted, in acetic acid 3% (w/v) by another 100-fold, to get a 100 µg/L solution. From this, solutions at lower concentrations could be prepared, diluting in 3% acetic acid.
Linearity of the response was assessed by testing all the concentrations reported in the JRC 'Protocol C': 0.5, 1, 2.5, 5, 10, 20 and 25 ppb. Calibration curves showed good linearity, with R^2 values ranging from 0.9482 to 0.9977, while limits of detection (LOD) were below 2 ppb (the specific migration limit set by Regulation (EU) No 2020/1245 for individual PAAs).
The analysis revealed no detectable levels of 2,6-TDA, 2,4-TDA and 3,3'-DMB in any of the tested samples. Aniline was found in significant concentrations in samples featuring an EVOH barrier (A and B), ranging from 20 to 43.5 ppb, possibly due to pigments or dyes used in these materials (Aznar et al., 2009). Lower levels of 4,4'-MDA were detected in two samples: A (0.4 ppb) and C (0.5 ppb).
Regulation (EU) 2020/1245, amending Regulation (EU) No 10/2011, sets the specific migration limit (SML) for PAAs at 2 ppb for individual PAAs and 10 ppb for total PAAs limit. In contrast, samples C and D featuring metallizations (MPE) and coextruded PE layers (COPE) layers respectively, showed only traces of aniline and 4,4'-MDA. Low levels of aniline in samples C and D may be attributed to the use of different adhesives in the manufacturing process, specifically, adhesives that do not contain aromatic isocyanates In both cases though, the food contact layer consisted of oriented polyethylene (OPE), providing improved mechanical and barrier properties. Further studies will be necessary to assess the presence of additional PAAs and ensure full compliance with regulatory limits and food safety requirements.

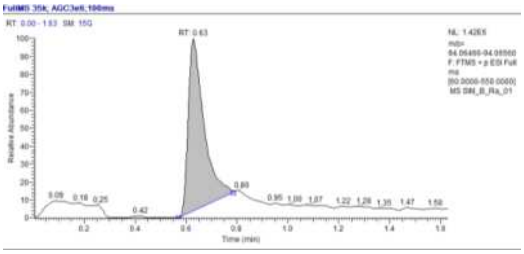


Fig. 1 *Chromatogram obtained by UPLC/HR-MS of acetic acid 3% after migration test showing aniline peak in sample A.*

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Functionalization of biopolymers via chemical methods for food packaging applications (P-II-4)

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Project activities focused on the synthesis of chitosan derivatives functionalized with hydroxycinnamic acids via enzymatic and chemical strategies, exploring three approaches:1) covalent binding of caffeic acid catalyzed by laccase; 2) condensation with caffeoyl chloride; 3) amide bond formation with ferulic acid by EDAC/NHS coupling. In the next phase of the work, the focus shifted to covalent functionalization of chitosan films with anhydrides and isocyanates of different chain length fatty acids, optimizing the experimental conditions to improve their efficiency.

Funzionalizzazione di biopolimeri tramite metodi chimici per applicazioni nel packaging alimentare.

Le attività del progetto hanno riguardato la sintesi di derivati del chitosano funzionalizzati con acidi idrossicinnamici tramite strategie enzimatiche e chimiche, esplorando tre approcci: 1) legame covalente dell'acido caffeico mediato da laccasi; 2) condensazione con il cloruro dell'acido caffeico; 3) formazione di legami ammidici con acido ferulico tramite EDAC/NHS. In seguito, l'attenzione si è spostata sulla funzionalizzazione covalente di film di chitosano con anidridi e isocianati di acidi grassi a diversa lunghezza di catena, ottimizzando le condizioni sperimentali per migliorarne l'efficienza.

Key words: Chitosan derivatives, biopolymer functionalization, hydroxycinnamic acids, fatty acid anhydrides, isocyanates

1. Introduction

This poster presents the main results and challenges faced during the second year of the PhD research, which focused on the synthesis of chitosan derivatives functionalized with hydroxycinnamic acids through enzymatic and chemical strategies, as well as the covalent functionalization of preformed chitosan films. In the first part, three synthetic approaches were investigated: i) covalent grafting of caffeic acid mediated by laccase from *Trametes versicolor*, ii) condensation between caffeoyl chloride and the primary amino groups of chitosan, iii) amidation of ferulic acid via *N*-hydroxysuccinimide (NHS) ester activation. The resulting conjugates were characterized in terms of chemical structure by FT-IR and NMR, and the grafting efficiency was evaluated using the Folin–Ciocâlteu assay.
In the second part, preformed chitosan films were functionalized with fatty acid anhydrides and isocyanates bearing alkyl chains of varying lengths. The objective was to acylate the reactive groups of chitosan, namely the primary amino and hydroxyl groups, to reduce the hydrophilicity of the material. Different solvents, reaction parameters, and pre-treatments were tested to optimize the efficiency of modification and improve the material properties. The obtained films were characterized by FT-IR, contact angle analysis, and measurements of Water Vapor Transmission Rate (WVTR) and Oxygen Transmission Rate (OTR), while additional analyses using Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), and X-ray Diffraction (XRD) are planned.

2. Materials and Methods

Chitosan (50 - 190 kDa, 75–85% deacetylation), caffeic acid (CA), ferulic acid (FA), and laccase from *Trametes versicolor* were used as received, along with other reagents such as DMAP, thionyl chloride, EDAC, NHS, and fatty acid derivatives (anhydrides and isocyanates with various alkyl chains). Three main strategies were employed for the covalent functionalization of chitosan. The enzymatic approach involved grafting CA onto chitosan using laccase in phosphate buffer (pH 6.5, 100 mM, 30 °C) for 24 h, followed by characterization via FT-IR, ^{1}H-NMR, and Folin–Ciocâlteu assay. In the chemical acyl chloride method, caffeoyl chloride was synthesized *in situ* and reacted with chitosan in acidic aqueous medium, using DMAP as the base. Reaction conditions, including DMAP equivalents, were optimized, and the products were isolated by acetone precipitation and methanol washing. In the third approach, FA was condensed with chitosan in water/ethanol using EDAC/NHS as coupling agents, with variations in FA/coupling agent's molar ratio, temperature, and reaction time. The products were recovered by precipitation and washing. Conjugates from all the employed methods were freeze-dried and characterized by TLC, FT-IR and ^{1}H-NMR. For film functionalization, preformed chitosan films (cast from 1% w/v solution into square Petri dishes, 120 × 120 mm) were soaked in organic solutions of fatty acid anhydrides or isocyanates (1–2% v/v) in different solvents (methanol, ethyl acetate, acetonitrile). The reactions were performed at room

temperature for 24 - 48 h, with or without alkaline pre-treatment to enhance the reactivity. The modified films were washed, vacuum-dried, and analyzed by FT-IR, further characterization (OTR, contact angle, SEM, AFM, XRD) is planned.

3. Results and Discussion

3.1 Assessment of chitosan derivatization

To verify the actual covalent functionalization of chitosan, spectroscopic analyses (FT-IR, ^{1}H-NMR) and thin-layer chromatography (TLC) were performed. The results indicated that the functionalization was successfully achieved through chemical strategies, whereas no effective grafting was observed using the enzymatic approach. Specifically, the laccase-mediated reaction, which was expected to exploit the reactivity of the quinone intermediate derived from caffeic acid, led instead to the formation of insoluble aggregates, likely due to uncontrolled polymerization of caffeic acid. It is worth noting that the enzymatic activity of the laccase used (5.6 U) was significantly lower than the activity reported for similar reactions (153 U) (Božič, 2012). In addition to this limitation, the large mass of enzyme required made the post-reaction purification particularly challenging, other than significantly expensive. In contrast, the chemical approaches proved to be effective. In particular, the grafting efficiency did not exceed a degree of substitution (DS) of 8.84% for the caffeoyl chloride method, while a maximum DS of 7.6% was obtained using the EDAC/NHS coupling with ferulic acid. The DS values were calculated from the ^{1}H NMR spectra according to the method reported in literature (Liu, 2017), using the ratio between the total peak area of aromatic protons from the styrene moiety (δ = 6.0 - 8.0 ppm) and the peak area of H-2 of chitosan (δ = 3.06 ppm), considering three aromatic protons for all phenolic acids in this study. In the case of the caffeic acid conjugate C-CAC, the grafting degree was additionally estimated by the Folin–Ciocâlteu assay. The grafting rate ranged from 67 to 110 mg CAE/g, depending on the experimental conditions. Both these results and DS align with literature (İlyasoğlu, 2019). However, two major drawbacks emerged: the significant amount of reagents required to functionalize relatively small quantities of chitosan and the low reproducibility of the results, in particular with the caffeoyl chloride method. The moisture sensitivity of the chloride acyl likely contributed to the poor reproducibility of the protocol. Attempts to perform the reaction in dry organic solvents (e.g., DMSO) were also ineffective, most likely due to the limited accessibility of the amino groups, as the reaction occurred under heterogeneous conditions (chitosan is not soluble in organic solvents). Alkaline pre-treatments of chitosan with sodium hydroxide, aimed at disrupting polymer chain organization and increasing surface area, did not improve the outcome.

3.2 Assessment of film functionalization

Functionalization trials using both anhydrides and isocyanates were successful, albeit to different extents. Anhydrides showed significantly lower reactivity compared to isocyanates. Effective grafting with anhydrides was only observed when the chitosan films were pre-treated with a strong base (i.e., NaOH) able to 1) neutralize the residual acetic acid, and 2) favor the acylation on both the aminic and primary hydroxy groups. This was confirmed by the appearance of specific IR absorption bands attributed to amide and ester bonds, particularly the amide I band around 1650 cm^{-1}. The pre-treatment with organic bases (i.e., triethylamine) was less effective, probably due to a lower ability to interact with functional groups of the matrix, compared to aqueous bases. As a consequence, chitosan protonated amino groups may not be sufficiently nucleophilic to react with anhydrides and form amid bonds. Similarly, hydroxyl groups appeared to lack adequate nucleophilicity to react with anhydrides and form esters. It has long been reported in the literature that the steric accessibility of these functional groups can play a critical role in such reactions and conditions (Muzzarelli, 1978).

In contrast, isocyanates exhibited much higher reactivity, which was influenced by the steric hindrance of the specific isocyanate used. Notably, even in the absence of pre-treatment, the primary hydroxyl groups on chitosan were sufficiently nucleophilic to react with isocyanates, leading to the formation of carbamate linkages. This was confirmed by FTIR analysis, showing the appearance of characteristic carbamate bands, including the C=O stretching around 1720 cm^{-1}, N–H bending near 1600 cm^{-1}, and C–N stretching in the 1340–1260 cm^{-1} region. This is advantageous as it preserves the primary amino groups, responsible for chitosan antimicrobial activity, while selectively masking hydroxyl groups that contribute to hydrophilicity. The obtained materials exhibited pronounced hydrophobic properties and were no longer soluble in aqueous acidic solutions, providing direct evidence of successful functionalization.

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Valorisation of Food Waste for the Development of Film Coatings for Food Packaging Applications (P-II-5)

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During the first two years of the PhD programme, research focused on the development of food packaging from agro-industrial waste, in particular coffee silver skin (CSS) and pine nut film (PSK). The work was divided into two phases: (1) formulation of film-forming coatings with biopolymers (PLA and PP) and biomaterials with CSS; (2) characterisation of mechanical properties, antioxidant activity and microbial load. The results showed high antioxidant capacity in coatings containing CSS and the absence of microbial contamination in all materials analysed.

Valorizzazione degli scarti alimentari per lo sviluppo di rivestimenti per imballaggi alimentari

Durante i primi due anni di dottorato, la ricerca ha riguardato lo sviluppo di imballaggi alimentari a partire da scarti agro-industriali, in particolare la cuticola d'argento del caffè (CSS) e la pellicola del pinolo (PSK). Il lavoro si è articolato in due fasi: (1) formulazione di rivestimenti filmogeni con biopolimeri (PLA e PP) e biomateriali con CSS; (2) caratterizzazione delle proprietà meccaniche, dell'attività antiossidante e della carica microbica. I risultati hanno mostrato un'elevata capacità antiossidante nei rivestimenti contenenti CSS e l'assenza di contaminazioni microbiche in tutti i materiali analizzati.

Key words: Food waste, Food packaging, Circular bioeconomy, Bioplastic, Environmental Sustainability

1. Introduction

This doctoral project explored using agro-industrial wastes, specifically Coffee Silverskin (CSS) and Pine Nut Skin (PSK), to create film-forming coatings for bioplastics like PLA and PP for food contact and develop biomaterials from CSS. The study also evaluated the mechanical properties, antioxidant activity, and microbial load of these coatings to assess whether sterilisation would be needed for industrial applications.

.2. Materials and Methods

2.1. Preparation of film coatings

Coatings were prepared using CSS and PSK by wet grinding with ball milling (3 g of solid in 40 g of liquid, 500 rpm, 2 h). For PSK, dry pre-grinding was performed, and wet grinding was carried out in a solution of water and PVA (0.75 g) to improve viscosity, adhesion and film-forming capacity. The PP and PLA films were pre-treated with plasma (30 s, 20% power). The PLA films were also preheated (1 h at 120 °C) to stabilise them. After applying the coating (thickness 50 µm), the films were dried and treated in an oven (2 h at 120 °C) with support glasses to improve water resistance through protein cross-linking.

2.2. Preparation of biomaterials and mechanical properties

350 g of coffee silverskin (CSS) was mixed with cellulose acetate and water to form a viscous mixture, moulded into 15 × 8 cm samples, air- and oven-dried at 50 °C, then pressed at 180 °C and 30 mbar. A PLA coating was applied on one side by thermo-pressing (180 °C, 30 mbar, 40 seconds) to improve water resistance. Mechanical tests (tensile strength, Young's modulus, elongation) were done in triplicate on CSS-only and PLA-coated samples using an Instron machine with a 50 mm test span and 2 mm/min crosshead speed.

2.3. Antioxidant Capacity

Antioxidants were extracted from 1 g of film using 25 mL of 96% ethanol under agitation for 24 h at room temperature, following a modified protocol from Guo et al. (2023). After filtration (45 µm), extracts were analysed using ABTS, DPPH, and FRAP assays. Trolox served as the standard, and results were expressed as µmol Trolox equivalents per gram of sample (µmol TE/g).

2.4. Microbiological test

To evaluate the residual microbial load of the coatings for food contact, total aerobic mesophilic bacteria, moulds, and yeasts were quantified. Surface sampling was performed with sterile swabs according to a zig-zag pattern. Samples were plated using the inclusion method and incubated at 30°C for 48 h (mesophiles) and 28°C for 48 h (moulds and yeasts). Results were expressed as CFU/cm².

3. Results and Discussion

3.1. Coating formulation

As described above, coatings were prepared using CSS and PSK. Studies have already been conducted in the

literature on the use of PLA with the addition of coffee silverskin, which appears to improve many properties of the material, such as its physical, functional and barrier properties (Sung et al., 2017; Capuano et al., 2024). As for PP, it appears to be a promising material for use in food packaging, given many of its excellent properties that have been studied (Krepker et al., 2018). From the samples obtained, it would appear that CSS integrates well with both PLA and PP, whereas PSK, even with the addition of the cross-linking agent PVA, did not show any improvement.

3.2. Mechanical properties

The addition of PLA to the CSS biomaterial increased elastic modulus and maximum elongation, indicating greater rigidity and deformability. However, pure CSS showed slightly higher average strength. In general, the CSS+PLA composite is more rigid and deformable, but less resistant than CSS alone.

3.3. Evaluation of the antioxidant capacity of coatings

Figure 1 shows that coatings containing CSS exhibited higher antioxidant activity, likely due to phenolic compounds, as the literature supports. Although specific data on PSK are lacking, pine nuts are known for their antioxidant potential. Experimentally, CSS-based coatings outperformed PSK-based ones. Among CSS formulations, CSS+PP showed higher antioxidant activity than CSS+PLA, likely due to a better interaction and compound stabilisation with the PP substrate. This suggests PP as a promising support for antioxidant-active films in food packaging.

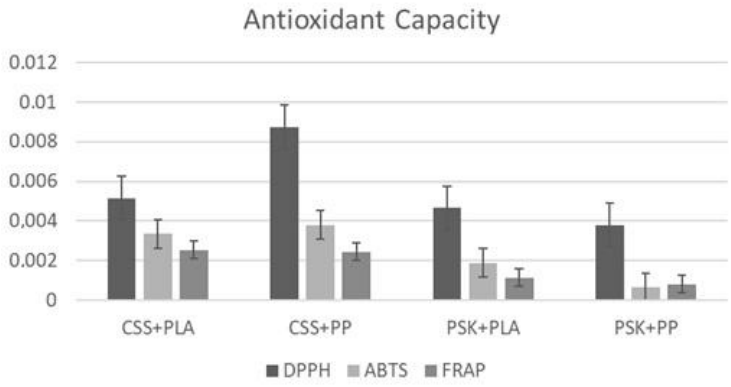


Figure 1. *The antioxidant capacity of different film coatings:Coffee Silverskin (CSS) on PLA and PP, Pine Nut Skin (PSK) on PLA and PP.The x-axis represents the different coating formulations, while the y-axis shows the antioxidant capacity, expressed as Trolox equivalents per gram of sample (µmol TE/g).*

3.4. Microbiological evaluation of coatings and biomaterials intended for food packaging

To ascertain their suitability for use as food packaging materials, a microbiological evaluation of the coatings was carried out using the plate inclusion method. The analyses, performed in triplicate, did not reveal any visible microbial growth. The absence of microbial contamination may be attributed to the processing steps, particularly the exposure to high temperatures and the plasma surface treatment applied before coating. From an industrial perspective, this finding could offer a significant advantage by reducing the need for additional sterilisation steps, as the material appears to be microbiologically safe immediately after processing.

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Advancing in Process Analytical Technology: e-sensing for food cold chain (P-II-6)

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Within the project activities, NIR spectroscopy was applied to study and monitor meat superchilling process. First, the penetration depth of the MicroNIR signal was evaluated under different thermal conditions. This allowed to understand how temperature and water state influence signal interaction with the meat tissues.
Then, a procedure of real-time monitoring for superchilling process by NIR spectroscopy was developed. PCA and aquaphotomics based multivariate control charts were able to assess the beginning of ice formation on the meat surface and the progress of the superchilling phase. Results confirmed the potential of NIR spectroscopy to support superchilling process optimisation.

Progressi nella tecnologia analitica di processo: e-sensing per la catena del freddo nel settore alimentare

Nell'ambito delle attività del progetto, la spettroscopia NIR è stata applicata per studiare e monitorare il processo di *superchilling* della carne. In primis, è stata valutata la penetrazione del segnale MicroNIR in diverse condizioni termiche per comprendere come temperatura e stato fisico dell'acqua influenzino l'interazione segnale-tessuto.
Successivamente, è stata sviluppata una procedura di monitoraggio in tempo reale del processo di *superchilling* mediante NIR. Carte di controllo multivariate basate su PCA e *aquaphotomics* hanno permesso di rilevare l'inizio della formazione di ghiaccio superficiale e l'avanzamento del processo.
I risultati confermano il potenziale della spettroscopia NIR a supporto dell'ottimizzazione di processo.

Keywords: Near Infrared Spectroscopy, PAT, Pork loin, Superchilling process.

1. Introduction

In line with the PhD thesis project, the second-year activities focused on the application of e-sensing technologies to monitor and optimise the superchilling process of pork loin. The study was divided into two main parts:
(A1) investigation of NIR signal penetration depth under different thermal conditions (refrigerated, superchilled, and frozen), to understand water state influence spectral signal attenuation within the tissue;
(A2) real-time monitoring of the superchilling process using NIR spectroscopy combined with the aim of developing Multivariate Statistical Control Charts (MSCCs) for identifying the superchilling phase and improving process control.

2. Materials and Methods

2.1 Study of NIR Signal Penetration Depth

Pork loin samples of dimensions 30 x 30 x h mm, with h = 30, 20, 10, 5 and 2.5 mm, were prepared from fresh pork loin (Italian market). To evaluate the penetration depth of the NIR signal, the samples were placed between the MicroNIR sensor (Viavi Solutions, USA), operating in the range 950-1650 nm, and a green polypropylene plastic sheet positioned on the opposite side. This setup aimed to verify whether the NIR signal could fully penetrate the tissue and detect the spectrum of the plastic. Spectra were continuously acquired every 120 seconds while the samples were gradually cooled, transitioning from refrigerated to superchilled and finally frozen states. Spectral data were pre-processed with Standard Normal Variate (SNV) transformation and subjected to Principal Component Analysis (PCA) in MATLAB (PLS Toolbox v. 8.5) to explore differences related to sample thickness and the detectability of the plastic film under varying thermal conditions.

2.2 Real-Time Monitoring of the Superchilling Process

Five pork loin cubes (30 x 30 x 30 mm, 32.5 ± 5 g) were prepared from fresh pork loins (Italian market). These samples underwent a superchilling process at -18°C with an air flow velocity of 1.3 m/s. Spectral data were collected every 120 s using a MicroNIR device (Viavi Solutions, USA) operating in the 950–1650 nm range, along with temperature monitoring with an Elitech GSP-6 sensor (UK). Spectral data were pre-processed using SNV transformation and analysed via PCA in MATLAB with the PLS Toolbox (v. 8.5). Aquagrams, generated following Tsenkova et al. (2018), tracked changes in water molecular structure during superchilling, enabling the identification of transitions from liquid to solid water states over time. PCA based and Aquagram based MSCCs were built and validated for identifying the superchilling phase and improving process control.

3. Results and Discussion

3.1 Assessment of NIR penetration depth in pork loin under different thermal states

The penetration depth study revealed that the MicroNIR signal could detect the spectrum of the green polypropylene (PP) plastic sheet only through the thinnest pork loin sample tested (h=2.5 mm). In thicker samples, the signal related to PP was no longer visible, indicating a penetration depth lower than 5.0 mm in fresh meat, as previously reported for other biological tissues (Padalkar and Pleshko, 2015). Moreover, the thermal state of the tissue influenced signal transmission: at the beginning of the process the PP signal was visible in the raw spectra. As ice crystals formed within the tissue, the PP signal gradually disappeared (Figure 1). This trend was further confirmed by PCA performed on SNV-transformed spectra (data not shown). The results highlight the short penetration path-length (≤ 2.5 mm) of NIR radiation in pork loins and its correlation with the physical state of water within the tissue. However, the penetration depth is adequate to study the thin frozen layer (1–3 mm thick) expected to form during superchilling (Kaale et al., 2013).

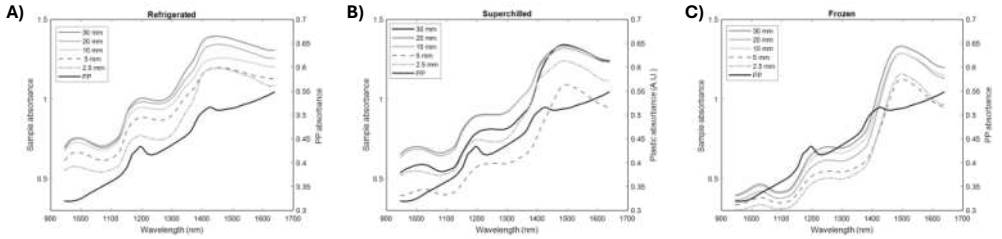


Figure 1. *NIR spectra collected from pork loin samples with different thicknesses and PP sheet: A) Refrigerated meat at initial time, B) Superchilled meat during ice crystallisation, C) Frozen meat at later time.*

3.2 Monitoring of water state transitions during superchilling of pork loin

Confirmed the suitability of signal penetration, NIR spectroscopy was applied to monitor water molecular structure changes in pork loin during superchilling. The online acquisition revealed progressive shifts in key absorption bands – around 980, 1200, and 1450 nm – related to physical states of water (Workman and Weyer, 2007). To better interpret these variations, PCA was performed on SNV-pretreated spectra (Figure 2A, 2B). PC1 effectively described the overall water physical transition, whereas PC2 captured more subtle structural changes occurring throughout the superchilling process. Complementing data exploration, aquaphotomics was used to explore water reorganization at a molecular level. The aquagram (Figure 2C), constructed using water matrix coordinates (WAMACs), reflected phase changes such as ice nucleation and crystal growth, in line with Kaale et al. (2013).

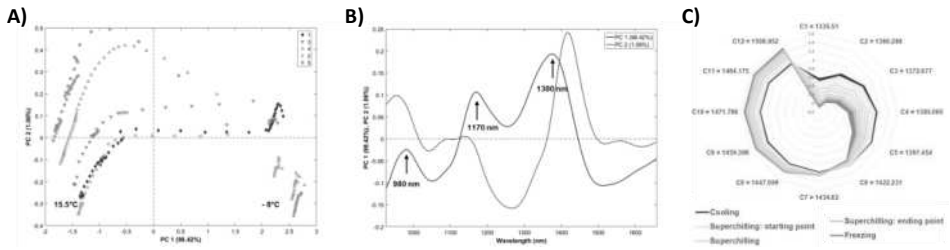


Figure 2. *Explorative data analysis of SNV- NIR spectra collected during the process: (A) PCA score plot, (B) PCA loading plot, (C) aquagram based on selected WAMACs.*

To translate these spectral patterns into a tool for process control, multivariate statistical control charts (MSCCs) were developed using both PC1 and C1 scores (data not shown). The charts successfully identified the superchilling process, showing strong agreement with time-temperature profiles. Together, these approaches confirm the suitability of NIR spectroscopy for real-time monitoring and optimisation of cold chain processes. Pilot plant applications are envisioned to validate the proposed approach.

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Optimizing Red Straw Wine Production: A Multi-Faceted Approach (P-II-7)

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Co-tutor: Prof. Ivano De Noni

The PhD project activities were carried out within the red straw wine framework, modelling grape dehydration for red straw wine production, with the aim of evaluating the impact of the interaction between grape variety, withering duration and the optimal harvest time to initiate the dehydration process.

Ottimizzazione della produzione di vino rosso da uve appassite: un approccio integrato

Le attività del progetto di dottorato sono state svolte nell'ambito della produzione di vino passito rosso, attraverso la modellazione del processo di disidratazione dell'uva, con l'obiettivo di valutare l'impatto dell'interazione tra varietà di uva, epoca di vendemmia e durata dell'appassimento sulla composizione di uva, mosto e vino.

Keywords: Strawine, enology, withering, phenols.

1. Introduction

In accordance with the PhD project (Baviera, 2024), this poster reports the main results of the activities concerning the activities reported as follows:
A2. Management of grape withering
(A2.1) Monitor of withering for different grape varieties. Assessment of the interaction between grape variety and withering levels on the composition of grapes;
(A2.2) Setting of harvest and withering duration. The selection of the optimal harvest date for the dehydration process involved analysing samples of different grape varieties at different ripeness level.

2. Materials and Methods

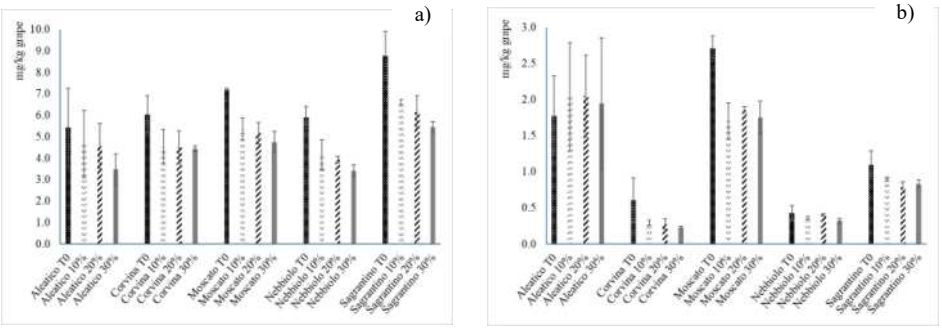
(A2.1) Five grape varieties ('Aleatico', 'Corvina', 'Moscato di Scanzo', 'Nebbiolo', 'Sagrantino') were withered in a drying room at the University of Verona under controlled conditions (14–15 °C, 40–50% RH). Samples were taken at T0 (fresh grape) and at 10%, 20%, and 30% weight loss (WL). The content of total flavonoids (TFs) and anthocyanins (ACNs) was assessed. The extraction of proanthocyanidins from skin and seeds was carried out by acetone/water 70/30 (v/v). The content and units of proanthocyanidins (PACs) was determined by acid cleavage in the presence of phloroglucinol (Kennedy and Jones, 2001).
(A2.2) 'Corvina', 'Moscato di Scanzo' and 'Nebbiolo' were harvested at 18°, 21°, and 23° Brix, then withered up to 20% WL. The content of TFs and ACNs was determined. Musts were produced and analysed for sugar content, titratable acidity (TA), pH, and readily assimilable nitrogen (RAN). Microvinification trials were carried out with skin maceration, and the alcoholic fermentation was daily monitored by weight loss. Organic acids (gluconic, tartaric, malic, lactic, acetic, citric, fumaric, succinic) were quantified by HPLC-UV as described by Fracassetti et al. (2019) in wine.
For both activities, grape samples were homogeneized for the assessment of total TFs and ACNs (Di Stefano et al., 1989).

3. Results and Discussion

3.1 (A2.1) Monitor of withering for different grape varieties

Dehydration kinetics varied across cultivars. 'Moscato di Scanzo' showed the fastest water loss (about 1.05% WL/day), completing withering in around 20 days. In contrast, 'Corvina' had the slowest rate (about 0.3% WL/day), requiring 55–70 days. While comparable levels of TFs were found among the varieties investigated (Figure 1a), 'Aleatico' and 'Moscato di Scanzo' were richest in ACNs, followed by 'Sagrantino' (Figure 1b). 'Corvina' and 'Nebbiolo' had the lowest concentration of ACNs (Figure 1a). As shown in Figure 1a, TFs decreased in all varieties by up to 40%, suggesting that oxidative degradation outweighs concentration from water loss (Scalzini et al., 2024). ACNs decreased at 10% weight loss for 'Corvina', 'Moscato di Scanzo' and 'Nebbiolo' grapes, while remained quite stable for 'Aleatico' and 'Sagrantino' (Figure 1b). Skin PACs dropped sharply from T0 to 10% and continued to decline to 30% in all varieties, except for 'Aleatico' grape showing the highest content of constitutive units at 10% WL (around 400 mg/kg) which follows a decrease below 100 mg/kg at 30% WL. Seed PACs remained mostly stable in 'Aleatico', 'Corvina' and 'Nebbiolo', though some fluctuations were noted, especially at 30% weight loss. On the contrary, seeds PACs exhibited a decreasing trend for 'Moscato di Scanzo' and 'Sagrantino' (data not shown).

Figure 1 ***a)*** *Total Flavonoids (TFs; mg/berry) and* ***b)*** *total Anthocyanins (ACNs; mg/berry) of 'Aleatico', 'Corvina', 'Moscato di Scanzo', 'Nebbiolo' and 'Sagrantino' grapes at T0, 10%, 20%, 30% weight loss.*



3.2 (A2.2) Setting of harvest and withering duration

Musts from 'Corvina', 'Moscato di Scanzo' and 'Nebbiolo' harvested at different Brix showed changes influenced by concentration/depletion effects in a variety-dependent patterns. The increase in sugar content, reflecting greater grape ripeness, was observed only between 18° and 21° Brix. (Table 1). At 23° Brix, 'Corvina' and 'Moscato' showed a slight sugar decrease, probably because of the growth of grey mould, which consumed sugars via respiration. All varieties showed a slight, pH increase during dehydration. Titratable acidity (TA) varied during the withering in a cultivar-dependent behaviour: TA remained fairly stable with a mild rise in 'Corvina', it dropped sharply at 23° Brix in 'Moscato di Scanzo' and it declined steadily in 'Nebbiolo' (Table 1). Readily assimilable nitrogen (RAN) increased in 'Corvina', decreased in 'Moscato di Scanzo', and declined in Nebbiolo from 18° to 21° Brix before stabilizing. The main organic acids were gluconic, tartaric, and malic acids, with traces of citric acid (lower than 0.5 mg/L). Gluconic acid was the most abundant and increased with ripeness, except in 'Moscato di Scanzo', which dropped from 14.1 to 11.2 mg/L between 21° and 23° Brix. Among the organic acids, gluconic acid showed a slight increase in concentration with rising Brix degrees. In contrast, tartaric and malic acids remained relatively constant in the 'Corvina' variety, while they fluctuated in 'Moscato di Scanzo' and 'Nebbiolo', following the same trend as titratable acidity (data not shown). Considering TFs, these compounds increased in 'Corvina' from about 4 to 6.5 mg/berry, they dropped from 3.5 to 1 mg/berry in 'Moscato di Scanzo' due to spoilage, and they remained in 'Nebbiolo' (around 5–5.5 mg/berry). ACNs levels followed decreasing trends in all the grape varieties investigated.

All the fermentation were completed within 12 and 21 days. Differences were found in the fermentation rates. 'Moscato di Scanzo' fermented quickly at 18° Brix (11 days, 24.9 g sugar consumed /day). 'Corvina' had constant and fast fermentation for all 18°Bx, 21°Bx and 23°Bx (about 13 days, 19.5 g sugar consumed/day). In contrast, 'Moscato di Scanzo' and 'Nebbiolo' showed slower fermentation for higher sugar content, respectively about 13.5 and 16.7 g sugar consumed /day.

Table 1 *Must chemical parameters: sugars (g/L), pH, titratable acidity (TA; g/L) and readily assimilable nitrogen (mg/L; RAN) of 'Aleatico', 'Corvina', 'Moscato di Scanzo', 'Nebbiolo' and 'Sagrantino' grapes at T0, 10%, 20%, 30% weight loss*.

Sample	**Sugars** (g/L)	**pH**	**TA** (g tartaric acid/L)	**RAN** (mg/L)
Corvina 18°Bx	215.8±0.6	3.0±0.0	5.1±0.0	161.7±0
Corvina 21°Bx	250.2±0.3	3.2±0.0	5.6±0.0	220.9±11.5
Corvina 23°Bx	238.9±1.6	3.3±0.0	5.7±0.1	257.3±0.1
Moscato 18°Bx	224.5±3.4	2.9±0.0	9.1±0.0	256.1±7.4
Moscato 21°Bx	282.9±7.2	3.1±0.0	10.3±0.0	214.9±0.0
Moscato 23°Bx	265.6±2.5	3.3±0.0	6.8±0.1	120.5±0.0
Nebbiolo 18°Bx	230.6±1.7	3.1±0.0	6.8±0.1	137.0±2.7
Nebbiolo 21°Bx	263.7±4.4	3.2±0.0	5.8±0.0	85.9±2.5
Nebbiolo 23°Bx	263.5±1.0	3.4±0.0	4.4±0.0	79.3±0.7

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Valorization of food by-products: technological strategies for tailored food Ingredients (P-II-8)

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The first activities of the PhD thesis project concerned the valorization of wheat bran, the major by-products of the milling industry, through the recovery of biomolecules. Compound extraction was performed using enzymatic-assisted extraction (EAE). For this purpose, to optimize the process, a Box-Behnken Design (BBD) based on the response surface methodology was employed. The extracts obtained were subjected to chemical analyses to identify and optimize process conditions to obtain protein extracts with favorable technological properties for further application as food ingredients.

Valorizzazione dei sottoprodotti alimentari: strategie tecnologiche per ingredienti alimentari personalizzati

Le prime attività del progetto di tesi di dottorato hanno riguardato la valorizzazione della crusca di frumento, il principale sottoprodotto dell'industria molitoria, attraverso il recupero di biomolecole. L'estrazione dei composti è stata effettuata mediante estrazione enzimatica assistita (EAE); a tal fine, per ottimizzare il processo, è stato impiegato un disegno Box-Behnken (BBD) con metodo della superficie di risposta. Gli estratti ottenuti sono stati sottoposti ad analisi chimiche per identificare e ottimizzare le condizioni di processo al fine di ottenere estratti proteici con proprietà tecnologiche favorevoli per ulteriori applicazioni come ingredienti alimentari.

Key words: Enzyme-assisted extraction, Box-Behnken design, wheat bran, techno-functional properties, new food ingredients.

1. Introduction

In accordance with the PhD thesis project previously described (Ferraioli, 2024), this poster reports the main results of the first two activities concerning:

(A1) **Optimization of EAE for wheat bran:** enzymatic extraction was performed using an alkaline protease to obtain the recovery of proteins and antioxidant compounds.

(A2) **Techno-functional characterization of the extracts:** optimized extracts were characterized by their technological properties (water and oil holding capacity, emulsifying ability).

2. Materials and Methods

The recovery of proteins and antioxidant compounds from wheat bran was carried out through EAEP with alcalase protease, comparing it with the aqueous extraction process (AEP). Wheat bran was mixed with milliQ water at the ratio of 1:10 (w/v), adjusting the pH to 8.60 with carbonate buffer pH 9. Box-Behnken design (BBD) by response surface method (RSM) was employed to investigate the effects of extraction parameters and determine optimum process conditions. The following conditions were explored: enzyme concentrations (E/S) (500-1500 U/g), temperature (20-30°C) and time (20-60 min). Protein yield, degree of hydrolysis (DH%), antioxidant activity (ABTS) and zeta potential were chosen as dependent variables. The optimal process conditions were defined to maximize protein extraction yield and minimize DH%, while modulating the antioxidant capacity. On the optimized extracts, water (WHC) and oil (OHC) holding abilities were studied, as well as emulsifying properties. Data were statistically elaborated through analysis of variance (ANOVA) and Duncan's test.

3. Results and Discussion

3.1 Protein yield and DH%

The temperature affects the efficiency of extraction yield (Fig. 1a), by softening tissues and increasing solubility, which contributes to enhancing protein and polyphenols recovery (Ilaiyaraja *et al.*, 2015). Meanwhile, results show that at 25°C, the lowest protein yield is achieved, followed by 20°C and finally by 30°C. Concerning the independent variable E/S, its effect on protein yield is more evident, as the higher this parameter, the higher the yield. The pivotal role of enzymatic treatment is underscored by the findings of control (CTR), where the absence of enzyme application results in a markedly lower protein yield. Results agree with the literature, where the use of a higher amount of enzyme, to a certain extent, determine higher DH% (Souza *et al*., 2019). The highest DH% (Fig. 1b) is achieved under conditions of 1500 U/g, 40 min, and 25 °C, suggesting that prolonged exposure and

higher enzyme concentrations at moderate temperatures facilitate extensive protein breakdown. E/S ratio is a key factor influencing the reaction rate. As the E/S ratio increases, DH% generally rises due to more enzyme molecules being available to catalyze the breakdown of peptide bonds, leading to enhanced protein solubilization.

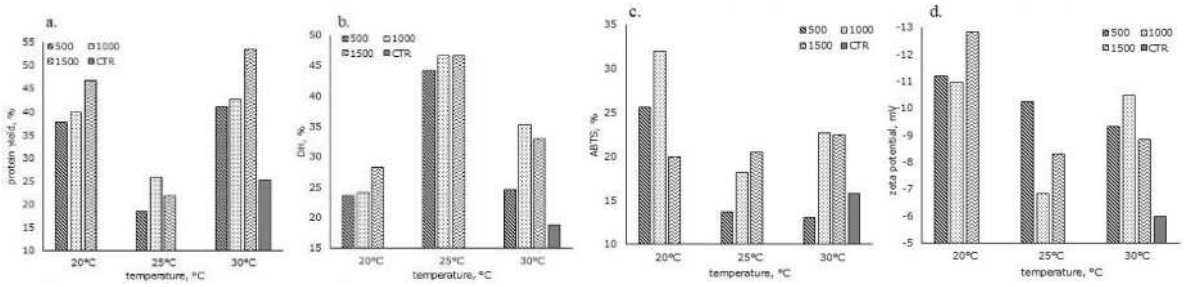


Figure 1: *Effect of enzymatic extractions with enzyme concentration (500-1500 U/g) and temperature (20-30°C) on measured responses (a. protein yield, b. degree of hydrolysis, c. ABTS and d. zeta potential), compared to untreated control CTR.*

3.2 Antioxidant activity and zeta potential

The highest antioxidant activity peaks under conditions of 1000 U/g, 20 min, and 20 °C (Fig. 1c), probably due to low molecular weight peptides. Those peptides are generally associated with enhanced antioxidant activity in contrast to larger peptides due to their smaller steric hindrance in the active center of the short chain that could easily approach and react to free radicals (Nwachukwu and Aluko, 2019). The pivotal role of enzymatic treatment is underscored by the findings that CTR results in a markedly lower amount of antioxidant compounds. Zeta potential of the hydrolysate samples is higher than that of the control one (Fig. 1d). This is due to the increase in the net charge on the protein hydrolysate. The increase in negative charges might be due to the increase in the number of hydrophobic amino acid side chains after enzymatic hydrolysis (Zaitoun *et al*., 2022).

3.3 Functional properties of optimized extracts

Enzymatic optimized extracts show higher WHC values than the control, particularly Op1 and Op3 (Table 1), likely due to the exposure of hydrophilic groups during hydrolysis. Op2 presents the highest OHC value (~6 g/g), suggesting greater exposure of hydrophobic residues. These effects support the idea that hydrolysis unfolds proteins, enhancing their binding properties. The emulsifying activity index (EAI) decreases with increasing enzyme concentration, likely due to the formation of smaller peptides less effective at stabilizing interfaces. In contrast, the emulsion stability index (ESI) peaks at Op2 (~65 min), indicating improved emulsion stability, likely due to medium-sized peptides capable of forming cohesive interfacial films. These results align with Bozkurt et al. (2021), who report similar findings with hydrolyzed glutenin.

Table 1: *Functional properties of optimized extracts Op1, Op2 and Op3 compared to the control (CTR). In order Water Holding Capacity (WHC); Oil Holding Capacity (OHC); Emulsifying Activity Index (EAI) and Emulsion Stability Index (ESI).*

Samples	E/S	Time	Temperature	WHC (g/g)	OHC (g/g)	EAI (m²/g)	ESI (min)
CTR	-	60	30	0.127 ± 0.006^{a}	2.737 ± 0.089^{b}	43.008 ± 0.241^{d}	8.875 ± 0.013^{a}
Op1	500	40	30	1.297 ± 0.019^{d}	3.585 ± 0.031^{c}	19.674 ± 0.440^{b}	14.836 ± 0.032^{b}
Op2	1000	20	20	0.93 ± 0.013^{b}	5.932 ± 0.220^{d}	28.853 ± 0.058^{c}	61.029 ± 3.730^{c}
Op3	1500	40	30	1.188 ± 0.580^{c}	2.063 ± 0.053^{a}	16.829 ± 0.109^{a}	11.149 ± 0.005^{a}

Data are means ± standard deviations of triplicate determinations (n = 3).
In response parameter columns, distinct letters denote statistically significant differences per Duncan's test.

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Emerging Processing Technologies for the Sustainable Production of Innovative Foods (P-II-9)

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Topic: Food Science and Biotechnology; Doctoral cycle: XXXIX; Year of the attendance: II
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The first two activities of the PhD thesis project are described. Firstly, the protein extraction and modification of bioproducts, in this study rapeseed cake (RC), was investigated. Pulsed electric fields (PEF) and ultrasound (US) were used as pretreatment. Secondly, the drying and rehydration kinetics of fresh green peas were investigated. A PEF pretreatment was performed to find out whether it has an influence on the drying and rehydration capacity and the quality characteristics of the peas.

Tecnologie emergenti di processo per la produzione sostenibile di alimenti innovativi

Le prime due attività del progetto di tesi di dottorato hanno riguardato, in primo luogo, l'utilizzo di tecnologie innovative per l'estrazione e la modificazione di proteine da sottoprodotti, specificamente da un panello di colza post-estrazione dell'olio. A tale scopo sono stati utilizzati campi elettrici pulsati (PEF) e ultrasuoni (US). In secondo luogo, è stata studiata la cinetica di essiccazione e reidratazione di piselli verdi freschi differentemente trattati e la loro modificazione qualitativa.

Keywords: Pulsed electric field, protein extraction, protein modification, drying kinetics, mathematical modelling.

1. Introduction

In accordance with the PhD thesis project previously described, this poster reports the main results of the first two activities concerning:

(A1) the investigation of protein extraction and physico-chemical and structural modification in rapeseed cake pre-treated with PEF and US;

(A2) the evaluation and modelling of the drying, rehydration capacity and quality properties of green fresh peas pre-treated with PEF.

2. Materials and Methods

(A1) The PEF pretreatment was carried out with a laboratory-scale PEF plant in batch mode. The RC was treated in distilled water at a ratio of 1:20 and the pH was adjusted with 1M NaOH. Each treatment was carried out with an electric field strength of 1.7 kV/cm and 28 kJ/kg at a pH of 11.5, 71 kJ/kg at a pH of 11.5, 24 kJ/kg at a pH of 7 and 65 kJ/kg at a pH of 7. After PEF pretreatment, the pH of all samples was adjusted to 11.5. The extraction process was then carried out by stirring the samples for 3 hours at 35°C. The pH was checked every 20 minutes and adjusted with 1 M NaOH if necessary. The solutions were centrifuged using the at 4000 rpm at 25°C for 20 minutes and the supernatant was collected. Then the pH was adjusted to 4.5 and kept overnight at 4°C in a refrigerator for isoelectric precipitation. Finally, the protein was extracted, the pH adjusted to 7.0 and freeze-dried. Finally, the protein samples were analysed: Colour, Fourier transform infrared spectroscopy, differential scanning calorimetry (DSC) (Jia et al., 2021), water holding capacity (WHC), oil holding capacity (OHC) (Zhang et al. 2017), emulsifying activity and stability (Georgiev et al. 2022), protein solubility (Boye et al. 2010). A further test was carried out with US. Frequency and duration of treatment were the independent variables. The frequency varied between 25 and 45 kHz and the duration of treatment was 20, 40 and 60 minutes. Later, the same alkaline extraction process as in the first experiment was performed and the protein was recovered. WHC, OHC and DSC analyses were performed to understand the thermal stability and physicochemical changes of the rapeseed protein.

(A2) The fresh green peas were mixed with water and placed in the treatment chamber. Tap water with a conductivity of 0.3 µS/cm, determined with the EC-Metre basic 30+ conductivity metre, was used as the conductivity medium in the treatment chamber. The device used as a fixed pulse width of 20 µs. The specific energy of the treatment was 25 kJ/kg and 50 kJ/kg and the electric field 1.8 kV/cm. Untreated and PEF-pretreated fresh green peas were dried in a hot air dryer. Three different drying temperatures were used: 50°C, 60°C and 70°C. The air velocity was 2 m/s. During drying, the weight of the sample was recorded every 10 minutes. Six different mathematical models were applied to the drying kinetics to evaluate the effects of PEF pretreatment on the drying process at each temperature. The drying curves were plotted as a function of the dimensionless moisture content (MR) during drying. MR was calculated as the moisture content at any point (Mt, kg water/kg dry matter) in time divided by the initial moisture content (Mo, kg water/kg dry matter). The regression analysis was performed with R Studio. To explain the goodness of fit of each model, the correlation coefficient (R2), root mean square error (RMSE) and sum of squared errors (SSE) were calculated. The

shrinkage of the samples was determined by calliper measurement and the colour changes by CVS analysis using Image-J software. In addition, the effective moisture diffusivity, activation energy and thermodynamic calculations were performed using equations (Doymaz and Kocayigit 2011). The rehydration capacity was measured by soaking about 2 g of the dried samples in distilled water (1:50 w/w) at room temperature. The weight of the samples was initially recorded every half hour for 3 hours and later every hour for 8 hours using a digital balance (sensitivity 0.001 g). The rehydration capacity was defined as the weight after rehydration (g) divided by the weight of the dried material (g). During rehydration, protein release from the peas was analysed by spectrophotometric analysis with a quartz cuvette at 280 nm.

3. Results and Discussion

3.1 Effect of pulsed electric field (PEF) as a pretreatment on isoelectric extraction of protein from Rapeseed Cake

PEF treatment in combination with pH adjustment significantly influenced the physicochemical properties of rapeseed protein (RP), affecting WHC, PS, thermal stability, secondary structure and rheology. RP is more soluble by treatment at alkaline pH with a specific energy of 65 kJ/kg (PEF4) (Fig1). The rheological analysis showed that PEF4 even leads to higher viscosity and shear stress (Fig2). The PEF treatment led to structural changes in the proteins, suggesting improved stability and functionality. US treatment at 45 kHz for 40 minutes led to maximum improvement in the WHC (from 4.56 g/g to 5.19 g/g) of the protein samples and DSC analysis revealed lower heat stability of the US-treated samples compared to the control samples.

***Figure 1**. Protein solubility of the PEF pretreated and control samples*

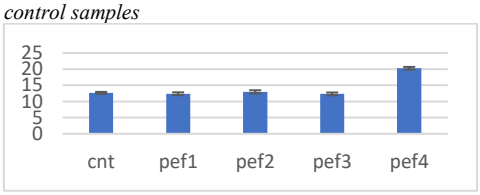
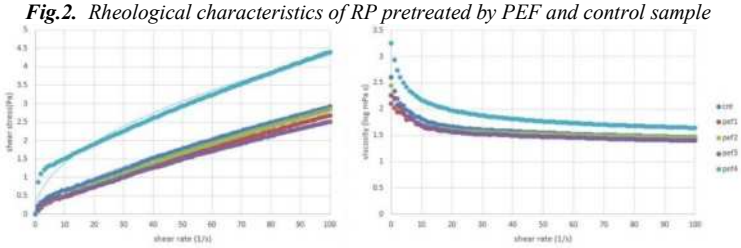


***Fig.2.** Rheological characteristics of RP pretreated by PEF and control sample*



3.2 Optimizing Green Pea Drying: Effects of Pulses Electric Field on Drying Kinetics and Sustainability

Depending on the drying temperature, PEF pretreatment improved the drying rate of peas by 20 to 80. A stronger improvement was observed at 70°C. A higher specific application resulted in faster drying. All mathematical models showed a good fit (R^2: 0.923–1.0). The activation energy ranged from 5.45 to 28.7 kJ/mol, with the PEF-treated samples requiring more energy to initiate diffusion. Thermodynamic calculations confirmed PEF and double PEF increase the resistance of proteins to heat (higher ΔH) while maintaining the positive entropy typical of unfolding ($\Delta S > 0$). The process remains thermodynamically favourable ($\Delta G < 0$), but higher PEF energy (PD) does not further improve stability, indicating a limit to the benefit. PEF pretreatment also dramatically improved the rehydration capacity of peas. However, the PEF-treated samples showed a high protein release to the rehydration water, especially those that had been dried at higher temperatures. The PEF pre-treatment drastically reduced shrinkage. Instead, the colour changes between the PEF-treated and control samples: L and a value decreased and b values all increased in the PEF-treated samples, indicating that PEF pretreatment increased darkening, the PEF-treated samples became greener and yellowing also increased.

Figure 2 : Drying kinetics of C, P, and PD samples.

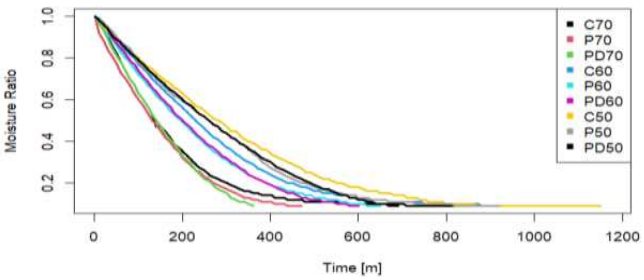
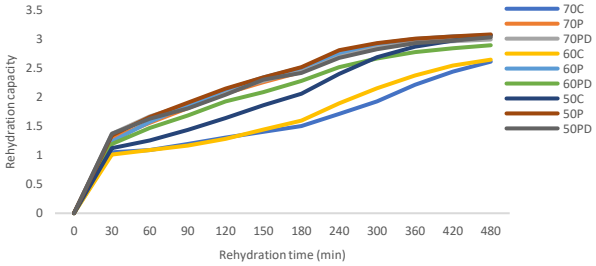


Figure 3 : Rehydration capacity of C, P, and PD samples.



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Bioactive compounds extracted through green technologies from lemon peels, fennel and tomato waste: their potential use as antioxidant (P-II-10)

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Tutor: Prof. Raffaele Romano

This manuscript presents the first three activities of the PhD thesis. In the first experiment, ultrasound- (UAE) and microwave-assisted extraction (MAE) were applied to fennel stems and bulbs to obtain bioactive-rich extracts. The second experiment used accelerated solvent extraction (ASE) with citric acid to recover pectin and other bioactive compounds from spent lemon peels. Lastly, a cascade extraction was performed on tomato seeds, combining supercritical CO_2 extraction (SFE) and microwave extraction. All processes were optimized using Design of Experiments (DoE) and Response Surface Methodology (RSM), and extracts were characterized via chromatographic and spectroscopic analyses.

Composti bioattivi estratti attraverso tecnologie green scarti di bucce di limone, finocchi e pomodoro: il loro potenziale utilizzo come antiossidanti

Questo manoscritto presenta le prime tre attività della tesi di dottorato. Nel primo esperimento, l'estrazione assistita da ultrasuoni (UAE) e da microonde (MAE) è stata applicata a steli e bulbi di finocchio per ottenere estratti ricchi di composti bioattivi. Il secondo esperimento ha impiegato l'estrazione accelerata con solvente (ASE) con acido citrico per recuperare pectina e altri composti bioattivi da bucce esauste di limone derivanti dalla produzione del Limoncello. Infine, è stata eseguita un'estrazione a cascata dai semi di pomodoro, combinando l'estrazione con CO_2 supercritica (SFE) e l'estrazione a microonde. Tutti i processi sono stati ottimizzati mediante Design of Experiments (DoE) e Response Surface Methodology (RSM), e gli estratti sono stati caratterizzati tramite analisi cromatografiche e spettroscopiche.

Key words: Green extraction, Chemometric Approach, Food Waste, Design of experiment, Food Processing.

1. Introduction

In accordance with the PhD thesis project, this poster reports the main results of the first three experiments:
(E1) Optimization of a green extraction process (MAE and UAE) from fennel bulbs and stems;
(E2) Optimization of a green extractive process (ASE) from Lemon peels derived from the production of Limoncello liqueur.
(E3) Assembly of a cascade extraction from tomato seeds for sequential recovery of all bioactive fractions.

2. Materials and Methods

(**E1**) A Box-Behnken Design (BBD) was used to evaluate the effects of extraction variables on chlorogenic acid recovery. Three factors were tested at three levels: Power (80–120 W), Time (10–30 min), and Solid/Liquid ratio (25–100 g/L) for UAE; Temperature (50–70 °C), Time (10–30 min), and Solid/Liquid ratio for MAE. Extracts obtained under optimal conditions were analyzed for total polyphenol content (TPC), antioxidant activity, VOCs, and ascorbic acid.
(**E2**) A Central Composite Design (CCD) with RSM was used to optimize pectin extraction, testing Temperature (50–70 °C), Time (5–20 min), and pH (1.5–2.5). Citric acid was used as a green acidifying agent compared to the normal acids used industrially to extract pectins (HCl or H_2SO_4). Optimal extracts were analyzed for ethanol, TPC, antioxidant activity, galacturonic acid, individual polyphenols, and vitamin C.
(**E3**) For CO_2 extraction, a CCD with RSM was applied to optimize yield (%) based on Pressure (150–350 bar), Flow (6–10 mL/min), and Temperature (20–60 °C). For microwave extraction, the same method was used with Temperature (25-70°C), Time (5-30 min), and Solid/Liquid ratio (10-50 mg/mL) as factors to optimize sugar content.

3. Results and Discussion

3.1 Optimization of UAE and MAE for bulbs and stems of fennel

ANOVA tests showed that for UAE of stems, significant factors ($p < 0.05$) included Power, Time, Time*Solid/Liquid ratio, and Solid/Liquid ratio²; for bulbs, Power, Time, Solid/Liquid ratio, Power², Power*Time, and Time². For MAE of stems, Temperature, Time, and their quadratic and interaction terms were significant; for bulbs, Temperature, Time, Temperature², Temperature*Time, and Solid/Liquid ratio² were significant. Optimal MAE conditions were 70°C, 30 min, 64 g/L for bulbs (BOMW) and 58.6 g/L for stems (SOMW). Optimal UAE conditions were 117.4 W, 17 min, 100 g/L for bulbs (BOUS) and 100 W, 30 min, 100 g/L for stems (SOUS).

Chlorogenic acid content (mg/L) was highest in SOMW (39.57), followed by BOMW (29.35), BOUS (24.18), and SOUS (3.03). SOMW also showed the highest antioxidant activity (~85% inhibition). Ascorbic acid was highest in SOUS (380 mg/L), followed by BOUS (184), SOMW (170), and BOMW (112). MAE yielded more chlorogenic acid than UAE, likely due to reduced ultrasound cavitation in stems with high fiber content, which increases solvent viscosity (Hao et al., 2022; Martinez-Solano et al., 2021).

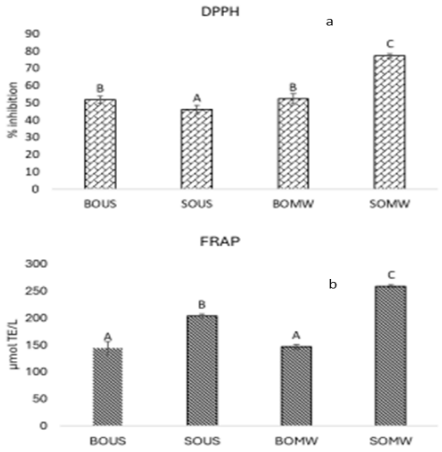


Figure 1: Antioxidant activity of extract obtained in optimal condition

3.2 Optimization of ASE for exhausted lemon peels

ANOVA tests revealed that linear factors such as Temperature and pH and the quadratic factor of pH all had p-values < 0.05, thus confirming their statistical significance. The optimal conditions that resulted in the highest pectin extraction yield were 64°C, 13 min and a pH of 2. The pectin extraction yield from the extract was 0.44% confirming the value predicted by the experimental model. Table 1 shows the values of antioxidant activity, individual polyphenols, ascorbic acid and ethanol present in the extract obtained in optimal conditions and the control obtained with citric and hydrochloric acid without the green technique. Increased pressure in ASE leads to facilitated cell wall rupture and consequently increased extraction and solubilization of bioactive compounds (Chaves et al., 2022).

Table 1: Chemical Characterization of the extract obtained in optimal condition (ASE-Optimal) and the control with citric acid (Cont-CIT) and Chloridic acid (Cont-HCl)

Sample	DPPH (IC50)	FRAP (µM TE/L Extract)	Ascorbic Acid (mg/L)	Hesperidin (mg/L)	Naringin (mg/L)	Rutin (mg/L)	Ethanol content (%)
ASE-Optimal	0.39 ± 0.02^{c}	97.61 ± 6.13^{a}	60.55 ± 2.47^{a}	36.75 ± 0.35^{a}	12.25 ± 0.21^{a}	71.83 ± 1.20^{a}	0.16 ± 0.01^{b}
Cont-CIT	0.43 ± 0.01^{b}	78.72 ± 2.39^{b}	24.1 ± 2.12^{b}	34.95 ± 0.63^{b}	10.50 ± 0.42^{b}	35.18 ± 3.25^{b}	0.21 ± 0.01^{a}
Cont-HCl	0.46 ± 0.04^{a}	57.10 ± 3.54^{c}	28.35 ± 0.64^{b}	32.9 ± 1.55^{c}	10.55 ± 0.22^{c}	27.84 ± 2.44^{b}	0.09 ± 0.01^{c}

3.3 Optimization of CO_2 extraction for tomato seeds

ANOVA showed that factors such as pressure and flow have the most significant influence on yield by both contributing to an increase in extraction efficiency.

The optimal extraction conditions were identified using a desirability function, which predicted a maximum yield of 20.71% at a pressure of 350 bar, a flow rate of 10 mL/min, and a temperature of 40.5 °C. The experimentally obtained yield was 20.61 ± 0.13%, demonstrating the robustness of the model (R^2 = 95.68%). The content of Stigmasterol, β-sitosterol, and α-tocopherol in the optimal extract was 1.25 mg/g, 3.03 mg/g, and 0.26 mg/g, respectively. For subsequent microwave-assisted sugar extraction, the only statistically significant factor is the solid/liquid ratio, which shows a negative correlation with the response, and thus as the level of the factor increases, sugar recovery decreases

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Characterization of doughs and flours for leavened bakery products: correlation with process parameters and finished product quality for continuous improvement (P-II-11)

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Tutor: Prof. Fernanda Galgano; Co-tutors: Dr. Maria Di Cairano, Dr. Cristiano Paganoni, Dr. Lucia C. Mecca

This PhD project aims to investigate how dough characteristics and process parameters influence the quality of leavened bakery products, specifically minicakes. Industrial data collection combined with rheological analyses has revealed correlations between dough behavior during processing and the occurrence of defects in the final product. A scientific and data-driven approach could be useful for predicting dough behavior and supporting process optimization and quality control of final product. The research activities are part of an industrial PhD project co-funded by Barilla G. e R. Fratelli S.p.A.

Caratterizzazione di impasti e farine per prodotti da forno a lievitazione naturale: correlazione con i parametri produttivi e con la qualità del prodotto finito, in ottica di miglioramento continuo

Questo progetto di Dottorato ha l'obiettivo di indagare in che modo le caratteristiche dell'impasto e i parametri di processo influenzino la qualità dei prodotti da forno lievitati, in particolare merendine. La raccolta di dati su scala industriale, combinata con analisi reologiche, ha evidenziato correlazioni tra il comportamento dell'impasto durante la lavorazione e la comparsa di difetti nel prodotto finito. Un approccio scientifico basato sull'analisi dei dati potrebbe risultare utile per prevedere il comportamento dell'impasto e supportare l'ottimizzazione del processo e il controllo qualità del prodotto finito. Le attività di ricerca rientrano in un progetto di Dottorato industriale co-finanziato da Barilla G. e R. Fratelli S.p.A.

Key words: Bakery products, dough, process parameters, rheology analysis

1. Introduction

The increasing consumer demand for leavened bakery products with high quality standards has brought attention to the optimization of dough characteristics and production processes (Lomonaco et al.,2025). In this context, the PhD project aims to investigate the relationship between raw materials, dough rheology, and process parameters, with a special focus on their effect on final product quality. Industrial production often introduces variability linked to factors such as dough temperature, dough consistency, water content and mechanical stress during forming (Jerome et al.,2019). These elements can affect the structural integrity of the product, and the amount of waste. The overarching goal of the research is to develop a data-driven and experimental framework that enables continuous process improvement and standardization in leavened baked goods. Understanding these interactions is essential to reduce defects, optimize resource use, and respond more effectively to consumer expectations.

2. Materials and Methods

The study was carried out in collaboration with the Barilla plant in Melfi (PZ), which produces leavened minicakes. Data were collected over a seven-month period (September 2024 – April 2025) and included observations from 103 dough batches, obtained through on-line control charts, operator reports, and automated sensor systems. Three main production stages were monitored:

Dough stage
- Parameters recorded: temperature, farinograph consistency, water content, presence and amount of rework, and kneading parameters;
- Equipment: Brabender® FarinoGraph®-TS, temperature probe.

Process stage
- Hourly control charts for monitoring forming operations;
- Parameters: extrusion speed, mold weight distribution (left vs right), forming deviations.

Finished product stage
- Sampling every 30 minutes: 3 multipacks per batch (200–300 minicakes/day)
- Parameters: weight, height, width, length, structural integrity (plate breakage), and consumer complaints;
- Equipment: Sensure® vision system, digital scales, dimensional gauges.

Data was analyzed through descriptive statistics and correlation matrices.

3. Results and Discussion

Only part of the results is reported here due to the company's confidentiality agreement.
The analysis of the data collected during the second year allowed the identification of key factors influencing the consistency of the dough and the quality of the finished product:

1. Dough temperature and consistency

The results confirm that dough consistency is influenced by temperature: as the dough temperature rises from approximately 12 °C to 17 °C, a progressive decrease in farinograph consistency is observed (Fig. 1). This behavior is generally attributed to a plasticization of the gluten-starch matrix, resulting in a softer dough structure (Della Valle et al., 2022). Temperature represents a critical process parameter. In this context, regulating dough temperature through controlled CO_2 injection during mixing contributes to the optimization of rheological properties, maintaining consistency within optimal processing ranges. This approach ensures batch-to-batch reproducibility and minimizes variability in dough behavior during downstream phases such as extrusion and shaping.

2. Impact of rework added in the dough

Figure 2 illustrates dough consistency as a function of rework addition levels (0%, 3%, and 4.5%) along with corresponding water adjustments. The results indicate that incorporating low levels of rework (up to 4.5%) does not significantly affect dough consistency (p= 0,696), provided that water content is appropriately increased to offset the absorption properties of the reworked material. Specifically, doughs with 3% maintain consistency levels comparable to the control. These findings suggest that proper water compensation effectively stabilizes dough consistency across different rework percentages, thereby ensuring process steadiness.

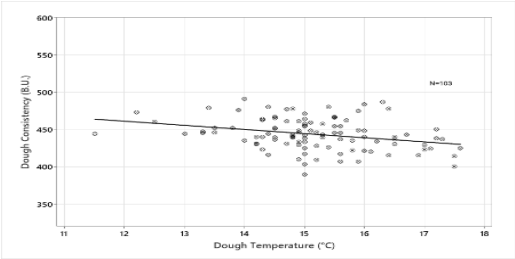


Figure 1 *Dough consistency vs dough temperature*

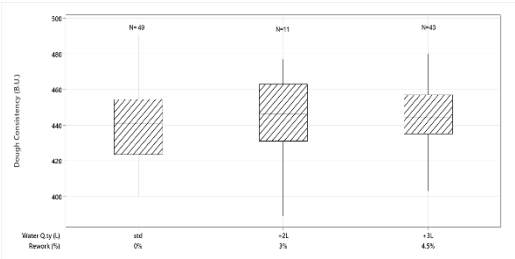


Figure 2 *Boxplot of the consistency of the dough relation to the content of water and reworked added to the dough.*

3. Breakage in final products

Figure 3 shows that higher average dough consistency values (440–460 B.U.) during the first five production days considered (about 10 doughs per day) coincided with increased product breakage, while lower consistency values in the following days correlated with fewer defects (sampling on 300 minicakes per day). This suggests that stiffer doughs may contribute to breakage due to structural stress. Although not conclusive, the trend highlights the importance of maintaining optimal consistency levels and adjusting process parameters to improve product uniformity. Further studies are needed to confirm this correlation.

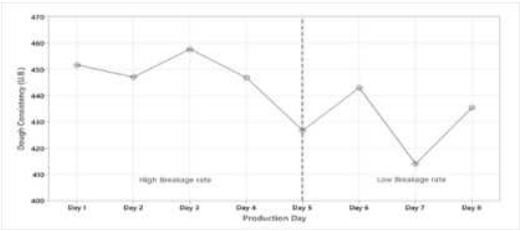


Figure 3 *Effect of consistency on breakage percentage of minicakes in different days of production.*

The study identified key process factors (temperature, water content, and rework) that influence dough consistency and product quality. These outcomes emphasize that understanding the correlations across different process stages is essential for ensuring product quality and minimizing defects.

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Impact of Supplier Variability and Storage Time on the Hydration and Mechanical Behavior of Durum Wheat Semolina (P-II-12)

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This study examines the effect of suppliers and annual variation on the hydration and mechanical properties of durum wheat semolina produced in a local pasta factory. Samples from different suppliers and two production years (2024 and 2025) were analyzed for moisture, color, particle size, water absorption, and dough texture. Statistical analyses (ANOVA and PCA) showed significant year- and supplier-related differences, especially in water absorption capacity, cohesiveness, and humidity. Results demonstrated that evaluating the characteristics of raw materials and their behavior can optimize pasta processing and improve product consistency.

Impatto della variabilità del fornitore e del tempo di conservazione sul comportamento di idratazione e sulle proprietà meccaniche della semola di grano duro

Questo studio analizza l'effetto della variabilità tra fornitori e anni di produzione sulle proprietà di idratazione e meccaniche della semola di grano duro, utilizzata da un pastificio locale. Campioni provenienti da diversi fornitori e da due anni consecutivi (2024 e 2025) sono stati analizzati per umidità, colore, granulometria, capacità di assorbimento d'acqua e consistenza dell'impasto. Le analisi statistiche (ANOVA e PCA) hanno evidenziato differenze significative legate sia all'anno di produzione che al fornitore, in particolare per quanto riguarda la capacità di assorbimento d'acqua, la coesività e il contenuto di umidità. I risultati ottenuti dimostrano che la valutazione sistematica delle caratteristiche delle materie prime e del loro comportamento tecnologico può contribuire a ottimizzare il processo di pastificazione e a migliorare la costanza qualitativa del prodotto finale.

Keywords: Semolina quality, Dough properties, Multivariate analysis, Raw material.

1. Introduction

In accordance with the PhD thesis project, this poster reports the main results of the first two activities concerning:
(A1) Physical and mechanical properties of durum wheat semolina;
(A2) Evaluating semolina variability related to different suppliers and periods using one-way ANOVA and PCA.

2. Materials and Methods

Durum wheat semolina samples were collected from different suppliers in two different periods, 2024 and 2025. Each sample was analyzed for color (CIELab), moisture content, water absorption capacity, and particle size distribution (600–150 µm range). Semolina was then hydrated to 45% moisture, and dough samples were prepared for textural analysis. Dough hardness and cohesiveness were measured after 30 minutes of resting using an Instron Universal Testing Machine (Model 5500 Series, Instron, Norwood, MA, USA). Statistical analyses were carried out using one-way ANOVA and principal component analysis (PCA) to evaluate the effects of year and supplier variability.

3. Results and Discussion

3.1 Determination of the physical and mechanical properties

Cohesiveness measures the extent to which the pasta can be deformed before it ruptures; it significantly influences pasta processing and final product quality (Ramos et al., 2023). The cohesiveness of dough prepared from durum wheat semolina significantly varied across both production years and among suppliers (Fig. 1). For the 2024 samples, cohesiveness ranged from approximately 0.31 to 0.56. The sample "CMM_2" exhibited notably higher cohesiveness. Conversely, the sample "LM_1" had the lowest cohesiveness. This may reflect weaker gluten interactions, possibly resulting from mechanical disturbance, structural heterogeneity, or quality deviation factors previously identified as influencing dough cohesiveness by Ramos et al. (2023). A similar variability was observed in 2025 samples, ranging between 0.34 and 0.56. The samples "CMM_4" and "LM_9" showed the highest cohesiveness, while "CAS_1" and "MS_3" displayed significantly lower values. Cohesiveness varied significantly even across samples from the same supplier within the same production year, indicating that factors other than the supplier, such as processing conditions, storage, or semolina batch variability, are important. This observed variation in cohesiveness has a significant impact on quality, emphasizing the necessity of supplier and quality management in ensuring product consistency.

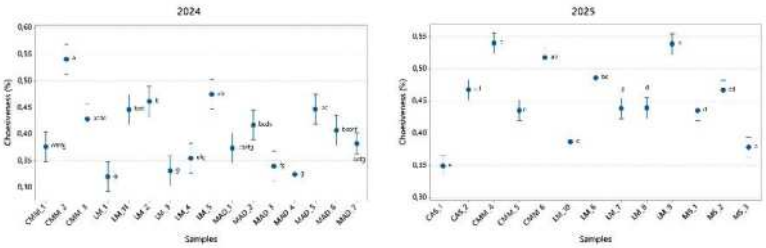


Figure 1 *Normalized values of dough cohesiveness measured in semolina samples across production years (2024 and 2025) and different suppliers.*

Water absorption capacity (WAC) is also a significant parameter that can affect the quality of the final product; it showed clear variability across different suppliers and production years (Fig. 2). The variability in WAC was higher in 2024 than in 2025; in 2024, two of the three providers (CMM, MAD) had significantly different behavior, but in 2025, just one supplier (LM) diverged from the trend. Some suppliers like "CMM_1" display notably higher absorption, because of the higher dietary fiber content, as demonstrated by Zhu et al. (2024). In contrast, "MAD_1" exhibited lower water absorption. This behavior may be related to differences in particle size distribution and granule structure, factors that have been shown to influence water absorption in semolina, as reported by Laurent et al. (2023).

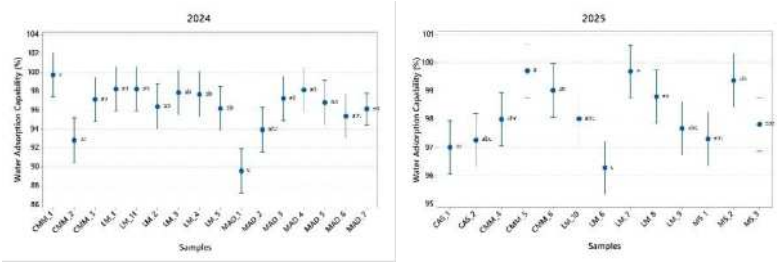


Figure 2 *Water absorption capacity (WAC) of durum wheat semolina was evaluated across production years (2024 and 2025) and different suppliers.*

Principal component analysis (Fig. 3) revealed a clear separation of samples by years and partially by supplier origin. The primary contributors to variability along the first two principal components were cohesiveness, WAC, moisture, and the b* color parameter. These variables are known to reflect semolina hydration behavior and dough structure, suggesting their key role in determining processing performance (Vignola et al., 2025). The PCA results also highlighted that, even within the same supplier, considerable variability exists across different production batches and times, reinforcing the need for regular quality assessment and predictive monitoring using rapid and non-destructive methods such as in-line near-infrared (NIR) spectroscopy.

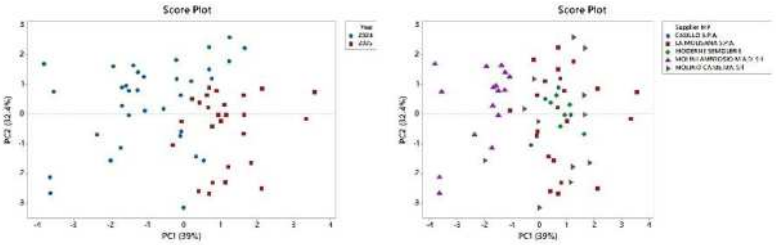


Figure 3 *Principal Component Analysis (PCA) of durum wheat semolina samples. Score plots showing the distribution of samples based on production years and suppliers, using as parameters WAC, Colour, Humidity, Cohesiveness, Hardness)*

In conclusion, the results of this study highlight the relevance of the characteristics of raw material variability during pasta processing and encourage further investigation into the relationships between characteristics such as granulometry and hydration kinetics on the final product behavior and quality.

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Characterization Of Proteins From Barley Malting By-Products For Food Applications (P-II-13)

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In this work, the protein concentrates obtained after alkali extraction of industrial malting rootlets were characterized in terms of nutritional profile (amino acid composition and protein digestibility) and techno-functional properties (solubility, foaming and emulsifying abilities, SDS page, water holding capacity and fat absorption capacity). Data were further compared with those obtained on common plant-derived proteins such as soy, pea and rice, to elucidate the potential application of protein from malt rootles in novel food formulations.

Caratterizzazione delle proteine da sottoprodotti della maltazione dell'orzo in ottica di utilizzo in formulazioni alimentari

In questo lavoro, i concentrati proteici ottenuti dopo l'estrazione alcalina su radichette di malto sono stati caratterizzati in termini di profilo nutrizionale (composizione degli amminoacidi e digeribilità proteica) e proprietà tecno-funzionali (solubilità, capacità schiumogene ed emulsificanti, water holding capacity and fat absorption capacity). I dati sono stati ulteriormente confrontati con quelli ottenuti su proteine di origine vegetale comuni, quali soia, piselli e riso, per chiarire la potenziale applicazione delle proteine da radichette di malto in nuove formulazioni alimentari.

Key words: alternative proteins, malt rootlets, techno-functional

1. Introduction

In accordance with the PhD thesis project previously described, this poster reports the main results of the activities concerning:

(A1) the characterization of barley malt rootlets proteins through the assessment of nutritional proprieties in terms of amino acids content and protein digestibility;
(A2) the study of their techno-functional properties.

2. Materials and Methods

Two types of malting by-products were considered: barley malt rootlets (MR) obtained after malt deculming step, and barley malt residual pellet (MRP). Proteins were extracted following alkali extraction and isoelectric precipitation, while three commercial protein powders (soya bean, pea and rice) (average protein content: 90% for soy and pea, and 80% for rice) were selected for the comparison of nutritional and functional properties. To determine the composition of amino acids, a prior 24 h hydrolysis was performed using hydrocloridric acid 6N and samples analyzed using HPAEC-PAD. In vitro protein digestion (Tinus et al., 2012) was performed using a multienzyme solution protocol. Relative protein solubility (PS) was determined at various pH values between 2 and 12, according to the protocol adapted from methods of Boye et al. (2010). Foaming properties were expressed as foaming capacity (FC) and foam stability (FS) as described by Barac et al., (2015) with some modifications. Emulsifying properties were analysed according to the methods described by Pearce & Kinsella, (1978). Water holding capacity (WHC) was determined according to AACC (2000c) method 56–30 with slight modification according to Boye et al. (2010). Fat absorption capacities were determined using the procedure by Lin et al. (1974) with slight modifications. Triplicate data were statistically evaluated by one-way analysis of variance (ANOVA) and Tukey's Multiple Comparison Test procedure at the 5% significance level ($p<0.05$).

3. Results and Discussion

The characterization of proteins-rich ingredients obtained from malting by-products evidenced significant differences in their functional and nutritional properties when compared to other plant-based proteins, such as soy, pea, and rice. MR and MRP, extracted using an alkaline method, showed a favorable amino acid profile, with appreciable lysine content (7.12% and 6.12% of total protein for MR and MRP, respectively), also when compared to FAO amino acid patterns (Fig. 1). Digestibility data indicated that MR and MRP were comparable to soy and pea proteins (~75%), emphasizing their potential for incorporation into plant-based food products.
Protein solubility trends of malt rootlets were similar to that of soy and pea proteins, although their foaming capacity and emulsifying stability were somewhat weaker, likely due to differences in their protein content and

structural characteristics. Nevertheless, MR and MRP demonstrated good emulsifying activity (107 and 152 m^2/g), on par with pea protein (Tab.1). Furthermore, MR and MRP exhibit higher water-holding capacity (~6 ml/g) than other protein isolates, suggesting their potential application in semi-solid food formulations.
In conclusion, industrial malting by-product may provide a sustainable and valuable protein source suggesting potential applications within the food industry. For example, its high water-holding capacity makes it well-suited for semi-solid formulations, while its emulsifying properties could contribute to improving plant-based dairy or meat alternatives. MRP can be effectively recycled to produce protein-rich ingredients, thereby enhancing the nutritional and functional profile of various food products and opening up opportunities for industrial scale-up in protein recovery.

Figure 1 . Essential amino acids (mg/g protein) in isolate proteins compared with the requirement pattern (FAO, 2013)

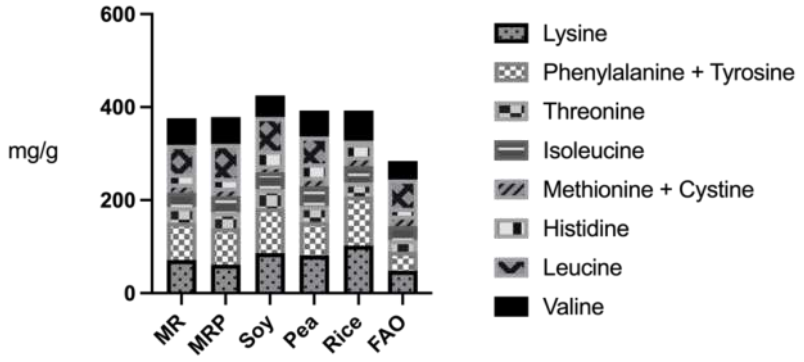


Table 1. Techno-functional properties of protein samples (mean value ± ds)

	EAI (m^2/g)	ESI (%)	FC (%)	FS (%)	WHC (ml/g)	FAC (%)
MR	106.84 ± 3.58^{c}	34.66 ± 5.21^{c}	$16.66\pm3.33_{c}$	$0\pm0_{b}$	$6.15\pm1.36_{a}$	$36.19\pm3.49_{cd}$
MRP	$153.51\pm7.32_{b}$	$19.29\pm3.49_{d}$	$15.55\pm5.09_{c}$	$0\pm0_{b}$	$6.10\pm0.45_{a}$	$29.73\pm5.52_{d}$
Soy	$28.68\pm3.31_{d}$	$91.68\pm5.14_{a}$	$145.55\pm6.93_{a}$	$97.28\pm1.36_{a}$	$4.38\pm0.13_{ab}$	$58.93\pm2.20_{a}$
Pea	$160.03\pm9.25_{ab}$	$48.90\pm7.80_{b}$	$60.0\pm8.81_{b}$	$96.51\pm3.11_{a}$	$2.60\pm0.03_{bc}$	$50.86\pm6.48_{ab}$
Rice	$179.23\pm16.58_{a}$	$25.47\pm1.56_{cd}$	$3.26\pm0.12_{c}$	$0\pm0_{b}$	$1.60\pm0.02_{c}$	$43.24\pm1.44_{bc}$

MR, proteins from malt rootelts; MRP, proteins from malt residual pellet; EAI, emulsifying activity index; ESI, emulsifying stability index; FC, foaming capacity; FS, foaming stability, WHC, water holding capacity; FAC, fat absorption capacity.

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Physicochemical and Techno-functional Characterization of Flours from Neglected Sicilian Black Chickpea Varieties (P-II-14)

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The first three activities of the PhD thesis project are described. Firstly, the research conducted among custodian farmers led to the identification and selection of two Sicilian neglected landraces of chickpea (*Cicer arietinum* L.): Rough Black Chickpea (RBC) and Smooth Black Chickpea (SBC). The two varieties were evaluated for their agronomic traits through field trials. The harvested seeds were milled to produce flours, which were characterized through chemical, physico-chemical, and techno-functional analyses.

Caratterizzazione chimico-fisica e tecno-funzionale di farine da varietà neglette di ceci neri siciliani

Le prime tre attività del progetto di tesi di dottorato sono descritte. La ricerca condotta presso agricoltori custodi ha permesso di identificare e selezionare due ecotipi locali di ceci (*Cicer arietinum* L.) negletti Siciliani: Cece Nero Rugoso (RBC) e Cece Nero Liscio (SBC). Le due varietà sono state valutate per le loro caratteristiche agronomiche mediante prove sperimentali in campo. I semi raccolti sono stati moliti per ottenere farine, successivamente caratterizzate attraverso analisi chimiche, fisico-chimiche e tecno-funzionali.

Keywords: Sicilian legumes, neglected varieties, chickpea flours, physicochemical characterization, techno-functional properties.

1. Introduction

In accordance with the PhD thesis project previously described (Torre, 2024), this poster reports the main results of the first three activities concerning: A1) Selection of neglected legume varieties and their agronomic evaluation in field trials; A2) Legume flour production from the selected varieties; A3) Legume flour characterization through chemical, physicochemical, and techno-functional analyses.

2. Materials and Methods

2.1 Agronomic field trials

The neglected Sicilian legume varieties, provided by Azienda Agricola C. da Risicata (Agira, EN), were manually sown in a randomized block design with three replicates. Rows were spaced 5 × 70 cm apart, corresponding to a density of 28.6 plants/m². Morpho-biometric measurements were determined at the beginning of legume flowering, while yield and yield components were assessed at harvest on a 1-m linear basis.

2.2 Proximate composition and techno-functional properties of chickpea flours

Legume seeds were milled using a Bühler DLFU Laboratory Disc Mill to obtain flour with a particle size of approximately 0.5 mm. Moisture and ash contents were determined according to the AOAC standard method (Lee, M.H., 1995). The fat content was determined by a CEM SMART 6™ solids analyzer and ORACLE™ NMR fat analyzer (Matthews, North Carolina, USA). The protein content was measured using the Dumas method with a nitrogen-to-protein conversion factor (Kp) of 6.25, according to Badia-Olmos et al. (2025). Carbohydrate content was calculated by difference. Colour analysis was performed using a colorimeter based on the CIE Lab* colour space system. Particle size distribution was assessed by mechanical sieving. Bulk density (BD), water absorption index (WAI), water solubility index (WSI) and water and oil absorption capacities (WAC, OAC), were determined through the method reported by Du et al. (2014).

3. Results and Discussion

3.1 Agronomic evaluation

The selected chickpea varieties exhibited promising agronomic performance under the field trial conditions, with the smooth black chickpea achieving a yield of 1.8 t ha^{-1}, coupled with favorable seed size and biomass production as shown in Table 1. These results highlight the value of recovering neglected varieties for resilient agricultural systems under climate change conditions.

Table 1 *Morpho-biometric and yield traits (mean ± SD).*

Plant height (cm plant^{-1})	24.2±3.0	25.2±0.9
Aboveground biomass DW (g plant^{-1})	11.3±1.0	17.3±1.7
Grain yield (t ha^{-1})	1.6±0.1	1.8±0.1
N. seeds plant^{-1}	14.4±0.4	22.0±1.8
N. seeds pod^{-1}	1.0±0.1	0.7±0.1

3.2 Chemical composition of chickpea flours

The chemical characterization of the chickpea flours (Table 2) revealed a moisture content of 7.9% and an ash content value close to 2.8%, suggesting a favorable mineral

profile. The protein content ranged from 19.32% to 20.17%, supporting their use as a plant-based protein source in functional foods. The fat content ranged from 4.33 to 4.67%, higher than that of most cereal flours, contributing positively to flavor and mouthfeel in formulated products (Grasso et al., 2022).

Table 2 *Proximate chemical composition (% w/w) of chickpea flours. Values are expressed as mean[a] ± standard deviation.*

Sample	Moisture	Ash	Protein[b]	Fat	Carbohydrate
RBC	7.89±0.05	2.84±0.02	19.32±0.12	4.33±0.13	73.51±0.23
SBC	7.88±0.01	2.87±0.01	20.17±0.02	4.67±0.10	72.29±0.31

[a] All data represents the mean of three determinations. [b] Protein content was calculated as Total Nitrogen × 6.25

3.3. Physicochemical and techno-functional properties of chickpea flours

3.3.1. Colour and particle size

Both chickpea flours showed high lightness (L* > 86) and a pronounced yellow hue (b* > 20), with low a* values (~1), indicating a bright, natural color suitable for various food applications, as reported in Table 3. Particle size analysis (Figure 1) showed that most flour particles were around 0,5 mm, reflecting an effective mechanical milling and sieving. The homogeneous particle size distribution ensures consistent hydration behavior and facilitates even mixing in food matrices.

Figure 1 *Particle size distribution curves of chickpea flours.*

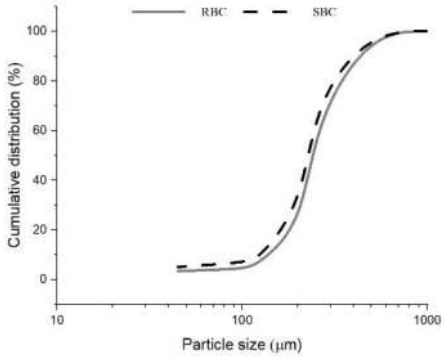


Table 3 *Colorimetry analysis of chickpea flours.*

Sample	L*	a*	b*
RBC	89.58±0.02	1.32±0.01	21.24±0.11
SBC	86.93±0.06	1.00±0.01	20.20±0.06

3.3.2. Techno-functional properties

Table 4 reports on the techno-functional properties of chickpea flour. As shown, they exhibited a water absorption index (WAI) value between 3.73 and 4.29 g g^{-1}, reflecting the ability of the starch-protein matrix to swell in the presence of water. This property supports their use in applications where hydration and textural development are critical, such as extruded snacks, soups, or dough-based systems.

Table 4 *Techno-functional properties of chickpea flours.*

Sample	BD (g mL^{-1})	WAI (g g^{-1})	WSI (g $100g^{-1}$)	WAC (g g^{-1})	OAC (g g^{-1})
RBC	0.71±0.01	4.29±0.42	19.24±0.81	1.82±0.27	1.80±0.19
SBC	0.72±0.01	3.73±0.33	22.43±0.78	1.77±0.35	1.76±0.22

The water absorption capacity (WAC) ranged from 1.77 to 1.82 g g^{-1}, confirming a good ability to retain moisture, a crucial aspect that translates into improved softness and a more pleasant mouthfeel in the final products. This property is attributable to the hydrophilic constituents, such as polysaccharides. Additionally, the intrinsic proteins of chickpea flours played a significant role in their high-water absorption capacity. The oil absorption capacity (OAC) ranged from 1.76 to 1.80 g g^{-1}, in line with those expected for flours containing moderately hydrophobic protein fractions. This property enhances flavour retention and contributes to the structure and caloric density of various food formulations, such as sauces, burgers, or baked goods. As suggested by Kaur et al. (2021), more hydrophobic proteins show superior binding of lipids, implying that non-polar amino acid side chains bind the paraffin chains of fats.

The results suggest that the analyzed chickpea flours could serve as a valuable base ingredient in protein-enriched formulations or meat analogues, and as a functional component in gluten-free or vegetarian diets (Grasso et al., 2022).

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Valorization of Grass Pea (*Lathyrus sativus L.*) Proteins through Microgels: Impact of pH on Structural Properties (P-II-15)

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The activities planned for the first year of the PhD project have been completed. Using grass pea (Lathyrus sativus L.) isolate, extracted in the laboratory by isoelectric precipitation, thermal induced gels were made at different pH (2, 3, 7, 9) which were then used to produce microgels. The isolate, along with gels and microgels, were characterized to assess how pH influenced the formation and the physical properties of such colloidal structures.

Valorizzazione delle proteine di cicerchia (*Lathyrus sativus L.*) attraverso i Microgels: Impatto del pH sulle proprietà strutturali

Le attività perviste per il primo anno sono state finalizzate. Utilizzando un isolato di cicerchia (*Lathyrus sativus L.*), ottenuto in laboratorio attraverso precipitazione isoelettrica, sono stati realizzati dei gel tramite trattamento termico a diversi pH (2, 3, 7, 9) e da questi sono stati ottenuti dei microgels. Sia gli isolati che i gel e i microgel sono stati caratterizzati per valutare come il pH abbia influenzato le proprietà fisiche di queste strutture colloidali.

Key words: Grass pea protein, thermal induced gel, pH, microgel, rheology, tribology.

1. Introduction

According to the PhD thesis project previously described (Iervese, 2024), the main results presented in this manuscript and accompanying poster are related to the following activities:
A1) **Characterization of the physico-chemical properties of native protein isolate.** In this activity solubility, particle size, ζ-potential, and surface hydrophobicity of the grass pea protein isolate (GPPI) were studied.
A2) **Preparation of microgels using the protein isolate and characterization.** Heat-induced gels were prepared from GPPI and analyzed for their viscoelastic properties, particularly in relation to pH. Subsequently, the resulting microgels were characterized in terms of particle size, ζ-potential, flow behavior, and tribological properties.

2. Materials and Methods

The grass pea (*L. sativus*) flour was provided by a farmer (Bisenti, Te, Italy). The extraction of the protein fraction from the flour was conducted following the protocol by Feyzi et al., (2018). The protein isolate (GPPI) was characterized for its solubility across a pH range of 2 to 12, according to the method by D'Alessio et al., (2023). The least gelation concentration (LGC) of the isolate was determined using the method by Kaur & Singh, (2007). Thermal properties of the protein isolate were evaluated by differential scanning calorimetry (DSC) (Pyris 8500, PerkinElmer, Shelton, USA). Isolates were adjusted to pH 2, 3, 7, and 9. The surface hydrophobicity of the pH-adjusted isolates was assessed using the fluorescent probe ANS (8-Anilino-1-naphthalenesulfonic acid). The pH-modified isolates were then subjected to thermal treatment at 95 °C for 1 hour to induce gelation. After refrigeration overnight, the amplitude and frequency test were performed on the gels (strain range: 0.01% - 100%, 1 Hz; frequency range: 0.01 Hz - 100 Hz, 0.1% strain; MCR 302, Anton Paar, Graz, Austria). Microgels were produced by mechanical shear (Panda Plus 2000, GEA Niro Soavi, Parma, Italy), following the protocol described by Sarkar et al., (2017) and analyzed via dynamic light scattering (DLS) (Litesizer 500, Anton Paar, Graz, Austria) to determine particle size and zeta potential. Their flow curves (shear rate range: 0.3 to 245 s^{-1}) were studied and modelled by Herschel–Bulkley. Tribological behavior was assessed according to Kew et al., (2023). Data were analyzed by one-way ANOVA, and analyses of differences between experimental groups were made using Tukey's multiple-comparison tests with a significance threshold of 0.05.

3. Results and Discussion

3.1 Characterization of grass pea protein isolate (GPPI)

The protein isolate exhibited the characteristic U-shaped solubility curve typical of legume proteins (Figure 1), with increased solubility at both acidic and alkaline pH extremes and a marked minimum in the pH range of 4.5–5. These findings are consistent with those reported in the literature. Thermal analysis revealed the onset of protein denaturation at approximately 87.91 ± 0.62 °C, with a denaturation peak observed around 88.88 ± 0.62 °C. The least gelation concentration (LGC) was determined to be approximately 16% (w/w), a value subsequently used to prepare thermally induced gels for microgel production. Surface hydrophobicity was assessed on the pH-modified protein isolates. A significantly higher surface hydrophobicity, over an order of magnitude greater, was observed

in the acid-treated samples (pH 2–3) compared to those at neutral or alkaline pH. This suggests a greater exposure of hydrophobic groups in the acidic isolates, likely due to increased protein denaturation under these conditions. Droplet size distributions (Figure 2) evidenced that the largest particle size was observed at pH 2, with a decreasing trend as the pH increased toward neutral and alkaline conditions.

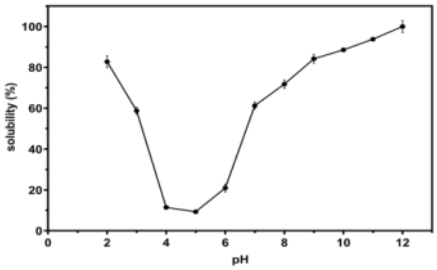


Figure 1*: solubility of GPPI at different pH (2 to 12)*

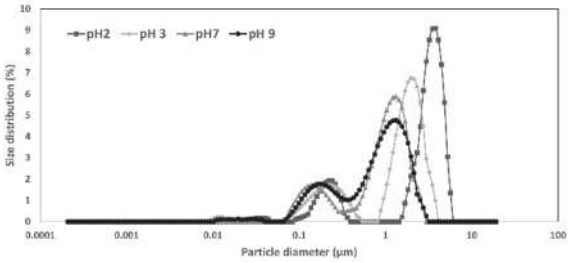


Figure 2*: Particle size distribution of GPPI at different pH*

3.2 Rheological behaviour of thermally induced gels

The thermally induced gels were first analyzed using amplitude sweep tests (fFigure 3) to determine the linear viscoelastic region (LVER); a strain amplitude of 0.1% was identified as appropriate for maintaining linear behavior. The rheological properties of the gels were clearly influenced by the pH at which they were formed. Specifically, gels prepared at acidic pH exhibited the highest mechanical strength, while those formed at alkaline pH were the weakest. Gels formed at neutral pH displayed intermediate properties. This pH-dependent behavior was further confirmed through frequency sweep analysis, which revealed consistent trends across the frequency range.

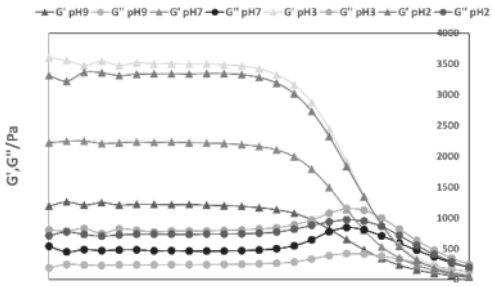


Figure 3 *Strain sweep of GPPI gel at different pH*

3.3 Characterization of microgels

The microgels were analyzed for particle size, which was again found to be dependent on the pH at which the parent gels were formed. Specifically, the hydrodynamic diameter at pH 2 was approximately 345 nm, while at pH 7 and pH 9 it was around 171 nm and 155 nm, respectively. Significant differences were confirmed also by zeta potential: at pH 2, the zeta potential values were closer to zero, indicating reduced electrostatic repulsion and a greater tendency for particle aggregation, which may explain the larger particle size observed at this pH. Flow curve analysis showed pseudoplastic behavior at pH 2, while samples at pH 7 and pH 9 exhibited Newtonian flow characteristics. Tribological measurements demonstrated superior lubricating properties in the sample with pH 9, which displayed the lowest coefficients of friction across all regimes. Conversely, the pH 2 sample exhibited the highest friction in the boundary regime, which gradually decreased to match the pH 7 sample in the mixed regime. In conclusion, pH modification of grass pea protein isolate (GPPI) induces molecular changes that had an impact on the rheological behavior of the thermally induced gels, leading to stronger gel formation at acidic pH. This pH dependency is also evident in the derived microgels, which exhibited significant differences in particle size and distinct rheological and tribological behaviors.

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Modulating White Wine Quality: The Effects of Ozone Treatments During Production (P-II-16)

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This experiment, part of my PhD thesis, explored alternatives to sulphur dioxide in winemaking for two Italian white grape varieties Fiano and Vermentino. Ozone was applied as a pre-fermentative treatment, either alone or combined with enological adjuvants, following the Purovino method, that also includes its use as a sanitizing agent on winery materials. Traditional vinifications were used as control. Instrumental and sensory analyses were carried out after bottling and throughout bottle aging to evaluate oxidative stability and overall wine quality.

Impatto del trattamento con ozono sulla qualità dei vini bianchi

Questo esperimento, condotto nell'ambito della mia tesi di dottorato, ha indagato l'uso di alternative al diossido di zolfo durante la vinificazione, concentrandosi su due vitigni bianchi italiani il Fiano ed il Vermentino. L'ozono è stato applicato come trattamento prefermentativo, da solo o con coadiuvanti enologici, seguendo il metodo brevettato Purovino, che prevede anche il suo utilizzo come agente sanitizzante sulle materiali di cantina. Vinificazioni tradizionali sono state utilizzate come controllo. Sono state eseguite analisi strumentali e sensoriali dopo imbottigliamento e durante l'affinamento in bottiglia per valutare l'impatto dell'ossidazione e la qualità complessiva del vino.

Key words: Ozone, Wine, Sensory analysis, Color, Volatile compounds

1.Introduction

Sulphur dioxide (SO_2) is widely used to preserve food and wine due to its effectiveness, but consumers increasingly seek wines with reduced chemical additives because of potential health concerns (Costanigro et al., 2014). Ozone is a promising alternative sanitizer for grapes, leaving no residues and promoting important metabolite production (Bellincontro et al., 2017; Río Segade et al., 2017). However, its effect on wine quality needs further study. This work investigated the use of alternatives to SO_2 in winemaking, focusing on their effects on two non-aromatic Italian white wine varieties: Fiano and Vermentino. The study explored the application of ozone assessing its potential to preserve wine quality and stability in comparison to traditional SO_2-based method. The aim was to compare the impact of each approach on the wines' chemical composition, antioxidant capacity and sensory profile over time.

2.Matherials and Methods

Three vinification strategies were evaluated: the use of ozone alone (O_3; Modesti et al., 2021), the application of ozone in combination with specific enological adjuvants following the Purovino method (PV; Menacrelli & Catelli, 2013) and conventional vinification with the addition of sulphur dioxide (SO_2). The wines obtained were analyzed after bottling (T0), after accelerated aging at 35 °C for five days (T1) and after 6 months of storage at 15°C (T6). At each sampling point, different analyses were performed. Total polyphenol content was determined using the Folin-Ciocalteu method and wine color was assessed using CIELab coordinates and absorbance at 420 nm. Antioxidant capacity was evaluated using the DPPH assay (Romanet et al., 2019). Volatile compounds were determined by HS-SPME/GC-MS (Slaghenaufi et al., 2018). Sensory evaluation was conducted by a trained panel using descriptive analysis (DA), using 10 cm unstructured line scales to assess color hue, in-mouth attributes (bitterness, astringency, acidity) and aroma intensity (citrus, yellow pulp fruit, pineapple, green apple, pear, rose) (Lawless & Heymann, 2010).

3.Results and Discussion

Significant differences in the polyphenol content were observed among the three treatments at all three sampling points in the Vermentino variety, with the ozone (O_3) treatment consistently showing significantly higher values. In contrast, for Fiano, significant differences were only found at T6, O_3 again displaying the highest concentrations. Regarding color parameters, b* values (yellow/blue color component) were higher for PV-treated samples at all times for the two varieties studied in agreement with the highest values of absorbance at 420 nm and sensory evaluation, suggesting increased browning. In terms of antioxidant capacity, Vermentino samples treated with sulfur dioxide (SO_2) revealed greater values across all time points, followed by O_3 and then PV. For Fiano, no significant differences were observed between the SO_2 and O_3 treatments, while PV consistently showed the lowest antioxidant capacity. These results suggest that the increased polyphenol content induced by ozone layer has not contributed to better preservation during wine shelf-life, particularly in Vermentino. Volatile organic compounds

(VOCs) showed only limited differences over time. Principal Component Analysis (PCA) was conducted to classify and differentiate treatments according to individual volatile compounds. In Vermentino, PCA (Figure 1a) explained 74.6% of the total variance, with a clear separation of wines by sampling time according to PC1. Notably, the O_3 treatment at T0 and T1 was characterized by the presence of certain terpenes (such as linalool and nerolidol), phenylethyl alcohol and several esters. The PV protocol at these time points was associated with isoamyl esters and isoamyl alcohol, while the SO_2 samples were linked to isobutanol and ethyl hexadecanoate. At T6, SO_2 samples were less correlated with VOCs while O_3 and PV were mostly associated with some terpenes (α-terpineol and hotrienol) and esters (ethyl isovalerate and ethyl 2-methylbutanoate). In-mouth sensory analysis for Vermentino indicated that SO_2-treated wines had the lowest bitterness. Conversely, O_3-treated samples consistently showed the highest perception of fruity descriptors such as green apple, pear, yellow pulp fruit and citrus across all time points. It is noteworthy that sensory faults were most prevalent in SO_2 treatments, while O_3 consistently had the lowest fault detection rate. For Fiano, the PCA (Figure 1b) explained 77.1% of the variance and, similarly to Vermentino, PC1 clearly separated the samples by time point, with T6 being associated with a smaller number of VOCs. SO_2 treatments were mainly characterized by esters and several higher alcohols. At T0, the PV wines were more strongly associated with terpenes like linalool, β-citronellol and nerol as well as methyl esters, while O_3 at T0 and T1 was mainly linked to esters. In terms of sensory evaluation, few significant differences in bitterness or astringency were noted in Fiano. Regarding sensory perceived aroma, yellow pulp fruit and citrus descriptors were the most prominent in O_3-treated samples at T0 and T1 (and in SO_2 at T0). At T6, O_3 again showed the highest intensity for all descriptors, including pear, yellow pulp fruit and citrus. Additionally, O_3 displayed the lowest percentage of sensory faults at T6, whereas SO_2 had the fewest faults at T0. These outcomes suggest that ozone treatment may positively influence the aromatic complexity and sensory quality of the wines, supporting its potential as an effective alternative to SO_2.

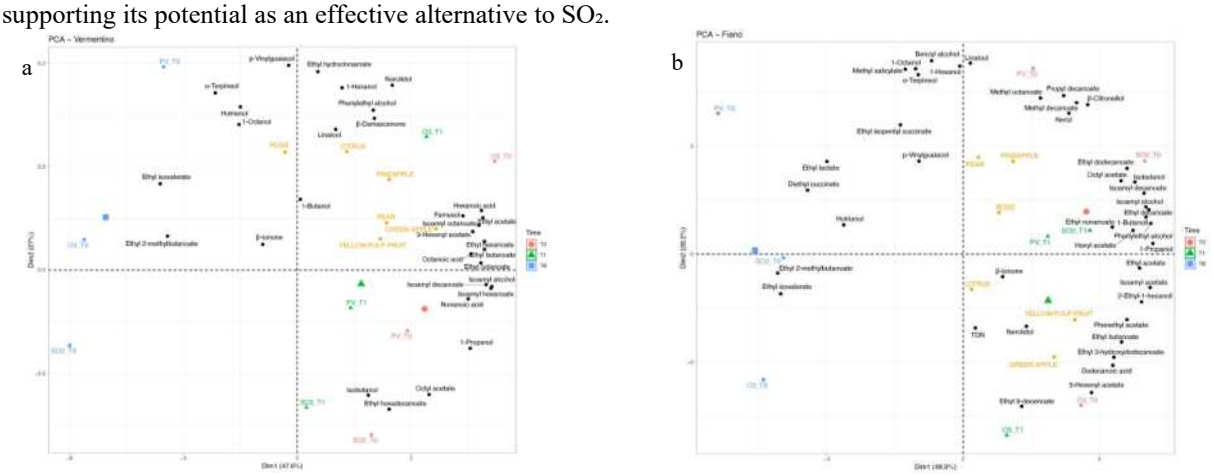


Figure 1. *Principal Component Analysis (PCA) of individual significant free VOCs (active variables) and aroma sensory descriptors (supplementary variables) for Vermentino (a) and Fiano (b) wines. Aroma descriptor intensities are also projected as supplementary quantitative variable.*

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Exploring the behavior of alternatives to montmorillonite clays in white wine protein stabilization (P-II-17)

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The first PhD experiment evaluated alternative clays (sepiolite and palygorskite) as potential substitutes for bentonite in white wine protein stabilization. Protein removal efficiency and wine quality parameters were compared at the minimum effective dose for each clay. A sensory analysis was also conducted.

Valutazione del comportamento di argille alternative alla montmorillonite nella stabilizzazione proteica del vino bianco

Il primo esperimento del dottorato ha valutato l'uso di argille alternative (sepiolite e palygorskite) come potenziali sostituti della bentonite nella stabilizzazione proteica dei vini bianchi. Sono stati confrontati l'efficacia nella rimozione delle proteine e i parametri qualitativi del vino alla dose minima efficace di ciascuna argilla. È stata inoltre condotta un'analisi sensoriale.

Key words: Protein stabilization, bentonite alternatives, white wine clarity, wine quality

1. Introduction

In line with the dissertation project [1] and building upon a literature review on the role of bentonite in wine production (Activity A1), submitted to *Comprehensive Reviews in Food Science and Food Safety*, this poster presents the main findings of Activity A2. This phase aimed to evaluate the performance of alternative clays to montmorillonite for protein stabilization in white wine, with a particular focus on their protein removal capacity, their impact on key wine quality parameters, and sensory perception.

2. Materials and Methods

A100 L batch of Moscato bianco must, selected for its potential protein instability, was fermented. The experiment aimed to evaluate the effects of two commercial bentonites, Plusgran Gel (PGG) and Mastervin Compact (MVC), and three clay materials: PS9 (99% sepiolite), G30 (95% palygorskite), and VHS (90% montmorillonite), applied at different concentrations in triplicate. An untreated sample served as the control. Dosages were selected based on preliminary trials and were as follows: 0.1, 0.2, 0.3, and 0.4 g/L for PGG, MVC, and VHS; and 0.4, 0.6, 0.8, and 1.0 g/L for PS9 and G30. All gels were prepared at 5% w/v. Protein stability was assessed following a reported method [2], with slight modifications. A volume of 18 mL of wine was filtered through a 0.45 µm cellulose acetate membrane and heated at 80 °C for 2 hours. After 3 hours at room temperature, turbidity was measured. Wines showing a difference in turbidity (ΔNTU) of less than 2 before and after heat treatment were considered stable. The treatment effects were compared at an equivalent stabilizing dose, evaluated through protein removal (HPLC) [3], basic oenological parameters, CIELab coordinates, volatile organic compounds (VOCs, via GC-MS) [4], phenolic content (via spectrophotometric index), while for the sensory analysis a general descriptive analysis (DA) was used, employing 10 cm unstructured line scales for in-mouth descriptors (*bitterness, astringency, acidity*) and aroma (*citrus, yellow pulp fruit, pineapple, green apple, pear and rose*) [6].

3. Results and Discussion

The stabilization dose required to achieve protein stability was established at approximately 10 g/hL for the two commercial bentonites (MVC, PGG) and the experimental montmorillonite (VHS). In contrast, the two alternative clays (G30, PS9) necessitated a sixfold higher dosage to reach a comparable level of stabilization.
The deproteinizing capacity, as evaluated by residual protein content under equivalent stabilization conditions, confirmed a significantly higher protein removal efficiency for MVC, PGG and VHS compared to G30 and PS9 (fig. 1). The determination of the Total Polyphenol Index (TPI) (fig. 2) revealed a highly significant correlation between protein removal and a reduction in TPI values (fig. 3), confirming previous findings [7], supporting the hypothesis that the removal of phenolic compounds is closely linked to protein removal by bentonite.
With respect to the technological parameters, no statistically significant differences were observed in pH across the treated samples. However, slight variations were detected in titratable acidity. In terms of colorimetric parameters, all products, with the exception of PGG, induced a significant reduction in absorbance at 420 nm when compared to the control. Notably, significant differences were observed among treatments, with the alternative clays consistently displaying the lowest absorbance values. Although significant differences were also detected in CIELab coordinates, the overall color differences were below the threshold for visual perceptibility ($\Delta E < 3$),

indicating that none of the treatments led to noticeable color alterations.

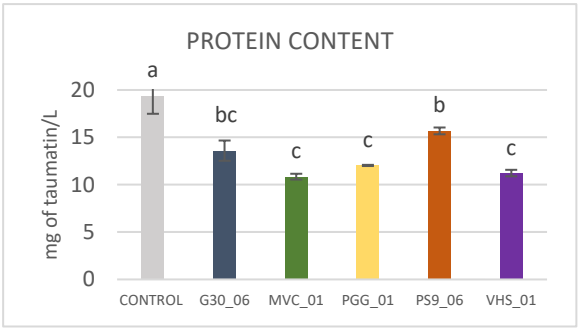


Figure 3: The residual protein content following treatment at the stabilization dose.

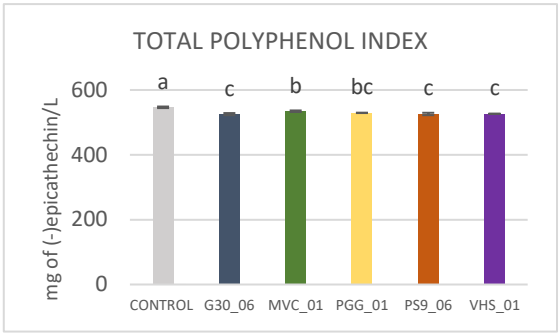


Figure 2: Impact of different products on TPI levels.

Among the 54 VOCs analyzed, only ethyl butanoate, methyl decanoate, octyl acetate, and dodecanoic acid showed statistically significant differences among the samples. This finding reinforces the view that bentonite, particularly when applied at low dosages, does not substantially impact varietal aroma compounds such as terpenes and C13-norisoprenoids, and in general it suggests that its negative effects on VOCs are minimal [8] (fig. 4). Sensory analysis showed no significant differences in acidity, consistent with analytical data. Astringency and bitterness differed significantly but were not correlated with TPI. Except for roses, all aromatic descriptors varied significantly; however, the four aroma compounds showing statistical differences were insufficient to explain these variations.

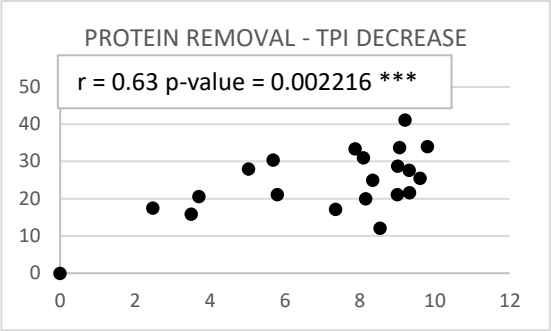


Figure 4: The highly significant correlation between protein removal and the decrease in the TPI.

The behavior of the different products proved to be highly specific and should be evaluated on a case-by-case basis to achieve optimal results [9]. These results highlight the challenge of finding effective bentonite alternatives, while confirming that, at low dosages, bentonite can stabilize white wine without compromising its key quality attributes.

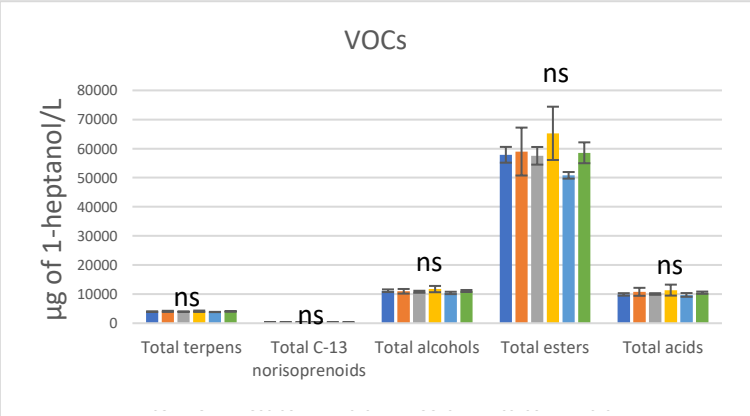


Figure 5: The total aroma content in the stabilized samples.

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Valorization of wine-related microbial bioresources for sustainability and innovation: case studies in the agri-food chain (P-II-18)

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The PhD project focuses on the exploration of wine-related microbial bioresources stored in VUCC-DBT (Verona University Culture Collection – dept. Biotechnology) and/or preserved in the strain collection of the Microbiology Laboratory belonging to 17 genera and isolated during vintages between 2015 and 2020. Specifically, the research aims at accurately identifying yeasts and bacteria and at characterizing them for possible valorization in the same production chain (wine) or other ones, considering also safety aspects. The information obtained will contribute to expand the catalogue of VUCC-DBT with well characterized wine-related bioresources as well to re-define specific screening protocols for selected biotechnological applications.

Valorizzazione di biorisorse microbiche di origine viti-enologica per la sostenibilità e l'innovazione: casi studio nella filiera

Il progetto di dottorato si focalizza sull'esplorazione delle biorisorse legate al vino, depositate nella collezione VUCC-DBT e/o nella ceppoteca del Laboratorio di Microbiologia appartenenti a 17 generi ed isolate durante le vendemmie tra il 2015 e il 2020. In particolare, la ricerca si focalizza sull'accurata identificazione molecolare di lieviti e batteri e sulla loro caratterizzazione per la potenziale valorizzazione sia nella filiera enologica che in altre filiere. Le informazioni ottenute contribuiranno all'espansione del catalogo della VUCC-DBT con biorisorse legate al vino ben caratterizzate che con la definizione di protocolli di screening per specifiche applicazioni biotecnologiche.

Keywords: microbial bioresources, microbial collection, valorization, lipids accumulation.

1. Introduction

As part of PhD projects, the activity described was developed to investigate lipid accumulation by *Metschnikowia pulcherrima*, to define reliable screening procedures, highlights promising strains, investigate the potential to formulate a feed supplement from the most promising strain of the collection also considering safety aspects related to non-QPS species (Larini *et al.*, 2025). *M. pulcherrima* is a promising non-conventional yeast explored for its tolerance and growing ability on different substrates, with an industrial potential as an oleaginous species. Oleaginous yeasts, in particular, can produce single-cell oils (SCO) as intracellular lipids for over 20% of their dry cell weight to store carbon and energy, an alternative resource for several industries, including biodiesel and feed supplementation. This accumulation is favoured by growth conditions with excess carbon and limited nitrogen, promoting de novo lipid synthesis (Caporusso *et al.*, 2021). Considering the high abundance of *M. pulcherrima* in the strain collection and its recent exploration for the accumulation of intracellular lipids in literature. In this study, qualitative plate methods were applied and compared with lipid extraction and gravimetric quantification to investigate their reliability on a limited number of test strains.

2. Materials and Methods

The test dataset was composed by Metschnikowia pulcherrima VUCC500 (VUCC-DBT) and nine type strains of the former species of *M. pulcherima*, recently reclassified (Sipiczki, 2022), with *Lipomyces starkeyi* DBVPG 6193 and *Yarrowia lipolytica* DBVPG 6053 (Lin *et al.*, 2011; Filippucci *et al.*, 2016), as positive controls. Strains were grown at lab scale in a high C/N medium with glucose as carbon source. The analyses were assessed in biological duplicate and technical triplicate for each test, and the formation of lipid droplets was observed under the microscope. A qualitative plate screening method using Rhodamine B dye (Niehus *et al.*, 2018) was evaluated by the observation of the changing of spots colour in both visible and UV light. Furthermore, a quantitative method was performed by assessing lipid accumulation with chloroform, methanol and water extraction of the dried biomass followed by a gravimetric quantification (Němcová *et al.*, 2021).

3. Results and Discussion

3.1 Investigation on lipid accumulation by *Metschnikowia pulcherrima*

The quantitative method was firstly assessed and compared with literature data by replicating the same accumulation condition both on positive strains and on seven of ten *M. pulcherrima* strains. Once it was found to be consistent, this method could also be applied to evaluate the most applicable qualitative method to screen a collection on lipid accumulation. Furthermore, quantitative data showed that *M. pulcherrima* VUCC500 could be an interesting SCO strain with a percentage of lipid accumulation of 21,4 ± 2,0% with glucose as a carbon source (Figure 1).

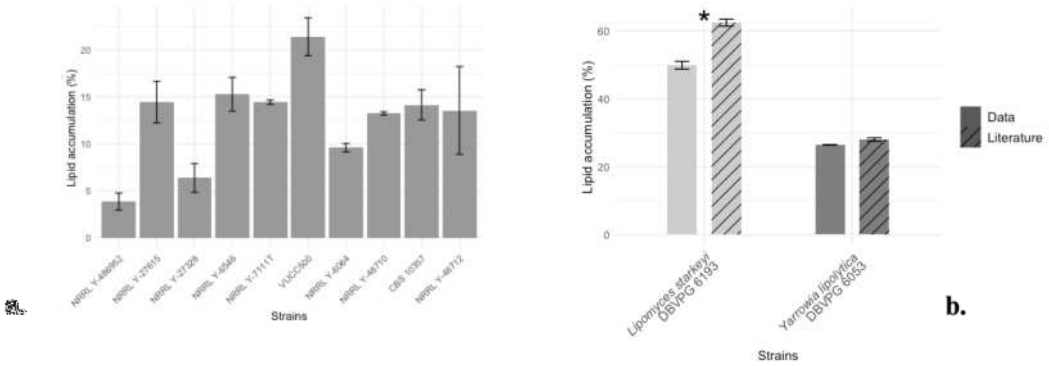


Figure 1 ***a.*** *Quantification of lipid accumulation on 10 strains of M. pulcherrima.* ***b.*** *Results of lipid accumulation on positive strains compared with literature data, * = p value < 0,05.*

However, qualitative protocols did not robustly reveal the oleaginous behaviour, in fact, by the comparison of the three qualitative methods (microscope, visible and UV light) only one strain on the total showed a positive accumulation trait in all the three parameters assessed (Table 1). Furthermore, by the comparison with quantitative results, this strain did not result to have an accumulation higher than 20%, normally defined as the threshold for the definition of oleaginous.

Table 1 *Results of the Rhodamine B plate screening protocol by Niehus et al. (2018). - indicates no accumulation, + an accumulation present but not in all cells, ++ an accumulation present in most cells and the brighter shade of colour.*

Species	Strains	Microscope	Visible light	UV light
Metschnikowia pulcherrima	NRRL Y-48695	++	-	-
	NRRL Y-27615	-	-	++
	NRRL Y-27328	++	-	-
	NRRL Y-6546	++	-	+
	NRRL Y-7111^T	++	-	+
	VUCC500	+	-	-
	NRRL Y-6064	++	-	+
	NRRL Y-48710	++	-	-
	CBS 10357	++	++	++
	NRRL Y48712	-	-	-

Based on results obtained, it will be necessary to test the accumulation with alternative low-cost carbon sources. On the other hand, it will be necessary to identify an alternative primary screening method to test the entire collection by applying the same setup. Finally, further analyses will be focused on safety aspects necessary for this non-QPS species.

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Techniques for Wine Dealcoholization and Microbiota Characterization (P-II-19)

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Growing market interest in non- and low-alcoholic wines (NoLo) has prompted scientific research into the development of innovative techniques capable of reducing ethanol content while preserving the sensory and functional properties of wine. This research project investigates both the efficacy of dealcoholization techniques, and the selection and characterization of microbial strains potentially involved in controlled fermentations or biogenic amine degradation, with a focus on compliance with European regulations and consumer safety.

Tecniche di dealcolizzazione del vino e caratterizzazione del microbiota

L'interesse crescente per i vini a basso o nullo contenuto alcolico (NoLo) ha stimolato la ricerca scientifica nello sviluppo di tecniche innovative per la riduzione dell'etanolo, salvaguardando al contempo le proprietà sensoriali e funzionali del vino. Il progetto di ricerca valuta l'efficacia delle tecniche di dealcolizzazione e la selezione/caratterizzazione di ceppi microbici per fermentazioni controllate o degradazione di ammine biogene, con attenzione alla conformità normativa europea e alla sicurezza del consumatore.

Key words: dealcoholization, wine microbiota, 16S rRNA sequencing, lees, fermentation, consumer safety.

1. Introduction

In accordance with the PhD thesis project previously described, this contribution reports the main outcomes of the experimental activities carried out during the second year of the program. Specifically, these activities focus on:

(A1.1) the identification and selection of microbial strains of potential biotechnological interest from winemaking residual matrices,

(A2.2) the development and validation of molecular techniques for the extraction and sequencing of microbial DNA from wine lees,

(A3.1) the initial groundwork for a comprehensive economic and consumer-oriented study on dealcoholized wine,

(A3.2) the update search for European regulatory frameworks concerning the safety, classification, and labelling of dealcoholized wine, with specific attention to permissible processing aids and alignment with OIV definitions.

Building upon the regulatory and technical review conducted during the first year—which defined the dealcoholization landscape, reviewed available techniques, and evaluated the impact on wine quality and health claims—this year's work marks the transition to laboratory experimentation. Although the initial objective was to isolate wild yeast strains from red and white wine lees, only bacterial colonies developed, prompting a shift in focus toward bacterial identification and microbiota profiling.

2. Materials and Methods

Several studies have demonstrated the effectiveness of using both culture-dependent and culture-independent methods to biotypize the isolated microorganisms (Bustos et al., 2004; Caporaso et al., 2012; González-Arenzana et al., 2017; Klindworth et al., 2013; Takahashi et al., 2014). Microbial isolation activities were carried out using residual oenological matrices, particularly red and white wine lees collected during the most recent winemaking season (2024). Direct plating was initially performed, starting from 10 mL of each thawed sample, using serial dilutions in sterile saline solution (9 g NaCl per liter of demineralized water), and a range of selective solid media targeting both prokaryotic and eukaryotic microorganisms. The media used included: de Man Rogosa Sharpe (MRS, Oxoid) supplemented with cycloheximide (Sigma-Aldrich, 0.1 g/L), incubated at 30 °C for 48 hours to isolate rod-shaped mesophilic lactic acid bacteria; M17 (Liofilchem) with cycloheximide (0.1 g/L), also incubated at 30 °C for 48 hours for coccoid mesophilic lactic acid bacteria; Yeast Extract-Peptone-Dextrose (YPD, Oxoid) with chloramphenicol (0.1 g/L) and Sabouraud Dextrose (SD, Oxoid) with chloramphenicol (0.1 g/L), both incubated at 25 °C for 48 hours to target yeasts and molds; and Rogosa (Oxoid), used for lactobacilli under incubation at 30 °C for 48 hours. For microbial enumeration, the pour plate technique was applied on MRS and M17 media, whereas the spread plate technique was used for YPD, SD, and Rogosa agar. Following the initial direct plating step, the same procedures were replicated using an enrichment strategy in liquid culture. Aliquots from each sample were inoculated into 150 mL of selective broth media (MRS, M17, YPD, and SD) and incubated under the same conditions. Once an increase in turbidity was observed, indicating microbial growth—samples were streaked again on agar plates. Although the primary objective was to isolate wild yeast strains suitable for controlled fermentations to reduce wine ethanol content, colony development was observed exclusively on media selective for bacteria. In order to obtain pure isolates, bacterial colonies were resuspended in liquid broth and

subjected to repeated streaking on selective media. The isolates are currently undergoing microscopy morphological evaluation and catalase assay and will be subjected to taxonomic and functional characterization.
In parallel, total microbial DNA was extracted from lyophilized lees using the FastDNA™ SPIN Kit for Soil (MP Biomedicals), with protocol adjustments to account for the complex wine matrix inspected by using an unculturable method. Quantification of extracted DNA was performed using Qubit™ Flex Fluorometer (ThermoFisher Scientific), while qualitative and structural integrity assessments were carried out using the Agilent 4200 TapeStation with the Genomic DNA ScreenTape assay. Samples that passed quality control were used to prepare 16S rRNA gene sequencing libraries according to the Illumina "16S Metagenomic Sequencing Library Preparation" protocol. This procedure includes amplification of the V3-V4 regions of the 16S gene amplicon, two magnetic bead-based purification steps using AMPure XP, sample indexing via Index PCR, and final library quality control using the D1000 assay on the TapeStation system. Validated libraries were normalized at 4nM, pooled and loaded onto the MiSeq platform (Illumina), generating in output FASTQ files containing raw sequence reads and associated base quality scores. These files constitute the primary output of the sequencing run and will serve as input for upcoming metabarcoding bioinformatics analyses aimed at taxonomic identification and the selection of strains of potential oenological relevance.

3. Results and Discussion

3.1 Identification and selection of microbial strains from winemaking lees residues

Preliminary results from microbial isolation activities revealed the exclusive growth of bacterial colonies on selective media, with no evidence of yeast development. The isolation process is currently ongoing and involves repeated cycles of colony resuspension and streaking on agar plates to obtain morphologically homogeneous pure cultures. Again, extracted DNA from pure cultures will be used to identify genus/species belonging based on sequence similarity starting from 16S curated bioinformatics database. These isolates will undergo further morphological and biochemical testing to evaluate their potential oenological application, particularly for their suitability in controlled dealcoholization processes and their ability to degrade biogenic amines.

3.2 Development and validation of molecular techniques for microbial DNA extraction and sequencing

Total microbial DNA was successfully extracted from both red and white wine lees. Quantification using the Qubit™ Fluorometer revealed an initial DNA concentration of 0.03 ng/µL for white lees and 0.8 ng/µL for red lees (following a 5:45 dilution in 10 mM Tris HCl). Quality and structural integrity were assessed using the Genomic DNA assay on the Agilent 4200 TapeStation platform, confirming sufficient DNA quality for library preparation. Following PCR amplification and magnetic bead-based purification, the libraries were quantified and verified using the D1000 assay (Agilent). The white lees library yielded 0.029 ng/µL, whereas the red lees library reached 1.46 ng/µL, indicating a notably higher amplification efficiency for the latter. The final pool of normalized libraries was mixed with the PhiX control and successfully sequenced on the Illumina MiSeq platform. The resulting FASTQ files were processed by downstream bioinformatics pipelines. Bioinformatic analysis of the 16S rRNA sequencing data returned high-quality reads, as confirmed by consistency across technical replicates (three aliquots per sample, each analyzed in duplicate). Taxonomic assignment identified a total of 12 distinct bacterial genera, with notable differences in both alpha and beta diversity metrics between red and white lees. Specifically, white lees were dominated by *Escherichia-Shigella* (up to 60%) and unclassified genera belonging to *Enterobacteriaceae* family (35%), while these taxa exhibited very low relative abundances in red lees samples (3% and 2%, respectively). Conversely, red lees were heavily dominated by *Pediococcus* (73%) and *Oenococcus oeni* (14%), both of which were detected at negligible levels (<1%) in white lees. These findings suggest significant differences in microbial ecology between lee types, which may be influenced by vinification variables such as grape variety, processing conditions, storage environments, and subsequent manipulations.

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From by-products to bioactive ingredients: the development of a functional snack using olive pâté and brewing spent grain (P-II-20)

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This study, part of the project "MASE-FAST-To-0: Zero Waste Agri-Food Supply Chains" focuses on the conversion of by-products from the olive oil and brewing industries (olive pâté and spent grain) into ingredients for the development of a functional snack. After the by-products were characterized and treated using various technological processes, four different prototypes were developed. These prototypes were high in dietary fiber, a source of protein, rich in unsaturated fats, and low in sugars. Sensory evaluation yielded positive results, with a quality comparable to that of a conventional snack, indicating potential for industrial-scale production.

Da sottoprodotti a ingredienti bioattivi: lo sviluppo di uno snack funzionale utilizzando patè di olive e trebbie esauste

Questo studio, parte del progetto "MASE-FAST-To-0: Filiere agro-alimentari a scarto zero", ha avuto l'obiettivo di convertire i sottoprodotti dell'industria olearia e birraria (patè di olive e trebbie esauste) in ingredienti per lo sviluppo di uno snack funzionale. Dopo la caratterizzazione e il trattamento dei sottoprodotti con diversi processi tecnologici, sono stati sviluppati quattro differenti prototipi ad alto contenuto di fibre, fonte di proteine, ad alto contenuto di grassi insaturi e a basso contenuto di zuccheri. I risultati sensoriali sono stati positivi, con una qualità comparabile a quella di uno snack tradizionale, suggerendo una possibile produzione su scala industriale.
Key words: circular economy, olive pâté, brewing spent grains, polyphenols, fibers, functional food.

1. Introduction

In accordance with the PhD thesis project previously described (Bonucci, 2024), this poster presents the results of the four activities: (A1) the olive pâté (OP) and brewing spent grains (BSG) characterization in phenolic composition, fiber content, nitrogenous compounds, organic acid profile, ash and moisture content. Application of different stabilization and treatment technologies: BSG were used wet (frozen) or dry (dried on fluidized bed at 50 °C for 1 hour); the OP was used debittered (by a chemical method) or non-debittered; (A2) the assessment of nutritional and functional characteristics of by-products to be exploited as bioactive ingredients; (A3) the development of laboratory-scale prototypes of a functional food product enriched with fiber and bioactive compounds; (A4) the formulations' analysis and optimization, assessing nutritional, chemical and sensory quality.

2. Materials and Methods

Four formulations, shown in Table 1, were made by combining wet/dried BSG with non-debittered/debittered OP and then compared with the CTRL.

Table 1 *Materials used for the formulation of the different snack prototypes.*

Recipe Code	Ingredients
13WBSG-NDP	Flour 0, 13% (d.m.) wet BSG, 30% non-debittered OP, EVOO, salt, dehydrated brewer's yeast.
13WBSG-DP	Flour 0, 13% (d.m.) wet BSG, 30% debittered OP, EVOO, salt, dehydrated brewer's yeast.
30DBSG-NDP	Flour 0, water, 30% (d.m.) dried BSG, 30% non-debittered OP, EVOO, salt, dehydrated brewer's yeast.
30DBSG-DP	Flour 0, water, 30% (d.m.) dried BSG, 30% debittered OP, EVOO, salt, dehydrated brewer's yeast.
CTRL	Flour 0, water, EVOO, salt, dehydrated brewer's yeast.

Moisture content, water activity, ash, total nitrogen, protein content, fat, carbohydrates, free sugars profile, total dietary fiber, β-glucan and arabinoxylan content, energy values, spread factor and weight loss, were performed according to Sileoni et al. (2022). The extraction and determination of phenolic compounds, their total amount, and the antioxidant activity, determined using DPPH, FRAP, ABTS tests, were conducted according to Esposto et al. (2021). All analyses were performed in duplicate. To assess whether the inclusion of non-debittered and debittered OP in the four prototypes led to significant differences ($p<0.05$) in perceived bitterness of the snacks, a paired comparison test (ISO 5495:2005) was conducted. To evaluate the overall sensory profile, a quantitative descriptive analysis was carried out. In both cases, sensory evaluations were performed by a panel of 11 trained panelists. To compare the results obtained in the experimentation and test the differences between the different products, the one-way ANOVA test was used, performed with SigmaStat v.2.0 software.

3. Results and Discussion

3.1 By-products characterization

The OP exhibited a moisture content of 73.8%, with dry matter comprising 24.36% oil and 32.83% fiber. Its fatty acid profile consisted of 72% MUFA, 17% SFA, and 11% PUFA. OP is rich in phenolic compounds, primarily hydroxytyrosol, tyrosol, oleacein, oleocanthal, and verbascoside, known for antioxidant, anti-inflammatory, antitumor, and antimicrobial effects (Bonucci, 2024). The debittering process reduced the total phenolic content from 1107.7 to 896.6 mg/kg, decreasing the amount of secoiridoids and increasing their hydrolytic derivatives. BSG had 80% moisture (wet) and 4.8% (dry), with dry matter containing 23.76% protein, 10.47% carbohydrates, 7.25% fat, 54.71% fiber, 1% β-glucans, and 24.12% arabinoxylans. The presence of fibers is highly relevant since they are recognized as functional molecules able to increase faecal bulk and improve gut health (EFSA, 2011).

3.2 Qualitative comparison between snack prototypes

As shown in Table 2, the inclusion of BSG and OP increased dietary fiber content beyond the 6% EFSA threshold for health claims and classification as a high-fiber food. The by-product-enriched formulations also qualify as a protein source (>12% energy from protein), with low sugar content (<5%) and high unsaturated fatty acids (>70%) (EC Reg. N. 1047/2012). Total phenolic compounds (TPC) were significantly higher than CTRL, particularly in samples containing debittered OP, despite it being found to contain fewer phenols than the non-debittered one. It may be assumed that oleacein and oleocanthal, more abundant in the non-debittered OP, exhibit stronger interactions with proteins and polysaccharides (De Toffoli, 2019), reducing their extractability and detection by the analytical method. In terms of antioxidant power, all tests were in accordance with the TPC, and 30DBSG-NDP was found to have the highest antioxidant capacity, potentially due to its higher hydroxytyrosol content.

3.3 Evaluation of sensorial profile

The paired comparison test revealed no statistically significant differences between the prototypes containing debittered and non-debittered OP. Therefore, considering the additional cost associated with the debittering process, further sensory evaluations were carried out using the prototypes formulated with non-debittered OP (13WBSG-NDP, 30DBSG-NDP) compared with the CTRL. The assessed sensory attributes included visual appearance, aroma, taste, flavour, texture/mouthfeel, trigeminal sensations, aftertaste, and overall acceptability. The by-product-enriched samples exhibited a darker brown colour, more pronounced malt and olive notes, increased bitterness, higher hardness and friability, as well as greater astringency and pungency. In terms of overall acceptability, 13WBSG-NDP received ratings comparable to the CTRL, suggesting its potential as a promising formulation for the functional food sector.

Table 2 *Characterization of snack prototypes.* *

	13WBSG-NDP	13WBSG-DP	30DBSG-NDP	30DBSG-DP	CTRL
Protein % (d.m.)	12.92 ± 0.03c	12.73 ± 0.04b	13.56 ± 0.03d	13.76 ± 0.02e	10.78 ± 0.03a
Fat % (d.m.)	9.77 ± 0.22c	9.13 ± 0.17b	12.47 ± 0.08e	10.54 ± 0.54d	6.92 ± 0.15a
Carbohydrates % (d.m.)	60.57 ± 0.65c	61.67 ± 1.19c	44.93 ± 0.77a	50.94 ± 1.24b	77.78 ± 0.42d
of which sugars % (d.m.)	3.63 ± 0.02a	4.18 ± 0.02b	4.58 ± 0.03d	4.24 ± 0.02c	4.14 ± 0.01b
Fiber % (d.m.)	14.05 ± 0.38b	13.69 ± 0.69b	25.85 ± 0.50d	21.42 ± 0.93c	3.18 ± 0.19a
β-glucans % (d.m.)	0.18 ± 0.01b	0.23 ± 0.01c	0.32 ± 0.01d	0.24 ± 0.01c	0.13 ± 0.01a
Arabinoxilan % (d.m.)	4.52 ± 0.10b	4.30 ± 0.17b	6.95 ± 0.14c	7.06 ± 0.15c	1.96 ± 0.09a
TPC (mgGAE/kg d.m.)	810 ± 87.2b	1208.7 ± 25.8c	1240.6 ± 53.7c	1328.8 ± 59.8c	422.3 ± 16.7a
DPPH (μmol TE/kg)	1178.73 ± 6.45b	1252.17 ± 2.77c	1411.65 ± 5.22d	1388.19 ± 0.31e	624.67 ± 4.61a
FRAP (μmol TE/kg)	6991.44 ± 37.23b	7660.92 ± 63.29c	8834.94 ± 28.54d	8685.77 ± 55.84d	2726.91 ± 2.23a
ABTS (μmol TE/kg)	809.06 ± 26.60b	931.92 ± 30.82c	1086.78 ± 6.71d	1072.01 ± 3.73d	318.84 ± 1.49a

** The results are the mean of two determinations ± the sd. Different letters indicate a statistically significant difference ($p < 0.05$).*

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Food System Innovation and Sustainability: Legume Cooking Water (P-II-21)

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The first and second year of the PhD focused on the evaluation of composition and techno-functional properties of legume cooking waters (CWs) in-lab developed or obtained by canned chickpeas, beans, lentils, and peas, compared to egg white (EW). CWs were analyzed also after applying two treatments: clarification and acidification. Legume species affected composition and techno-functional properties. Clarification reduced apparent viscosity and improved foaming capacity; Acidification increased apparent viscosity and overrun. All CWs showed higher emulsifying ability but lower stability than EW. Results highlighted the potential of CWs as new ingredients for the formulation of tailored foods (e.g., for vegan, eggs-allergic).

Innovazione e sostenibilità dei sistemi alimentari: acqua di cottura dei legumi

Nei primi due anni del dottorato sono state valutate composizione e proprietà tecnologiche delle acque di cottura dei legumi (CW), sviluppate in laboratorio o ottenute da ceci, fagioli, lenticchie e piselli in scatola, confrontate con l'albume (EW). Le CW sono state valutate anche dopo due trattamenti: chiarificazione e acidificazione. L'origine botanica influenza composizione e proprietà tecnologiche. La centrifugazione riduce viscosità apparente e migliora la capacità schiumogena, mentre l'acidificazione aumenta viscosità apparente e overrun. Tutte le CWs mostrano una maggiore capacità emulsionante ma minore stabilità rispetto EW. Dai risultati emerge il potenziale delle CWs come nuovi ingredienti per formulazioni alimentari personalizzate.

Key words: legume cooking water, foaming properties, emulsifying capacity, acidification, clarification

1. Introduction

In accordance with the PhD project aims and the Gantt chart activities (A#) previously described (Sala, 2024), this poster reports the main results of the first two years of research. The literature survey (A1) revealed a growing interest in legumes by-products and highlighted the possibility of (re)use CWs as new food ingredients to reduce the environmental impact (e.g., water consumption) of legume products. According to the scientific gaps evidenced, the composition of chickpea-CW obtained at different cooking conditions or from canned chickpeas, and their techno-functionalities were evaluated (A2). Effects of common ingredients were also assessed (A2-2). In addition to previous Gantt activities, to broaden the knowledge developed, CWs from different canned legumes were considered, also after applying clarification (S) and acidification (A) processes. Trials on production and characterization of tailored products are on-going (A3).

2. Materials and Methods

This study investigates the chemical and techno-functional properties of CWs in-lab developed or obtained by canned chickpeas (C), beans (B), lentils (L) and peas (P) in comparison to egg white. CCW was produced as reported by Tufaro and Cappa (2023) with few modifications. On these matrices the effect of salt, sugar and lactic acid addition was studied. S-samples were obtained by centrifugation at 4200 rpm for 4 min at 20°C. A-samples were prepared adding lactic acid (pH=4). Protein content was determined by Kjeldahl. Protein profiles were analyzed by gel electrophoresis. Sugars were quantified by high-performance liquid chromatography (L-7000, Merck Hitachi, Darmstadt, Germany) and total starch by a Megazyme enzymatic assay kit. The legume CWs were also evaluated for dry matter, pH and apparent viscosity at 7°C and 20°C by a rheometer (MCR 102 rheometer, Anton Paar, Austria). Foaming properties were evaluated by whipping 150 g of CW at 7°C (Tufaro & Cappa, 2023) for their optimal whipping time defined by preliminary trials. Foams were characterized in terms of overrun, hardness (TA.HD.Plus, Stable Micro Systems, UK) and bubble distribution over time (up to 120 min). Emulsions were prepared by homogenizing CW (20°C) and sunflower oil with a high-shear homogenizer (UltraTurrax T25, IKA, Germany) at 17500 rpm for 1 min, following a modified method of Serventi et al. (2018) to evaluate the emulsifying ability (EA) and stability (ES).

All data were subjected to one-way analysis of variance (ANOVA), followed by the Least Significant Difference (LSD) test to identify significant differences between the samples ($p \leq 0.05$) using Statgraphics Centurion 18.

3. Results and Discussion

3.1 Effect of cooking conditions and common ingredients addition on CCW features

CCW obtained at 100°C had significantly ($p<0.05$) higher dry matter and apparent viscosity, but 15% lower overrun than CCW obtained at lower temperature. The addition of caster sugar and powdered sugar reduced overrun by 33% and 7%, respectively, while foam capacity improved with the addition of salt (2-21%) and lactic

acid (72-140%).

3.2 Effect of legume species and treatment on CW features

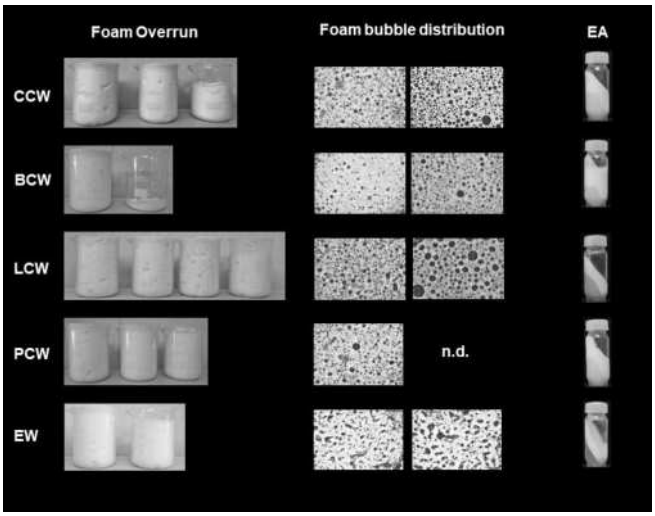
CWs from different legumes had 0.44-3.68 g/100 g sugar and 1.24-1.70 g/100 g protein contents, while starch was below the detection limit. PCW showed a 49-88% higher maltose content than the other CWs, whereas CCW had the highest protein content. Gel electrophoresis shows that proteins are mainly soluble. CWs significantly ($p<0.05$) differed in dry matters and apparent viscosities (Table 1), while pH of CW and CWS samples were 6.2-0.1. Centrifugation reduced apparent viscosity (by 10-37%), whereas acidification led to an increase (4-26%). Similar results were obtained at room temperature (20°C).

Table 1 *Effect of different treatments on dry matter, apparent viscosity of all legume cooking water (CW) on the left, technological imagines of different legume CW without treatment.*

Sample	Dry matter (g/100 g)	Apparent viscosity 7°C (mPa*s)
CCW	$7.35\pm0.01^{b,D}$	$15.1\pm1.5^{c,B}$
CCWS	$7.17\pm0.01^{c,C}$	$9.5\pm0.1^{c,A}$
CCWA	$6.92\pm0.02^{b,B}$	$27.4\pm0.2^{c,C}$
CCWSA	$6.72\pm0.02^{b,A}$	$14.0\pm0.1^{c,B}$
BCW	$7.33\pm0.07^{b,B}$	$179.0\pm1.9^{d,C}$
BCWS	$4.99\pm0.10^{b,A}$	$122.3\pm0.7^{d,A}$
BCWA	$7.04\pm0.08^{b,B}$	$200.4\pm1.1^{d,D}$
BCWSA	$5.05\pm0.43^{a,A}$	$130.7\pm0.9^{d,B}$
LCW	$4.51\pm0.01^{a,A}$	$3.1\pm0.1^{a,B}$
LCWS	$4.41\pm0.14^{a,A}$	$2.8\pm0.1^{a,AB}$
LCWA	$5.36\pm0.16^{a,C}$	$3.9\pm0.2^{a,C}$
LCWSA	$5.07\pm0.01^{a,B}$	$2.8\pm0.1^{a,A}$
PCW	$7.79\pm0.04^{c,AB}$	$8.9\pm0.1^{b,C}$
PCWS	$7.76\pm0.05^{d,A}$	$7.1\pm0.1^{b,A}$
PCWA	$7.90\pm0.06^{c,C}$	$9.3\pm0.4^{b,C}$
PCWSA	$7.87\pm0.04^{c,BC}$	$8.1\pm0.2^{b,B}$
EW	11.60 ± 0.07	6.8 ± 0.1

Note: in the same column, values followed by different letters are significantly different ($p<0.05$). Lowercase letters refer to different legumes equally treated; uppercase letters refer to the same legume differently treated.

Figure 1 *Foam overru, foam bubble distribution (t0 on the left, t120 on the right) and emulsion activity (EA) of different legume cooking water (CW).*



Note: n.d., not determinable.

Overrun values varied from 479% (BCW) to 2112% (CCWSA) depending on the legume type (Figure 1) and the process applied. Among the different legumes, LCW had the highest overrun (1921%). Clarification enhanced overrun (6-33%) due to the removal of insoluble substances that interfere with foam creation. Except for LCW, acidification increased overrun up to 53%, due to the modification of protein net charge which weakens the hydrophobic interactions (Lafarga et al., 2019). BCW had the finest structure (>80% of small-sized bubbles; Figure 1) and resulted more stable (data not shown); LCW had the highest number of medium-large bubbles. Bubbles coalescence was evident in all the samples (Figure 1) but only PCW collapsed after 60 min at 5°C. The treatment applied had no effect on the bubble's distribution of LCW and PCW, while somehow affected BCW and CCW.

In general, CWs showed higher EA (48-67%) and lower ES (33-88%) than EW (45% and 102%, respectively). BCW had the highest EA but the lowest stability. The treatment applied does not affect EA, while ES values increased after centrifugation for CCW (11%) and LCW (13%) and in all the samples after acidification (up to 21%).

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Insights into the impact of ultra-high pressure treated rice husk microfibrillar cellulose on the viscoelastic and thermomechanical properties of model ice cream mixes (P-II-22)

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The second year of this PhD project focused on characterizing model ice cream mixes stabilized with microfibrillated cellulose (MFC). These systems were compared to formulations containing conventional soluble fibers in terms of their stabilizing capacity and ability to impart similar viscoelastic and thermomechanical properties. MFC showed a remarkable thickening effect and provided thermal stability comparable to that of soluble fibers at equivalent concentrations.

Approfondimenti sull'impatto della cellulosa microfibrillare da lolla di riso trattata ad altissima pressione sulle proprietà viscoelastiche e termomeccaniche di miscele modello di gelato

Il secondo anno del presente progetto di dottorato si è concentrato sulla caratterizzazione di miscele modello di gelato stabilizzate con cellulosa microfibrillare (MFC). Tali sistemi sono stati confrontati con formulazioni contenenti fibre solubili convenzionali in termini di capacità stabilizzante e di conferimento di proprietà viscoelastiche e termomeccaniche analoghe. La MFC ha mostrato un notevole effetto addensante e ha garantito una stabilità termica comparabile a quella delle fibre solubili, a parità di concentrazione.

Keywords: Rice husk, microfibrillar cellulose, thermomechanical properties, model ice cream mixes.

1. Introduction

From a colloidal perspective, ice cream is a frozen foam comprising ice crystals, partially destabilized fat droplets, and air cells dispersed in a continuous freeze-concentrated (serum) phase. Due to its colloidal complexity, ice cream can undergo significant structural element changes during freezing, hardening, and frozen storage that are inextricably associated with impairing its textural quality. Stabilisers such as gums (galactomannans, alginates, carrageenan, and chemically modified cellulose) and oligosaccharides are well-established for their ability to impart body and texture, control meltdown, and inhibit texture deterioration due to ice recrystallisation (Soukoulis and Fisk, 2016). In the context of the circular economy, the production of functional hydrocolloids (thickeners, gelling and interface stabilizing agents) implementing exclusively physical processing methods is highly desirable. Microfibrillar cellulose (MFC), commonly produced via mechanical processing of cellulose biomass, has gained much popularity in recent decades due to its peculiar technological properties, renewability, and biodegradability (You et al., 2024). Regarding ice cream, MFC has found applications primarily as a thickening, texturing, and cryoprotective agent, particularly in low and non-fat formulations (Yu et al., 2021).

2. Materials and Methods

Cellulose extraction from rice husk was performed following the method previously described by Johar et al. (2012), while the MFC was obtained by passing a suspension of the extracted cellulose in water (1% b.w.) through a high-pressure homogenizer (PandaPLUS 2000, Gea, Italy) at 1200 bar for 9 cycles. Seven different model mixes, four at decreasing concentrations of MFC (0.2, 0.1, 0.075, 0.05% b.w.) and 3 with a single gum at a concentration of 0.2% b.w. (Xanthan gum, Guar gum, and Locust bean gum, XG, GG, and LBG, respectively) were produced. Atomic Force Microscopy (AFM) (Innova®, Bruker, U.S.) and Scanning Electron Microscopy (SU7000, HITACHI, Germany) were used to characterize the MFC physical and structural properties, providing insight into fibril structure, diameter, and organisation. The MFC colloidal properties were evaluated by measuring the Zeta potential (ζ) and Intensity-weighted mean hydrodynamic diameter (Z-Average) with a Zetasizer (Malvern Panalytical Ltd, U.K.)

Regarding the ice cream mixes, the size and distribution of coconut oil fat globules were assessed before and after the ageing process using a Mastersizer 3000 laser diffraction particle size analyzer (Malvern Panalytical Ltd, U.K.) using water as the dispersing medium. The refractive indices applied for water and coconut oil were 1.330 and 1.449, respectively, with an absorption index of 0.01.

Rheological tests were carried out in both liquid and frozen states with an MCR 302 rheometer (Anton Paar GmbH, Austria). In the liquid phase (5 °C), flow and frequency sweep tests were conducted using a concentric cylinder geometry, and LAOS (Large Amplitude Oscillatory Shear) tests were performed at 1 s^{-1} with strain amplitudes from 0.01 to 1000%. In the frozen state, a serrated parallel plate geometry was used to monitor viscoelastic changes from –40 to 5 °C via oscillatory thermo-rheometry (OTR). LAOS tests were also performed at -6°C at 1 s^{-1} with strain amplitudes from 0.001 to 1000%. Sedimentation stability was assessed with a LUMiSizer (LUM GmbH,

Germany) at 4000 rpm for 30 minutes at 25 °C, revealing phase separation trends. Finally, the internal structure of the ice cream matrix was visualized using Confocal Laser Scanning Microscopy (CLSM, Carl Zeiss AG, Germany).

3. Results and Discussion

3.1 Physicochemical and morphological aspects of RMFC

High-pressure homogenization (HPH) of MFC suspensions at a concentration of 0.73% b.w. led to the formation of an entangled network of cellulose microfibrils with measured diameters between 7 and 15 nm, as revealed by diameter analysis on AFM images. Dynamic Light Scattering (DLS) measurements ~~further~~ showed a hydrodynamic diameter of approximately 4.98 µm, consistent with the presence of microfibril clusters. Moreover, HPH caused a notable reduction in surface charge density—likely due to the exposure of hydroxyl groups on the fibrils surface.

3.2 Microstructural, steady state flow behaviour and dynamic rheological properties of the RMFC stabilised ice cream mixes

From CLSM micrographs and particle size data, MFC formed an entangled filamentous network that, unlike soluble fibers, sterically hindered lipid droplet size reduction. This effect resulted in significantly larger fat globule diameters ($d_{4,3}$ = 19.3 µm) compared to systems stabilized with LBG (3.6 µm), GG (0.9 µm), and XG(6.5 µm). Despite the absence of visible macroscopic phase separation in the MFC-containing mixes after storage at 4 °C for one-week, creaming phenomena were evident in all systems, with galactomannan- and XG-stabilized mixes being the most affected. The steady-state rheology showed that incorporating MFC led to a concentration-dependent increase in the consistency coefficient and a decrease in the flow behavior index, highlighting a stronger thickening capability relative to the soluble fibers. From the frequency sweep test, samples containing ≥0.075% MFC exhibited elastic-dominant behavior over the 0.01–10 Hz frequency range, with relaxation times below 0.02 s. Conversely, MFC at 0.05% and all soluble fiber formulations showed clearly viscoelastic responses, with relaxation times between 0.02 and 0.06 s.

3.3 Thermomechanical properties of the ice cream model in frozen state and at draw temperature (-6°C)

The thermomechanical behavior of the MFC-stabilized ice cream models in the frozen state was investigated through OTR and LAOS testing. All formulations exhibited a distinct α-relaxation peak ($\tan \delta_a$) in the OTR spectra. Notably, MFC-containing samples (except MFC 0.2%) showed significantly lower α-relaxation temperatures (–27.7 °C) compared to those containing soluble fibers (–25.2 °C), suggesting a reduction in molecular mobility of the unfrozen phase. These results were consistent with DSC-derived melting temperature (Tm') values, reinforcing the hypothesis that MFC at concentrations up to 0.1% does not substantially hinder the dynamics of unfrozen water. Conversely, the increase in both $\tan \delta_a$ and Tm' observed in the MFC 0.2% system could indicate the formation of a composite-like structure, likely due to the development of an interpenetrating cellulose network.
No clear trend was observed between the ice fusion temperature (secondary $\tan \delta$ peak) and MFC concentration. LAOS testing at –6 °C further revealed that the onset of non-linear viscoelastic behavior occurred within the strain range of 0.006–0.02%. Within the moderate amplitude oscillatory shear regime, all samples maintained linear viscoelasticity up to 0.4% strain. A pronounced yielding behavior, characterized by distorted elliptical Lissajous loops, became evident at strain amplitudes between 1 and 4%. Ice cream models containing soluble fibers displayed a broader linear viscoelastic range and stronger gel-like characteristics. In contrast, MFC-based systems were more sensitive to the applied shear stress, with their structural deformation being clearly dependent on MFC concentration.
According to our findings, MFC is a promising sustainable ice cream thickener alternative. Regarding its thickening power, MFC outperformed LBG, GG, and XG at the tested concentration range. On the other hand, only the use of MFC at 0.2% showed an effect on Tm' (calculated via OTR and DSC) similar to that caused by soluble fibre-based stabilisers. Further investigation is required to fully clarify the mechanisms by which MFC influences glass transition phenomena, thereby advancing the understanding of its functional role in complex food matrices.

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Process and Product Innovation for the Valorization and Sustainability of Confectionary Productions (P-II-23)

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(DM 118/2023 funding by PNRR)

The scientific community faces a major challenge to minimize food waste and reuse food processing byproducts. Nuts, which are widely used in confectionery, generate by-products rich in bioactive compounds that can be used for enhanced new productions. The project aims at valorizing nuts by-products to be used as new food ingredients to produce traditional confectionary products. New peeling methods, such as ultrasound, are being investigated to reduce water consumption and obtain almond skin with higher content in bioactive components.

Innovazione di Processo di Prodotto per la Valorizzazione e Sostenibilità di Prodotti Dolciari

La comunità scientifica si trova ad affrontare una sfida importante per ridurre al minimo gli sprechi alimentari e riutilizzare i sottoprodotti della lavorazione degli alimenti. Dalla frutta secca, ampiamente utilizzata in pasticceria, si generano sottoprodotti ricchi di composti bioattivi che possono essere valorizzati in nuove produzioni. Il progetto mira a valorizzare i sottoprodotti della frutta secca per utilizzarli come nuovi ingredienti alimentari per la produzione di prodotti dolciari tradizionali. Si stanno studiando nuovi metodi di pelatura, come gli ultrasuoni, per ridurre il consumo di acqua e ottenere una buccia di mandorla con un maggior contenuto di componenti bioattivi.

Key words: Food processing by-products, Almonds, Ultrasound technique, Innovative processes, Sustainable processes.

1. Introduction

Almonds are consumed in various forms (fresh, roasted, peeled or not) and are widely used in cooking and food production. The industrial processing of almonds includes cleaning, hulling, shelling, sorting, sizing, and in some cases processing like roasting, slicing, or dicing, depending on the final product. Almond skins (4-8% of the shelled almond weight) are generally removed by blanching, which produces both the skin and the blanching water as by-products (Lacivita *et al.*, 2024). Almond skins are rich in polyphenols which different studies suggested that can be used as antioxidant additives in the food industry or as functional ingredients in supplements (Mandalari *et al.*, 2010). With the blanching operations, nevertheless, many polyphenols are lost in the boiling water. Consequently, the antioxidant capacity of almond skins, which is mainly attributed to their phenolic content, can vary depending on the specific conditions used to their removal (Mandalari *et al.*, 2010). To meet the principles of environmental sustainability, the development of innovative peeling technologies is crucial. In literature various techniques are reported and highlight novel alternatives like infrared, ohmic-assisted, and ultrasound-assisted peeling (Zhou *et al.*, 2022). Ultrasound (20 *kHz*-1 *MHz*, typically <100 *kHz*) uses cavitation to create microbubbles, generating forces that disrupt cell walls, facilitating effective, non-thermal peeling (Zhou *et al.*, 2022). In view of this, the present study aims to use ultrasound as an innovative technology for peeling almonds, reducing the amount of water normally used. The phenolic content of skins, water used for ultrasound peeling and the water used for boiling were determined and compared.

2. Materials and Methods

Shelled almonds from the cultivars Fascionello from Sicily (FA) and Nonpareil from California (NA) were provided by Dolceamaro srl, the cultivar Tuono from Molise (TA) was provided by the Nutfuit Italia Soc. Agr. a.r.l. (Monteroduni, Italy). From each sample, according to Oliveira et al. (2018) ten representative fruits were selected, and kernel length (L), width (W), and thickness (T) were measured using a digital caliper (Electronic digital caliper – Stainless hardened). From these measurements, L/W, L/T and W/T ratios were calculated to obtain a shape index. On the almond samples three distinct almond peeling treatments were employed:
- Ultrasound Water Immersion (UWI): Almonds were fully immersed in water (10 *mL*) during the treatment (laboratory scale ultrasound bath (Bransonic B2210E-MT ELA-9408, Connecticut, U.S.A.) at 125 *W*). The treatment was performed for 3 and 6 minutes to test the improvement of the peelability;
- Ultrasound Water Spray (UWS): Almonds were placed on a polyethylene film covering the water surface of the ultrasound bath and were sprayed with water (simulating a conveyor belt);
- Blanching (BL): Almonds underwent a blanching process for 3 minutes at 95 °*C* (almonds:water 1:3).
The treatments were followed by hand peeling of the skin. Skins were dried at room temperature and then ground in a Janke & Kunkel mill (Ika Labortechnik, Wilmington, N.C., U.S.A.). The total phenol content (TPC) was determined with the Folin-Ciocalteu method according to Garrido et al. (2008) on skins and aqueous bulk from UWI and BL treatments. The study involved the drying of aqueous bulk from UWI and BL water, the extraction

of residues, and sonication of ground skins and water residues. The mixture was centrifuged and filtered to determine TPC. The Folin-Ciocalteu test was carried out, and a spectrophotometric measurement was made at 765 *nm*.

3. Results and Discussion

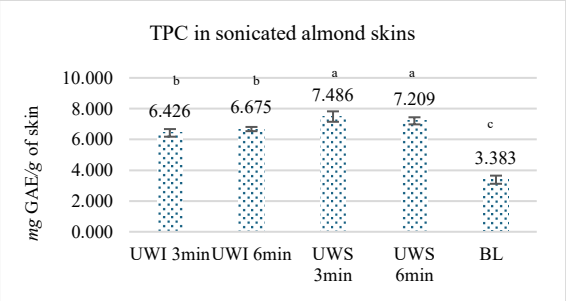
The morphological characteristics of the almond samples are listed in Table 1.

Table 1 Almond dimensions. Different lower-case letters within columns indicate statistically significant differences ($p < 0.05$) for each measurement

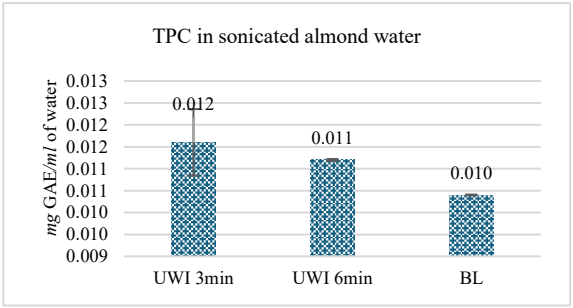
Samples	*Nut Length (mm)*	*Nut Width (mm)*	*Nut Thickness (mm)*	*L/W*	*L/T*	*W/T*
NA	*20.14 ± 2.02 [b]*	*11.09 ± 0.59 [c]*	*6.96 ± 0.52 [ab]*	*1.82 ± 0.19 [a]*	*2.90 ± 0.33 [b]*	*1.59 ± 0.10 [c]*
FA	*25.36 ± 1.25 [a]*	*15.71 ± 0.43 [a]*	*6.20 ± 0.37 [b]*	*1.61 ± 0.10 [b]*	*4.09 ± 0.17 [a]*	*2.54 ± 0.15 [a]*
TA	*21.94 ± 1.23 [b]*	*13.47 ± 0.28 [b]*	*7.41 ± 0.60 [a]*	*1.63 ± 0.10 [b]*	*2.96 ± 0.29 [b]*	*1.82 ± 0.13 [b]*

Each value is expressed as the mean ± standard deviation (n=10). L – Length, W – Width, T – Thickness.

Following the almonds' shape determination, subsequent analyses focused on those sourced from Molise (TA).



Graph 1 Comparison between TPC in sonicated almond skins for 3 and 6 minutes in both sonicated treatments and in blanched almond (UWI=Ultrasound Water Immersion, UWS= Ultrasound Water Spray, BL=blanched) Each value is expressed as the mean ± standard deviation.



Graph 2 Comparison between TPC in sonicated almond water for 3 and 6 minutes (UWI=Ultrasound Water Immersion) and blanched almond (BL=blanched). Each value is expressed as the mean ± standard deviation.

Graph 1 shows TPC in sonicated and almond skins (UWI, UWS, and BL) at 3-minute and 6-minute treatments and in the blanched skin. The sonicated peeled almonds showed higher TPC than those of blanched almonds (3.383 ± 0.27 *mg* GAE/*g* of skin). On the other hand, peels obtained from water-sprayed sonication (UWS) had higher phenolic contents (7.486 ± 0.34 *mg* GAE/*g* of skin and 7.209 ± 0.23 *mg* GAE/*g* of skin respectively for 3 and 6 minutes treatment) than those obtained by immersion sonication (UWI) (6.426 ± 0.25 *mg* GAE/*g* of skin and 6.675 ± 0.14 *mg* GAE/*g* of skin respectively for 3 and 6 minutes treatment). The peelability after UWI and UWS treatments remains unchanged, but sonicated peeled almond skins had higher phenolic content than BL due to phenolic solubilization during blanching and potential degradation during hot processing. Graph 2 shows the TPC in sonication water and in blanched water. No statistically significant differences have been highlighted among the waters from sonicated almonds and from blanched almonds. Among different peeling mechanisms for almond skins, ultrasound water spray treatment increases the TPC recovery. This suggests a promising way to produce a high-TPC by-product as a novel ingredient; Moreover, future research will explore the effectiveness of modifying ultrasound condition (output power and working frequencies variations) on different varieties and the connection between morphological characteristics of the almond kernel and ultrasound peelability.

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Innovative approaches for the development and performance evaluation of new frying mixtures through physical, chemical and sensory analyses (P-II-24)

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The project integrates physical, chemical and sensory data to evaluate the impact of oil degradation during frying on the perceived quality of both oils and fried products. In the initial phase of the project, 9 frying oils were characterized through the analysis of fatty acid composition (FAME), free fatty acids (FFA), peroxide value (PV), total polar compounds (TPC), oxidative stability index (OSI time), and volatile compounds (VOCs). Concurrently, sensory evaluation (QDA®) was performed on four selected oils and French fries cooked therein, while consumer preference test (hedonic evaluation, Check-All-That-Apply, CATA, and Just-About-Right, JAR) was conducted on French fries to assess the perceptible changes associated with semi-continuous frying.

Approcci innovativi per la messa a punto e la valutazione delle performance di nuove miscele di frittura attraverso analisi chimico-fisiche e sensoriali

Il progetto integra dati chimico-fisici e sensoriali per valutare l'impatto della degradazione degli oli durante la frittura sulla qualità percepita dell'olio e del prodotto fritto. In una prima fase, 9 oli da frittura sono stati caratterizzati mediante analisi degli acidi grassi (FAME), acidità (FFA), perossidi (PV), composti polari (TPC), tempo di induzione all'ossidazione (OSI time) e composti volatili (VOCs). Parallelamente, è stata eseguita una valutazione sensoriale (QDA®) su quattro oli e sulle patatine fritte ottenute mediante il loro impiego, mentre un test sulle preferenze dei consumatori (valutazione edonica, Check-All-That-Apply, CATA, e Just-About-Right, JAR) è stato condotto su un campione di patatine fritte, al fine di analizzare la percezione delle modificazioni indotte dalla frittura semi-continua.

Key words: Deep frying oils, oil degradation, oxidative stability, sensory analysis of fried products, Quantitative Descriptive Analysis (QDA®), consumer preference, Check-All-That-Apply (CATA), Just-About-Right (JAR).

1. Introduction

In accordance with the PhD thesis project, this poster reports the main results of the first three activities:

(A1) Chemical characterization of different types of vegetable oils and blends intended for frying, aiming to identify oxidation markers;
(A2) Training a panel of tasters (QDA®) to objectively assess the sensory quality attributes of frying oils and fried products;
(A3) Investigating the perception threshold of rancidity and other sensory attributes in French fries through consumer test, to gather information on preferences and acceptability of fried products.

2. Materials and Methods

Nine frying oils and oil blends were selected: sunflower (SO), high-oleic sunflower (HOSO), blend A (A), blend B (B), extra virgin olive oil (EVO), olive, palm, peanut (PEA), and soybean (SOY) oil. Each oil underwent a semi-continuous frying protocol over five days using French fries. The daily procedure included 1 hour of preheating, three 4-minute frying sessions spaced by 15-minute and a final 1-hour heating phase. Oil samples were collected at the end of each day. Three complete frying cycles were performed for each oil. FAME were determined by gas chromatography-flame ionization detection (GC-FID) (Semeniuc et al., 2020), while VOCs were analyzed by GC coupled to a mass spectrometry detector (GC-MS) (Tura et al., 2022); PV (method NGD C35), FFA (Reg. CE n. 2568/91), TPC (Circ. MINISAN 11.1.91 n.1.) and OSI time (AOCS Official Method Cd 12b-92) were also determined. Sensory evaluation was conducted using Quantitative Descriptive Analysis (QDA®) with a trained panel of 10 assessors. A standardized vocabulary was developed; only descriptors elicitated by more than 5% of assessors were retained for the final sensory sheets. Reference materials were used to calibrate panelists on attribute recognition and intensity. Panelists daily evaluated four oil samples and four corresponding French fries' samples, using dedicated sensory sheets. A consumer test involving 210 participants was conducted to assess French fries sampled on frying days 1, 3, and 5. Participants rated visual, olfactory, textural and taste liking using a 9-point hedonic scale, and performed Check-All-That-Apply (CATA) and Just-About-Right (JAR) assessments to capture perceived attributes and to rate the intensities of selected sensory descriptors.

3. Results and Discussion

3.1 Chemical characterization of different types of deep-fried oils

Analysis of the first frying cycle were completed for all tested oils and blends, and data are summarized in a traffic-

light table highlighting key analytical parameters. FFA increased over time but remained within legal limits. PV increased in all oils, displaying different trends over time; HOSO and SO peaked at T3 then declined, suggesting peroxide degradation, while EVO, OLIVE, PEA, and PALM showed a continuous rise in PV, consistent with lipid oxidation propagation. Regarding the blends, Blend A showed a sinusoidal PV trend, while blend B maintained the lowest PV levels. VOCs analysis revealed progressive aldehyde accumulation, especially hexanal and nonanal. Blend B exhibited the lowest aldehyde concentrations, while SO showed high levels of 2,4-decadienal (*E,E*) at T5 due to its high linoleic acid content. OSI was highest in blend A, followed by EVO, PEA, PALM, B, HOSO, and OLIVE; SOY and SO showed the lowest stability (<10 h). Blend B had the lowest TPC at T5, indicating minimal degradation, while SOY and PALM exceeded the 25% legal threshold. Overall, blends A and B demonstrated superior stability throughout frying.

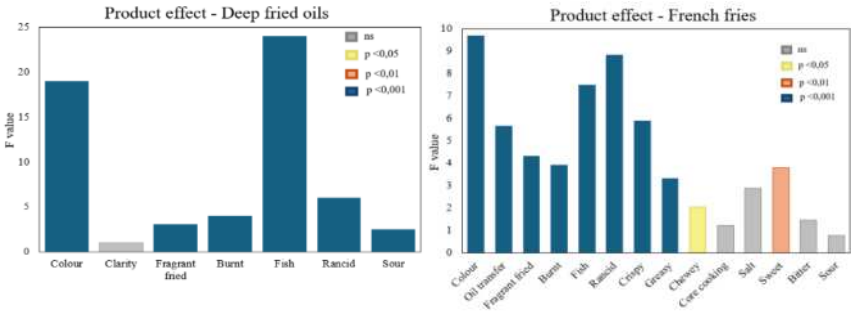
Table 1 *Visual evaluation of oil stability during frying.*

OIL	FFA	PV	VOCs	OSI	TPC
SO					
HOSO					
Blend A					
Blend B					
EVO					
OLIVE					
PALM					
PEA					
SOY					

3.2 Quantitative Descriptive Analysis (QDA®) of frying oils and French fries

The panel showed high discriminative ability ($p < 0.001$) across oils for attributes such as colour, fragrant fried, burnt, fish, and rancid, but not for clarity. For French fries, significant differences ($p < 0.001$) were found based on oil type for the following attributes: colour, oil transfer, fragrant fried, fish, rancid, crispy, greasy, chewy ($p < 0.05$), and sweety ($p < 0.01$). No significant differences emerged for core cooking, salty, bitter, and sour. The panel was aligned ($p > 0.05$) and repeatable ($p > 0.5$), effectively capturing sensory variations related to oil composition and frying duration.

Figure 1 *Sensory Panel's Discrimination Capacity for Oils and French Fries*



3.2 Hedonic-CATA and JAR scale to evaluate French fries' sensory perception and acceptability

From CATA results (n=70 subjects/day), significant differences (Cochran's Q test, $p < 0.05$) emerged for three attributes (fragrant fried, crispy and sweet), which declined notably from T3. JAR evaluation of four key attributes showed significant increases in perceived rancid and greasy, while fragrant fried and crispy remained stable. This suggests that attributes such as fragrant fried and crispy may retain similar perceived intensity (JAR) but be selected less frequently (CATA) as the oil deteriorates and these attributes become less prominent within the overall sensory profile. Moreover, the increase in rancid and greasy scores on the JAR scale, despite not being significant in CATA, indicates a perceptible rise in these notes that is not dominant enough to influence descriptor selection frequency. The overall liking (ANOVA, $p < 0.05$) did not show significant differences from T1 to T5, confirming that consumer acceptance remained stable and high during semi-continuous deep-frying. This finding suggests that moderate changes in certain sensory attributes did not negatively affect the overall perception of the fried product.

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Sensory analysis and emotional profile as a tool for the valorisation of sustainable and innovative food chain (P-II-25)

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The main objective of this PhD research project during the second year has been to develop and validate a new method for red wine valorization. This method integrates chemical data (complete chemical profile and VOCs), sensory analysis (panel tests by trained judges), and emotional profiling (wearable biosensors and self-reported questionnaires).

Sensory analysis and emotional profile as a tool for the valorisation of sustainable and innovative food chain

L'obiettivo principale di questo progetto di ricerca di dottorato nel corso del secondo anno è stato sviluppare e convalidare un nuovo metodo per la valorizzazione del vino rosso. Questo metodo integra dati chimici (profilo chimico completo e COV), analisi sensoriali (test di panel condotti da giudici qualificati) e profilazione emotiva (biosensori indossabili e questionari auto-compilati).

Key words: Sensory analysis, emotions, food quality, panel testing, EEG; electrocardiogram, wearable sensors, VOCs, GC-MS

1. Introduction

Wine is a cultural product capable of arousing emotions. Measuring emotions and figuring out how much they could influence preferences or purchase decisions of consumers is a new trend in sensory and consumer research [1, 2]. In accordance with the PhD thesis project, this poster reports the main results of the first two activities concerning:

(A) to set up and validate an affordable method to measure the emotions elicited by red wines.
(B) to identify possible reliable correlations between wine composition and the emotions elicited.

2. Materials and Methods

Eight red wine samples with different grape variety, vintage (2003-2020) and Product Designation of Origin were used for the multidisciplinary analysis reported on table 1. Due to the multidisciplinary approach combining sensory analysis, implicit and explicit measurements of emotions and chemical analysis of the wine product, the detailed materials and methods are represented on table 2.

Table 1. Wines selected for the tasting sessions

	WINES - RED							
Code	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
	DOC Maremma Toscana	IGT Toscana Rosso	DOCG Brunello di Montalcino	DOC Valpolicella Classico	DOCG Brunello di Montalcino Riserva	DOCG Amarone della Valpolicella Classico	DOCG Ghemme	IGT Costa Toscana

Table 2. Materials and methods for integration of sensory analysis and emotional profiling on red wine

Sensory-emotional experimental design	
Sensory methods	**Panel testing** on trained assessors [3]. **Self-report questionnaire (0–9-point scale) evaluated 24 attributes:** **16 quantitative:** color saturation, purple hues, garnet hues, olfactory intensity, roundness, structure, acidity, alcohol, astringency, bitterness, floral, fruity, vegetal, spicy aromas, off-flavors, persistence [2]. **8 hedonic:** attractiveness, finesse, frankness, harmony, finesse after-taste, frankness retro-nose, aromatic richness, hedonic level [2].
Emotional methods (explicit)	**Self-report emotional questionnaire (0–9-point scale)** with 6 emotions: fear, anger, sadness, joy/happiness, disgust/contempt, surprise [2].
Emotional methods (implicit)	**Autonomic nervous system (ANS) reactions** with biomedical signals using electrocardiogram (ECG) and galvanic skin response (GSR) applied only in olfactory phase [4].
Chemical analysis	**Chemical composition** with FT-IR spectrometer WineScan FT 120, UV-VIS spectrophotometry, enzymatic kit and OIV methods [2]; **Aromatic profile - VOCs** with headspace solid phase micro-extraction (HS-SPME) coupled with gas chromatography mass spectrometry (GC–MS) [2].
Statistical analysis	**Sensory data elaboration** and **descriptive statistic** with significant differences (Friedman's ANOVA) and verification of significance (non-parametric LSD post-hoc test) (Big Sensory Soft 2.0) [2]; **Multifactorial comparisons** with correlation matrix of 372 variables; Principal Component Analysis (PCA); and two-way Hierarchical Cluster Analysis (HCA) (JMP Pro 16.0.0 software) [2].

OIV: International Organization of Vine and Wine
VOC: Volatile Organic Compounds

Chemical characterization of wines showed ethanol concentration of wines (% v/v) ranging from 12.62% (samples

D) to 14.87% (sample F), with pH values between 3.38 (sample C) and 3.73 (sample H), and all samples showed complete alcoholic and malolactic fermentation. Volatile acidity values were all within the legal maximum allowed (1.2 g/L as acetic acid), but only three wines (A, C and D) presented lower values than 0.7 g/L. Chemical profile by HS-SPME-GC-MS identified 49 volatile aroma compounds, each associated with specific odor descriptors.
Sensory analysis performed by a trained panel using Quantitative Descriptive Analysis, revealed statistically significant differences ($p< 0.05$) in 14 of 24 sensory attributes. The highest hedonic index scores were presented by samples C (7.78), D (6.65) and A (6.61), meanwhile sample H present the lowest with 4.31. Interestingly, the wines evaluated by the panelist with the lowest hedonic indexes (G and H) were the oldest ones (2011 and 2003, respectively).
On figure 1 are represented the emotions evocated by wine trough self-reported questionnaires; samples C, A, and D elicited the strongest positive emotions (Joy and Positive Surprise), whereas the other wines mainly evoked negative emotions; sample H did not elicit Joy, consistent with its lowest hedonic index. Figure 2 shows the Pearson correlation between explicit emotions and wine aromatic profile giving the top of 25 aroma compounds most strongly associated with the emotions of Joy/Happiness and Disgust/Contempt. Ethyl isovalerate, phenethyl alcohol, and ethyl dodecanoate—compounds associated with sweet, fruity, and floral descriptors—exhibited strong positive correlations with Joy/Happiness and negative correlations with Disgust/Contempt. In contrast, ethyl lactate and ethyl pentanoate, associated with overripe and yeast-like notes, demonstrated inverse relationships.
To illustrate the integration between the wine chemical characterization, sensory analysis attributes, and emotions (explicit measurements) a principal component analysis (PCA) has been used. Figure 3 shows a clearly difference between the 8 wines. Wines with greater fruitiness and chemical smoothness as samples C, A and D evoke positive emotions, while those with a stronger, tannic profile tend to trigger more negative or intense emotional responses as sample B, E, F, G and H.
Regarding the emotions adquired by ECG and GSR, the analysis demonstrated the presences of autonomic nervous system (ANS) differences in response to sensory stimuli related to emotional wine, with higher sympathetic values at extrema and vagal increase in the presence of neutral sensory compounds. Therefore, preliminary findings support the hypothesis that certain molecules may act as emotional markers, capable of eliciting distinct affective responses even in trained panelists; further the method will be adapted to be applied in different food supply chains.

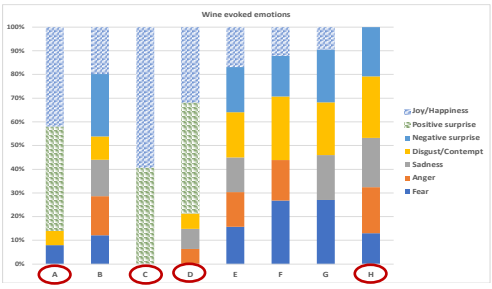


Fig. 1. Relative percentages of the mean emotions evoked by each wine sample during the sensory evaluation.

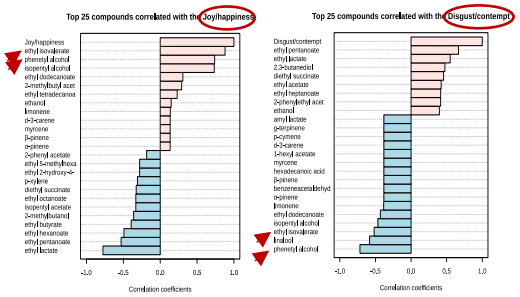


Fig. 2. Correlation (Pearson R) of top 25 aromatic compounds determined by GC–MS with the best and worse emotions: Joy/happiness and Disgust/contempt.

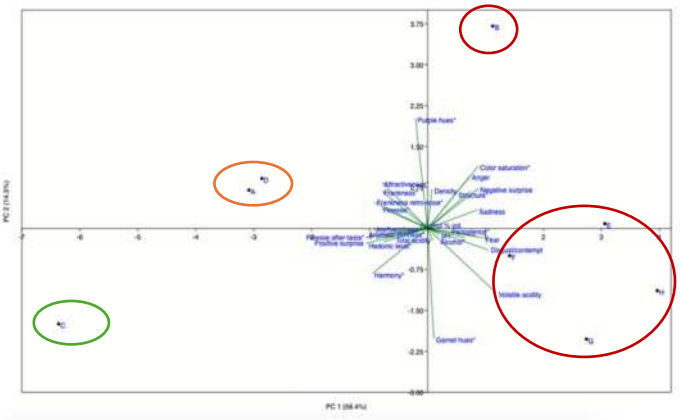


Fig. 3. Principal component analysis (PCA) of chemical characterization, sensory analysis attributes, and emotions (explicit method measurement).

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Attitude to sustainability and health and acceptability of baked goods by consumers (P-II-26)

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The first two activities of the PhD thesis project are described. Data collection activities, designed to address the overarching research objectives, have yielded valuable insights into how sustainability and health-related labels influence consumer perceptions and choices regarding baked products. In the first study, an innovative method for consumer segmentation based on attitudes toward sustainability and health was applied to study the impact of organic and ancient variety of soft wheat (Verna) labels on consumer perception. The second study employed a combination of explicit, implicit, and indirect measures to examine the influence of attitudes toward sustainability and of health and front-of-pack labels Ecoscore, Nutrinform Battery and Nutriscore on consumer expectations.

Attitudine alla sostenibilità e alla salute e accettabilità dei prodotti da forno da parte dei consumatori

Sono descritte le prime due attività del progetto di tesi di dottorato. Le attività di raccolta dati, progettate per rispondere agli obiettivi generali della ricerca, hanno fornito preziose evidenze sull'influenza delle etichette ambientali, di varietà e nutrizionali sulla percezione e sulle scelte dei consumatori riguardo ai prodotti da forno. Nel primo studio è stato applicato un metodo innovativo di segmentazione dei consumatori basato sulle attitudini verso la sostenibilità e la salute per comprendere l'impatto dell'etichetta "biologico" e "Verna" sulla percezione dei consumatori. Il secondo studio ha impiegato una combinazione di misure esplicite, implicite e indirette per analizzare l'influenza delle attitudini verso la sostenibilità e la salute e delle etichette fronte pacco Ecoscore, Nutrinform Battery, Nutriscore sulle aspettative dei consumatori.
Key words: expectations, implicit method, Text Highlighting, Ecoscore, Nutriscore, organic.

1. Introduction

In accordance with the PhD thesis project previously described (28th Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, 2024), this poster reports the main results of the first two activities concerning:

A1) **Data collection using a multidisciplinary approach** to investigate consumer expectations, segmented according to their attitudes toward sustainability and health. During the first year of the PhD, Study 1 involved the application of the Text Highlighting method to assess consumer attitudes toward sustainability and health-related information, and to explore how these attitudes influence product expectations. At the beginning of the second year, Study 2 focused on evaluating consumer attitudes toward environmental (Ecoscore) and nutritional (Nutrinform Battery and Nutriscore) labels, and their role in shaping expectations, combining explicit, implicit, and indirect measurement techniques.

A2) **Statistical analyses** were conducted using univariate and multivariate methods to test the effects of key variables across different experimental conditions and to explore the relationships among liking, sensory properties, emotional responses, and contextual factors. In addition, together with psycho-attitudinal questionnaires, text analysis was employed to segment consumers based on their attitudes toward sustainability and health.

2. Materials and Methods

In both studies, the impact of expectations generated by sustainability and health-related information on product perception was assessed using the classic procedure (Cardello, 2007). The first study involved 118 consumers evaluating two organic crackers (modern Altavista wheat vs. ancient Verna wheat) under blind, expected, and informed conditions (with the organic or organic and Verna labels). The second study involved 401 participants, in a preliminary phase (64 participants) and a main study (337 participants); four biscuits were evaluated using a between-subjects design combining Ecoscore (A/E) and Nutrinform Battery (high/low sugar and fat). A subgroup of 74 participants from the blind condition also completed an Implicit Association Test (IAT) (Greenwald et al., 1998) to assess emotional associations with Ecoscore and Nutriscore labels. Individual differences were measured through questionnaires (Food Neophobia Scale, Health and Taste Attitude Scale, and Sustainable Food Choice Questionnaire (Verain et al. 2021) and a Text Highlighting task (TH) (Jaeger et al., 2022). In Study 1, the TH text focused on modern and ancient organic flours; in Study 2, on front-of-pack labels (Ecoscore, Nutriscore, Nutrinform Battery).

3. Results and Discussion

3.1 Text Highlighting method to segment consumers for sustainability and health attitudes and

their role in studying expectations (Study 1)

Data were coded following Jaeger et al. (2022), assigning values of 1 (important), -1 (less important), and 0 (not highlighted). A K-means cluster analysis based on highlighting frequencies identified two clusters: one with 68 participants, the other with 50. Significant differences between clusters were found using Kruskal-Wallis's test. Based on the highlighted content, the clusters were interpreted as reflecting concerns with "Sustainability" (68 participants) and "Health" (50 participants) (Table 1). The two clusters showed distinct patterns of expectation assimilation. The "Health and Tradition" assimilates completely ($p<0.0001$) (informed evaluations aligning with expected judgments and higher than blind ones) for descriptors such as "sustainable product idea," "natural product idea," and "novelty idea" when assessing ancient Verna crackers. In contrast, the "Environmental and Social Sustainability" cluster demonstrated only partial assimilation ($p < 0.0001$), with informed judgments were higher than blind evaluations but not matching expected ones. These findings highlight product-specific consumer attitudes, reflected in varying expectation effects across clusters.

Table 1 *Information significantly contributing to cluster characterization. The assigned cluster corresponds to the group with the highest mean value in the Kruskal-Wallis's analysis, i.e., participants who most frequently marked the information as important.*

Information ***(S**ustainability Cluster)*	**p-value**	**Information** *(Health Cluster)*	**p-value**
Environmental impact	<0.0001	Anti-inflammatory effects	<0.0001
(lower release of) greenhouse gases	0.0004	Lower yields	<0.0001
Cost reduction	0.002	Higher content of minerals	0.0002
Biodiversity	0.006	Higher content of short-chain fatty acids	0.0003
Organic farming	0.008	Val d'Orcia from organic farming	0.037
Higher yields	0.008	Nutraceutical compounds	0.005

3.2 Consumer perception of Ecoscore and Nutrinform Battery labels (Study 2)

Study 2 shows that the Ecoscore (ES) label influences perceived sustainability but has limited impact on product liking. Products labeled with ES A are seen as more sustainable, especially by younger consumers (19–35), and raise expectations of liking. However, under informed conditions, these products are not rated as more liked than in blind tastings, indicating a gap between sustainability expectations and sensory experience. In contrast, ES E has a negative effect, particularly when paired with a negative Nutrinform Battery (NB) label (high in fats and sugars), leading to a significant drop in liking in three out of four samples. This suggests that unfavorable environmental and nutritional cues can jointly reduce consumer acceptance. The NB label combined with ES E and a poor nutritional profile; it contributes to lower appreciation. A negative NB label also consistently reduces perceived healthiness across products. Conversely, a positive NB label with ES A enhances perceived healthiness, though this does not fully translate into higher liking. This indicates a misalignment between health expectations and sensory quality, emphasizing the need for coherence between front-of-pack labels and product experience. The NB label also shapes sensory expectations, with lower fat and sugar content leading consumers to anticipate a less buttery, less sweet, more cereal-like, and grainier product profile.

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Validation of an Analytical Method for the Identification and Quantification of Heterocyclic Amines in Foods (P-II-27)

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A method was proposed for the extraction and quantification of Heterocyclic Amines (HAs) in foods using high-performance liquid chromatography (HPLC)-diode array detection (DAD). This method was applied to both a standard HAs solution and food matrices. The validated method was then used to determine the amount of these hazardous compounds in red meat samples**,** prepared at different cooking temperatures.

Validazione di un metodo analitico per l'identificazione e la quantificazione delle ammine eterocicliche negli alimenti

È stato proposto un metodo per l'estrazione e la quantificazione delle ammine eterocicliche (HA) negli alimenti utilizzando la cromatografia liquida ad alte prestazioni (HPLC)- diode array detector (DAD). Il metodo è stato applicato sia a una soluzione standard di HAs sia a matrici alimentari. Il metodo validato è stato poi utilizzato per determinare la quantità di questi composti pericolosi in campioni di carne rossa cotti a diverse temperature.
Key words: Heterocyclic amines, hazardous compounds, meat, food safety.

1. Introduction

This manuscript reports the main results of the initial activities of the PhD thesis project, focusing on:

- The development and validation of efficient and specific extraction and instrumental methods for the quantification of HAs in several food matrices.
- The analysis of HAs levels in red meat samples (prepared using different cooking temperature and cooking time).

2. Materials and Methods

The HPLC-DAD method was developed and validated for 11 HAs compounds:, 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8DiMeIQx), NorHarman2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline (7,8DiMeIQx), NorHarman, 2-amino-3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline (4,7,8TriMeIQx), Harman, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC). Standard methanol solutions of each HA was prepared and chromatographic gradients were optimized for adequate separation of all 11 analytes. HPLC analyses was conducted using a Vanquish HPLC system (Thermo Scientific) equipped with a DAD and a Gemini C18 100A column (250×4.6 mm; 5µm) (Phenomenex). The solvents were (A) 10 mM ammonium acetate with 0.1% formic acid and (B) acetonitrile, flow rate 0.8 mL/min, column temperature 25°C. The gradient program was: t_0 2% B; t_5 2% B; t_{14} 15% B, t_{19} 27%B; t_{29} 55%B, t_{31} 100%B, t_{41} 100%B, t_{46} 2%B. Injection volumes varying from 1 to 5µL, DAD range 200-600 nm, detection wavelengths 258 and 322 nm. Red meat samples were cooked on a thermal plate at 200°C and 300°C for different time (5 -30 minutes) to investigate the formation of hazardous compounds (HAs and α-DC). HAs were extracted from 2 g of homogenized meat using a modified QuEChERS method (Hsiao et al., 2017). Purification was performed in two steps, initially employing $MgSO_4$ and CH_3COONa, followed by further clean-up with a specific kit. Finally, the purified extracts were reconstituted in methanol. All compounds were identified by comparing retention times and UV spectra with those of standards and by splitting each peak with standards. Quantification of hazardous compounds was performed using external calibration curves.

Results and Discussion

The analytical performance of the developed HPLC-DAD method for HAs was validated. All 11 HA standards showed complete baseline resolution, with retention times (t_R) from 14.247 to 29.897 minutes (Figure 1). For quantitative analysis, calibration curves were established for each analyte by varying injection volumes (1-5 µL) at 1 ppm. Linear regression analysis yielded R^2 values consistently above 0.9987, confirming the method's suitability for quantitative determinations, comparable to validated literature procedures (Hsiao et al., 2017). Table 1 summarizes validation parameters, including maximum absorption wavelengths (λ_{max}), t_R, retention factor (k), separation factor (α), Limit of Detection (LOD), Limit of Quantitation (LOQ), calibration curve equation, and correlation coefficient (R^2) for each HA. The total separation time was approximately 30 minutes. All α values were consistently greater than 1 (1.02 to 1.36), indicating suitable separation as per Hsiao et al. (2017). The

developed method allowed for the simultaneous separation and quantification of 11 different heterocyclic amines with remarkable efficiency, even at low concentrations. Crucially, clear separation of structurally similar compounds, such as DiMeIQx isomers and Harman/Norharman, was achieved. The LOD values (0.48 to 8.97 ng/g) and LOQ values (1.59 to 29.91 ng/g) confirm the method's high sensitivity, making it suitable for trace analysis of HAs (Chang et al., 2018). The linearity ($R^2 \geq 0.9987$) further ensures the reliability of quantifications.

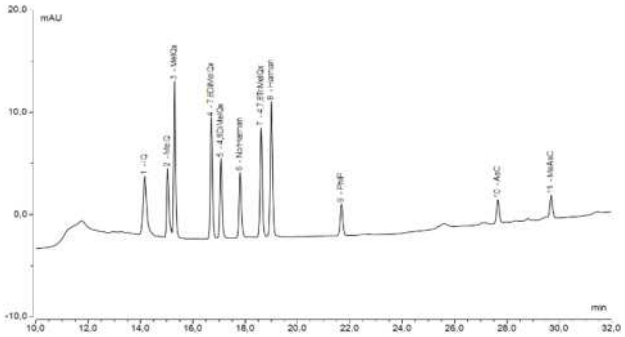


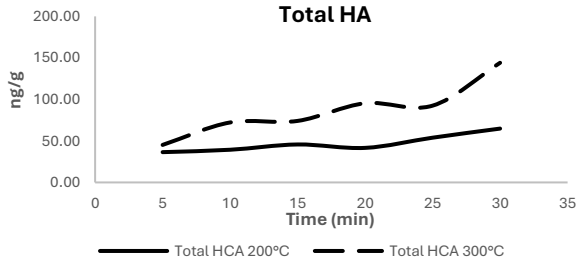
Figure 1 *HPLC-DAD chromatograms of heterocyclic amine (HA) standards.*

Table 1 *Separation data, analytical performance characteristics, calibration parameters, LOD and LOQ for Heterocyclic Amines (HAs) analyzed by HPLC-DAD.*

Compounds	t_R[a](min)	k[b]	α[c]	Linear regression equations	R^2	LOD[e] (ng/g)	LOQ[f] (ng/g)	Max λ (nm)
IQ	14,247	2,02	-	y = 3,7535x+0,0117	0,9992	8,97	29,91	253, 265, 326
MeIQ	15,107	2,20	1,09	y = 2,5327x-0,0094	0,9987	0,76	2,53	256, 268, 333
MeIQx	15,313	2,24	1,02	y = 6,9572x-0,1568	0,9998	0,48	1,59	263, 332
7,8DiMeIQx	16,727	2,54	1,13	y = 5,1428x-0,0646	1,0000	1,97	6,57	263, 328
4,8DiMeIQx	17,100	2,62	1,03	y = 3,4996x-0,0501	1,0000	8,28	27,59	264, 340
NorHarman	17,833	2,78	1,06	y = 3,0862x-0,0364	0,9999	6,55	21,84	250, 301, 371
4,7,8TriMeIQx	18,647	2,95	1,06	y = 4,9343x+0,0470	0,9999	1,44	4,79	264, 336
Harman	19,023	3,03	1,03	y = 6,2946x-0,0974	0,9999	0,74	2,48	246, 300, 364
PhIP	21,790	3,62	1,19	y = 1,6013x-0,0144	1,0000	2,28	7,60	236, 312
AαC	27,873	4,91	1,36	y = 1,2851x-0,0058	1,0000	2,23	7,43	236, 264, 340
MeAαC	29,897	5,33	1,09	y = 1,1762x-0,0233	1,0000	7,09	23,64	237, 264, 344

[a]t_R= retention time; [b]k= retention factor (t_R-t_0/t_0); [c]α = separation factor (t_{K2}/t_{K1}); [d]R^2 = Coefficient of Determination; [e]LOD = limit of detection base on 3*σ/slope; [f]LOQ = limit of quantitation base on 10*σ/slope.

Figure 2 *Trend of total HA concentrations (ng/g) in red meat cooked at 200°C (solid line) and 300°C (dashed line) for different time.*



The Figure 2) shows the trend of the total amount of HAs in cooked red meat. Six HAs were identified in meat samples: IQ, MeIQ, MeIQx, 4,8DiMeIQx, NorHarman and PhIP. Among these compounds IQ mainly contribute to total concentration, representing about 42.9% of the total HAs. At 200°C, HA concentrations show a gradual increase with cooking time, rising from about 36 ng/g to over 64 ng/g. At 300°C, however, the increase is more rapid, with levels starting at about 45 ng/g and exceeding 144 ng/g at the end of cooking time. This highlights how high temperatures dramatically accelerate the formation of HA, resulting in substantially higher concentrations. It is critical to note the high reactivity of HAs, which can undergo further transformations during cooking. Kinetic studies are underway to better understand the complex reaction pathways.

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Chang, CC., Kao, TH., Zhang, D. et al. (2018) Application of QuEChERS Coupled with HPLC-DAD-ESI-MS/MS for Determination of Heterocyclic Amines in Commercial Meat Products. *Food Anal. Methods* **11**, 3243–3256.

Hsiao HY, Chen BH, kao TH (2017) Analysis of Heterocyclic Amines in Meat by the Quick, Easy, Cheap, Effective, Rugged, and Safe Method Coupled with LC-DAD-MS-MS. *J. Agric. Food Chem.* **65**, 9360-9368.

Application of high-resolution nuclear magnetic resonance (HR-NMR) to evaluate the antioxidant properties of natural compounds used to extend the shelf life of food products rich in unsaturated lipid components (P-II-28)

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Tutor: Francesco Capozzi; Co-tutor: Elena Babini, Carlo Mengucci

The preservation of food products rich in unsaturated lipids remains a significant challenge in the agro-food industry due to the susceptibility of these lipids to oxidative degradation, which compromises food quality and safety. Oxidation processes lead to rancidity, off-flavors, and reduced nutritional value, thus limiting shelf life. Traditionally, synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been widely used to delay lipid oxidation. However, growing consumer demand for natural and clean-label ingredients has driven research toward identifying and characterizing natural antioxidant compounds derived from plant sources, including polyphenols, flavonoids, and vitamins.
Compared to traditional analytical techniques such as gas chromatography (GC) and high-performance liquid chromatography (HPLC), HR-NMR provides a more comprehensive profile of the sample without the need for extensive sample preparation or derivatization. While GC and HPLC often require separation steps and the use of solvents or reagents that can introduce variability or artifacts, HR-NMR analyzes the intact sample, preserving its native state. Additionally, HR-NMR allows for non-destructive analysis, which is advantageous for longitudinal studies and quality control.

1. Introduction

Recent studies have demonstrated the capability of HR-NMR to evaluate antioxidant efficacy by tracking the formation and consumption of oxidation markers such as hydroperoxides and aldehydes in oils and emulsions. For example, research by Smith et al. (2021) highlighted the use of HR-NMR coupled with PCA to quantify changes in linoleic acid oxidation in the presence of rosemary extract. Similarly, Chen and colleagues (2020) applied HR-NMR and multivariate analysis to characterize the interaction between natural polyphenols and unsaturated lipids in model food systems. Despite these advances, challenges remain in standardizing HR-NMR protocols for routine antioxidant evaluation and correlating NMR-based findings with sensory and shelf-life data. Moreover, the complexity of natural antioxidant mixtures and the influence of food matrix components require further methodological developments. This doctoral research aims to contribute to this growing body of knowledge by applying HR-NMR spectroscopy, combined with advanced normalization methods and multivariate analysis, to assess the antioxidant properties of natural compounds specifically designed to extend the shelf life of food products rich in unsaturated lipid components. The focus is on optimizing analytical workflows to improve data interpretability and reliability.

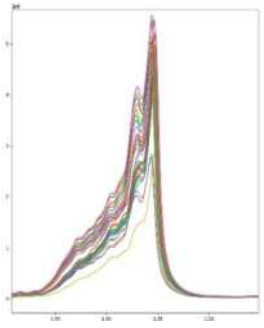
2. Materials and Methods

All the samples were contained inside edible pills: 6 drops of oil from a capsule were added to 500 µL of $CDCl_3$. The mixture was centrifuged at 14000 RCF for 5 min at 4°C, then diluted 4:1 with $CDCl_3$ and the centrifuge is repeated. The NMR analysis was performed exploiting the 1D zg without presaturation sequence. Experimental Calanus oil (BK) samples were obtained from Icelandic project partners. Several methods were used for oil extraction. Cold extraction (samples BK, BK1, BK5C, BK5D, BK5E, BK6A, BK6B): about 10 g of Calanus extracted from viscera, were centrifuged at 5100 rpm at 4°C for 12 min in a refrigerated centrifuge 3 times. Hot extraction (sample BK2): samples were placed in 50 mL tubes and placed in a heat bath at 70°C for 20 min, then cool to room temperature for 15 min before being centrifuged at 5100 rpm at 22°C for 12 min two times. Enzymatic extraction (sample BK3): 0.290 g of the enzyme Corolase was added to the Calanus samples (0.5% of the protein content). The mixture was placed in a heat bath at 75°C for 30 min to increase enzyme activity. The tubes were then placed on ice for 13 minutes until the heat bath reached the inactivation temperature of Corolase. The samples were kept at 90°C for 5 minutes before being extracted and allowed to cool on ice. Once the samples had cooled, they were placed in the centrifuge at 5100 rpm at 4°C for 12 minutes (the oil was recovered after two centrifuges). The different phases were stored at -18°C. Acetone extraction (sample BK4): samples were placed in 50 mL tubes and acetone was added to the sample in a 2:1 ratio. The mixture was shaken manually to ensure homogenization. The samples were kept on ice during the entire process. The tubes were centrifuged twice at 5100 rpm at 4°C for 12 min. After using a rotary evaporator, solids were collected, weighed and placed in the freezer at -18°C. The product was a mixture of water and oil, which required an additional separation step by centrifugation. Bligh & Dyer extraction (BK5A and BK5B):

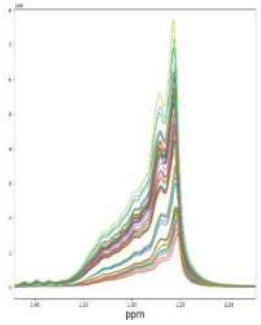
Calanus paste, chloroform and methanol were homogenized for 2 min. KCl was added, and the mixture was further homogenized for another minute. The samples were then centrifuged for 20 minutes at 2500 rpm at 4°C. The lipid-containing lower chloroform phase was taken and filtered. The contents of the evaporation flask were transferred to a 50 mL volumetric flask. The volumetric flask was filled with chloroform to the 50 mL mark. The contents were poured into 50 mL plastic vials and stored at -80°C pending further analysis.

3. Results and Discussion

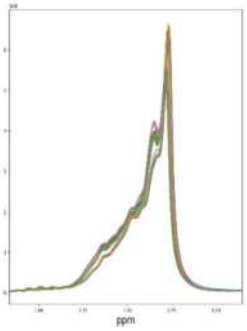
The research has advanced significantly, leading to several key findings. First, the application of Pivot normalization to the NMR data has proven to be more effective than the commonly used Probabilistic Quotient Normalization (PQN) in highlighting qualitative differences between the various collected samples.



Without normalization

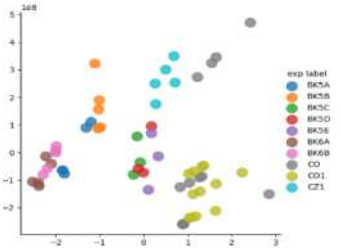


PON Normalization

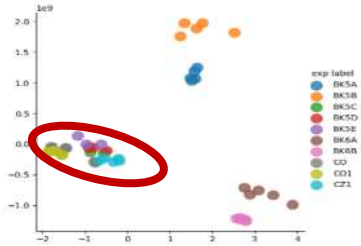


Pivot normalization

In particular, Pivot normalization significantly improved the outcomes of Principal Component Analysis (PCA), resulting in a much clearer and more distinct clustering of the samples. This enhanced normalization approach has contributed to better interpretability and discrimination within the dataset.



Score plot with PQN normalization



Score plot with Pivot normalization

Second, a comparison is currently underway between NMR and gas chromatography results, with the aim of evaluating the potential of the NMR-based method as an alternative for quantitative analysis.

During a three-month research stay at **Orta Doğu Teknik Üniversitesi (ODTÜ)** in Ankara, Türkiye, additional work focused on the relaxometric analysis of plant-based protein hydrogels. I personally prepared the hydrogels, which were formulated with two different protein compositions: one containing 20% pea protein, and another combining 15% pea protein with 5% hemp protein. Gels were prepared at six different pH values (5.5, 6, 6.5, 7, 7.5, and 8) to investigate the influence of pH and protein composition on relaxation behavior and gel structure. In addition to relaxometry, water-holding capacity (WHC) and oil-holding capacity (OHC) were also measured. The same set of analyses was extended to emulsified gels, which I also prepared, incorporating seed oil through ultrasound-assisted emulsification. This allowed for a comprehensive evaluation of their structural and functional properties.
Further results will be collected and presented by Turkish colleagues in the coming months.

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A smart hydrogel sensor for food spoilage detection: visual pH monitoring in milk and chicken samples (P-II-29)

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Tutor: Prof. Barbara Panunzi

A new pH-sensitive chromophore, named $T(OH)_2$, was synthesised. $T(OH)_2$ underwent chemical-physical characterisation. The chromophore was blended into an agar-based hydrogel to create a soft-matter sensor, named T-gel. Analyses of the mechanical properties, morphology, and optical characteristics were performed on the sensing system. Its colorimetric response was tested in both solution and gaseous phases. T-gel was applied to monitor the freshness of perishable foods such as milk and chicken meat samples.

Un sensore intelligente ad idrogel per il rilevamento del deterioramento degli alimenti: monitoraggio visivo del pH in campioni di latte e pollo

È stato sintetizzato un nuovo cromoforo sensibile al pH, denominato $T(OH)_2$. $T(OH)_2$ è stato sottoposto a caratterizzazione chimico-fisica. Il cromoforo è stato incorporato in un idrogel a base di agar per creare un sensore soft matter, denominato T-gel. Sul sistema sono state eseguite analisi delle proprietà meccaniche, della morfologia e delle caratteristiche ottiche. La sua risposta colorimetrica è stata testata sia in soluzione che in fase gassosa. Il T-gel è stato applicato praticamente per monitorare la freschezza di alimenti deperibili come campioni di latte e carne di pollo.

Keywords: hydrogel, chromophore, pH, food freshness, food monitoring.

1. Introduction

Following the PhD thesis project previously described (Milzi, 2024), this poster reports the main results of the first activities concerning:

(A1) Study of new optical smart sensors for monitoring and detecting food analytes and parameters;

(A3) Synthesis and characterization of a novel responsive dye. Based on the novel chemosensor, design and building of a visual soft matter sensing tool for intelligent packaging. Chemo-physical characterisation and study of the colorimetric performance of the sensor. Application of the sensing tool to real food samples for monitoring pH and VOCs (Diana,2024).

2. Materials and Methods

$T(OH)_2$ was synthesised via a condensation reaction between (2-((5-aminothiophen-2-yl) diazenyl)-5-nitrophenol, 10 mmol) and (4-formyl-3-hydroxyphenyl 4-(hexyloxy) benzoate, 10.5 mmol) in chlorobenzene under reflux for 3 h. NMR and MALDI-TOF MS analysis confirmed the structures. UV–visible absorption spectra were recorded by a JASCO F-530 spectrometer (scan rate 200 nm/min, JASCO Inc., Easton, MD, USA) on $T(OH)_2$ mixtures of water and acetone (3:1 and 1:6 v/v) using Britton-Robinson buffer solution. To obtain T-gel samples, 6% agar was dissolved in boiling Milli-Q water, 2% by weight of $T(OH)_2$ (0.02 M in acetone) was added, the mixture was boiled for 3 min and cooled in cylindrical molds. SEM analyses were performed using a scanning electron microscope, Thermo Phenom XL by Zeiss (Carl Zeiss NTS GmbH, Oberkochen, Germany). Swelling and water retention ratios were calculated according to Lv (2019). DSC experiments were performed with a Hitachi DSC 200 under nitrogen purge gas. For compression tests an Instron 5566 was used at a crosshead speed of 1 mm/min up to an extension of 2 mm (breaking of gel) with a 100 N load cell, with samples 30 mm in diameter and 12 mm in height. Colorimetric responses were evaluated by soaking T-gel tablets in buffered solutions with pH values between 2 and 12 for 10 min. The hydrogel's ability to detect NH_3 and HCl vapors was tested by exposing the samples for intervals of 5-30 s in sealed vials, repeating the cycles to assess reversibility. For the monitoring of food spoilage in chicken meat and milk stored at 4°C and 25°C, the tests were performed in triplicate. The CIE 1931 colour space coordinates were evaluated by the smartphone app RGB colour detector.

3. Results and Discussion

3.1 Optical properties of $T(OH)_2$ and characterization of T-gel

$T(OH)_2$ indicator reversibly works between pH=2 and pH=12. The intensely colourful solutions of $T(OH)_2$ give a strong absorption band in the visible spectrum both in mainly-water (water/ acetone 3:1) and mainly organic (water/acetone 1:6) solutions. In mainly-water solutions, the colour changes from a yellow tone to a green and finally a blue tone depending on the pH (Figure 1A). The absorbance spectra change accordingly. In mainly organic solutions, the colour gradually turns from yellow to orange and finally to magenta depending on the pH (Figure 1B).



Figure 1. *$T(OH)_2$ samples in (A) mainly-water (water/acetone 3:1) and (B) mainly organic (water/acetone 1:6) buffered solutions as a function of pH (from 1 to 12).*

The T-gel samples show the melting point at 88.0°C, while gel formation occurs at 30.1°C, following the data reported in the literature (Fernandez, 2008). SEM morphological analysis reveals a rather rough surface, characterised by an uneven distribution of micropores, rounded in shape and varying in size. This porous structure gives T-gel a functional advantage, acting as a permeable and "micro-spongy" medium in which protonation/deprotonation equilibria quickly and efficiently occur. In terms of swelling behaviour, the samples immersed in water show a rapid absorption capacity. After only 10 min, the swelling ratio (SR) reaches 105 g/g; after 1 h, it increases to 1026 g/g, and after 6 h, equilibrium is reached with an SR of approximately 3900 g/g. The sample at swelling equilibrium (SR = 3900 g/g, pH=7, T=25°C) was then subjected to water retention (WRR) testing. After one day of exposure to air at 25°C and 40% humidity, the gel retains approximately 80% of its initial water content; after three days, the WRR is still 65%. These values demonstrate excellent water retention capacity. Mechanical testing revealed that the hydrogel structure remains intact up to an applied stress of 120 kPa, corresponding to 20% compression, confirming their robustness and potential suitability for practical handling.

3.2 T-gel applications

T-gel samples underwent preliminary sensitivity tests to acid and basic vapours, specifically gaseous ammonia (NH_3) and hydrochloric acid (HCl). Exposure to these vapours generated a marked colorimetric response, evaluated and quantified by calculation of the CIE coordinates. Samples exposed to HCl showed a progressive increase in yellow hue, and samples exposed to NH_3 showed an intense magenta/purple colouration. These results confirm the reactivity of T-gel to environmental pH changes in the gas phase, with a fast visual response easily perceptible to the naked eye. Tests on real food samples were performed on milk and chicken samples. By immersion in milk, T-gel samples showed a specific colorimetric response directly correlated to pH for real and model samples. The fresh samples (pH=6.5–6.7), the altered samples (pH=4.4), and the mastitic samples (slightly alkaline conditions, pH=7.5) showed a distinctive colouration (see Figure 2A). By interaction with the nitrogen-based volatile organic compounds (VOCs) produced by chicken meat during the spoilage, the colour of the sensor underwent a marked change. For samples kept at room temperature, the yellow-orange colour of T-gel detected up to the third day, turns to dark purple by the fourth day (while pH of the meat increases from 5.8 to 7.9) (see Figure 2B). At 4°C (correct storage conditions), the colour transition was slower, occurring between the eighth and tenth days.

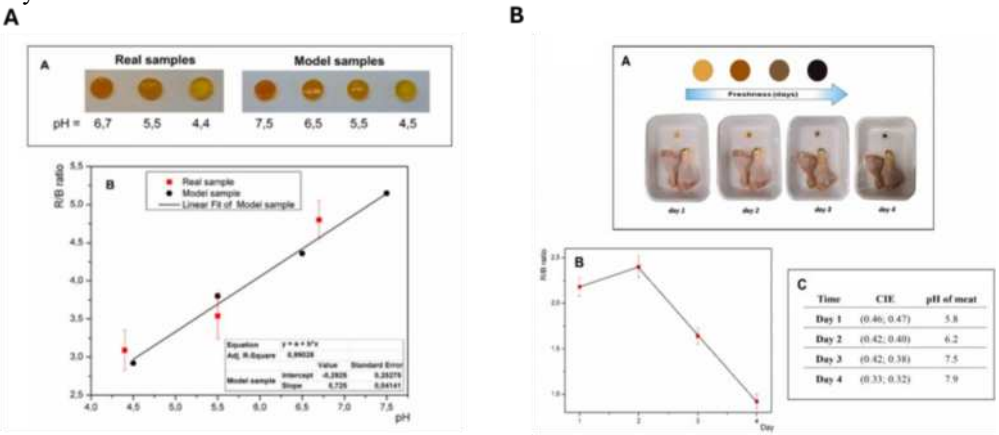


Figure2. *(A)Visual monitoring of milk samples at different pH. (B): Visual monitoring of chicken samples at 25°C and corresponding CIE colour parameters as a function of pH.*

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Study of anthocyanins from red cabbage as potential natural alternatives to synthetic antioxidants (P-II-30)

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Tutor: Prof. Matteo Scampicchio Co-Tutor: Prof.ssa Giovanna Ferrentino

This PhD project first investigates the antioxidant activity and reactivity of anthocyanins from red cabbage. Anthocyanins from red cabbage were extracted, identified and quantified. To evaluate the antioxidant activity of these compounds an AAPH-incubating method using HPLC-HRMS/MS was developed. This approach allowed the screening of the most reactive anthocyanins present in the extract and to establish a structure-activity relationship through reaction rate assessment. Findings were confirmed using HPLC-DAD-CAD. Secondly, red cabbage anthocyanins were tested in the presence of carotenoids to determine whether anthocyanins can offer protection against thermal and oxidative degradation. These results provide insights into anthocyanin use in food preservation.

Studio degli antociani del cavolo rosso come potenziali alternative naturali agli antiossidanti sintetici.

Questo progetto di dottorato indaga inizialmente circa l'attività e la reattività antiossidante degli antociani del cavolo rosso. Dopo l'estrazione, identificazione e quantificazione, è stato sviluppato un metodo basato sull'incubazione con AAPH utilizzando HPLC-HRMS/MS per valutare l'attività antiossidante. Questo approccio ha permesso di individuare gli antociani più reattivi nell'estratto e di stabilire una relazione struttura-attività attraverso l'analisi della velocità di reazione. I risultati sono stati confermati con HPLC-DAD-CAD. Inoltre, è stata studiata l'efficacia protettiva degli antociani del cavolo rosso ossidativa in presenza di carotenoidi contro degradazione termica e ossidativa. I dati ottenuti evidenziano il potenziale degli antociani nella conservazione alimentare.

Key words: Anthocyanins, red cabbage, antioxidant activity, AAPH, high-resolution mass spectrometry

1. Introduction

This poster reports the main results of the first two activities of this PhD project concerning:

(A1) The assessment of antioxidant activity and reactivity of anthocyanins using an AAPH-incubating method with HPLC-HRMS/MS and method confirmation with HPLC-DAD-CAD

(A2) The evaluation of red cabbage anthocyanin-mediated protection of carotenoids against thermal and oxidative degradation

2. Materials and Methods

Red cabbage (purple-leaved variety of *Brassica oleracea*) was purchased from a local market. Red cabbage was chopped and mixed with a Tristar BL-4009 and freeze-dried with a high-performance Epsilon 2–6D LSC plus laboratory freeze-dryer (Martin Christ, Osterode, Germany). Ultrasound assisted extraction (UAE) of anthocyanins was performed using a probe system (Hielscher UP400st, 400 W and 24 kHz, Hielscher Ultrasonic GmbH, Teltow, Germany) and a solution of H_2O-ethanol 1:1. The choice of the extraction solvents was based on literature (Ghareaghajlou et al, 2021). At the end of the extraction the extract was freeze-dried again. Anthocyanins identification, quantification and reaction with AAPH was performed using Dionex Ultimate 3000 UHPLC device coupled to electrospray ionization source (ESI) and Q ExactiveTM Orbitrap high resolution mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Confirmation was carried out with the Agilent 1260 Infinity HPLC system coupled with a diode array detector (DAD, Agilent Technology, USA) and with a CoulArray detector (CAD). Carotenoids analysis was performed using Dionex Ultimate 3000 UHPLC device coupled with atmospheric pressure chemical ionization (APCI) and Q ExactiveTM Orbitrap high resolution mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA).

3. Results and Discussion

3.1 Identification, quantification, and antioxidant activity of anthocyanins from red cabbage

Thirteen anthocyanins were identified based on the HRMS/MS fragmentation. Only cyanidin derivatives were found due to the presence of a fragment signal at 287.0545 m/z in the MS/MS spectra, corresponding to cyanidin aglycone. The base structure was cyanidin 3-diglucoside 5-glucoside with the glucoside residues being either nonacylated, monoacylated or diacylated with sinapic acid, ferulic acid, and/or p-coumaric acid. All anthocyanins have been reported in previous studies (Si Tan et al., 2023). Cyanidin derivatives were quantified with the building

of a calibration curve with standard cyanidin chloride. The main anthocyanins were cyanidin-3-(sinapoyl)-diglucoside-5-glucoside (Cy3(sin)diG5G), cyanidin-3-diglucoside-5-glucoside (Cy3diG5G), cyanidin-3-(p-coumaroyl)-diglucoside-5-glucoside (Cy3(p-coum)diG5G), cyanidin-3-(feruloyl)-diglucoside-5-glucoside (Cy3(fer)diG5G). This result is consistent with what has already been reported in literature (Si Tan et al., 2023; Strauch et al., 2019). The reaction between red cabbage extract and AAPH (Eq 1-2) was carried out at 37 °C and pH 7.4 (Zhuang et al., 2023). Every 40 minutes and up to 200 minutes aliquots were taken and immediately injected into the HPLC-HRMS/MS.

1. $AAPH+O_2 \rightarrow 2\ ROO^{\bullet}$
2. $AH+ROO^{\bullet} \rightarrow ROOH + A^{\bullet}$

The decrease in peak areas for each anthocyanin was first plotted with as concentration decay as function of time, and the reaction rate (μM/min) was calculated for each compound. When comparing acylated anthocyanins the order was the following:

Cy3(disin)diG5G> Cy3(sin)diG5G> Cy3(sin)G5G> Cy3(fer)(sin)G5G> Cy3(difer)diG5G> Cy3(fer)diG5G> Cy3(p-coum)diG5G> Cy3(sin)triG5G> Cy3(fer)triG5G

The compound showing the highest reaction rate was Cy3(disin)diG5G. Anthocyanins conjugated with sinapic acid, and two glucoside units showed higher antioxidant activity than cyanidin derivatives conjugated with ferulic acid and p-coumaric acid and two glucoside units. The double acylation increases the antioxidants activity as both Cy3(disin)diG5G and Cy3(difer)diG5G showed a higher reaction rate compared to their corresponding monoacylated compounds (Sadowska-Bartosz et al., 2024). For nonacylated anthocyanins, in literature it is said that these have lower antioxidants activity when compared to acylated and diacylated anthocyanins (Sadowska-Bartosz et al., 2024). However, in this case Cy3diG5G was one of the anthocyanins showing the highest reaction rate. An interpretation of the differences reported could be due to the multiplicity of secondary reactions that could affect the estimation of relative rate constants, especially when a matrix as complex as a natural extract is being considered (Sadowska-Bartosz et al., 2024). The results obtained by the reaction with AAPH were confirmed with HPLC-DAD-CAD analysis. There are no reports in literature for the analysis of red cabbage extract using HPLC-DAD-CAD. The results showed that the compound with the lowest half-wave potential, thus the antioxidant with more electron-donor capacity, was Cy3(disin)diG5G, the same compound that had shown the highest reaction rate during the reaction with AAPH. This pattern is confirmed for all other cyanidin derivates present in the extract.

3.2 Evaluation of red cabbage anthocyanin-mediated protection of carotenoids against degradation

Five carotenoid standards (beta-carotene, beta-cryptoxanthin, astaxanthin, lutein, zeaxanthin) were analysed with HPLC-HRMS/MS to evaluate the influence of the presence/absence of the red cabbage extract, temperature, and the presence/absence of oxygen. Three different temperatures were considered: 45°C, 25°C, 5°C. The peak areas of carotenoids were monitored over time at 0.5, 1, 2, 4, 6, 24 and 48 h. Also, the red cabbage extract was analyzed in presence and in absence of carotenoids at different temperatures and in presence/absence of oxygen. Preliminary results show that temperature has a significant impact on the concentration of all the compounds analyzed and that the best conditions to protect carotenoids from degradation is 5°C, with anthocyanins and without oxygen. Preliminary results show that the impact of the adding of anthocyanins is particularly evident at 45°C. At this temperature, both with and without oxygen, if no anthocyanins were added after 48 h the concentration of carotenoids dropped to zero.

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Characterization of performances of various natural antioxidants in the formulation of food, feed and pet food, to identify the best processing conditions (P-II-31)

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This PhD thesis research project is aimed to identify new antioxidant substances able to prevent and slow down the lipid oxidation in food, feed and pet food matrices, thereby extending their shelf life. The focus will be on antioxidant molecules of natural origin, ideally suitable for extraction from by-products. At the same time, this project will conduct a study of the antioxidant additives currently used in the cited sectors, in order to identify the optimal usage conditions and ensure the best performances.

Antiossidanti naturali tailor made: valutazione comparativa di antiossidanti nell'elaborazione di alimenti, mangimi e pet food e determinazione delle condizioni d'uso ottimali

Questo progetto di tesi di dottorato si propone di individuare nuove sostanze antiossidanti che possano contrastare l'ossidazione lipidica in matrici food, feed e pet food, prolungandone quindi la shelf life. L'attenzione si concentrerà sulle molecole antiossidanti di origine naturale e, idealmente, convenientemente estraibili da sottoprodotti. In parallelo, questo progetto mira allo studio dettagliato degli additivi antiossidanti già in uso in questi settori, al fine di determinare per ognuno le condizioni d'uso che possano garantire le performance migliori.

Key words: Lipid oxidation, antioxidants, pet food, feed and food.

1. Introduction

Lipid oxidation is a major factor limiting the shelf life of low-moisture, fat-containing processed foods such as pet food and animal feed. Even in small amounts, oxidized lipids can drastically compromise product quality by producing off-flavors, reducing palatability for both humans and animals. Primary and secondary oxidation products are directly responsible for shelf-life reduction and are closely regulated due to their impact on safety and sensory quality (Hu and Jacobsen, 2016). Since oxidation is an irreversible and self-propagating process, the only effective strategy is prevention, typically using antioxidants. These can be synthetic (e.g., BHA, BHT, TBHQ) or natural (e.g., tocopherol-rich extracts, rosemary extract). In recent years, increasing consumer demand for "clean label" products has driven the food, pet food, and feed industries to look for natural alternatives. However, these often require investigation to ensure both technological effectiveness and compatibility with specific matrices and production processes. Among the emerging natural sources, cashew nutshell liquid (CNSL), an oily extract obtained as a by-product of cashew nut processing (*Anacardium occidentale* L.), is attracting interest. CNSL contains alkylphenolic compounds such as anacardic acid, cardanol, cardol, and 2-methylcardol. Industrial CNSL, derived from thermally treated nuts, is rich in cardanol; it can be transformed into a renewable resource for the chemical industry (distilled cardanol). CNSL show promising antimicrobial, anti-inflammatory, and antioxidant properties (Andrade et al., 2011; Attanasi and Filippone, 2003; Gómez-Caravaca et al., 2010).
During the development of my PhD project, the original work plan has been slightly revised to optimize the research workflow based on early findings. The updated Gantt chart reflects these adjustments while preserving the project's objectives and scientific approach (Tab. 1).

Table 1 *Gantt diagram for this PhD thesis project (updated).*

Activity	Month	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Bibliographic research***																		
A2)	***Characterization of innovative antioxidants***																		
	1) Chemical charactertization																		
	2) Evaluation of antioxidant activity																		
A3)	***Tests on model systems and comparison with other antioxidants***																		
	1) Study of antioxidant concentration																		
	2) Comparison of antioxidant activity																		
A4)	***Tests on finished products and comparison with other antioxidants***																		
	1) Study of product formulation																		
	2) Shelf-life testing																		
A5)	***Evaluation of analytical methods***																		
A6)	***Assessment of sustainability and scale-up potential***																		
A7)	***Preparation of thesis, scientific articles etc.***																		

This poster reports the main results of the first two activities concerning:

A2) Selection of one or more innovative natural antioxidant compounds of potential interest (CNSL and distilled cardanol), characterization and preliminary evaluation of their antioxidant activity;

A3) Development of a "model system" composed of lipid matrices (sunflower oil), enriched with the innovative antioxidant under investigation and with traditional antioxidants (BHA, BHT, TBHQ, tocopherol-rich extracts, rosemary extract), for a comparative evaluation of their antioxidant effectiveness.

2. Materials and Methods

The determination of the peroxide value (PV) was carried out by iodometric titration in accordance with the method reported in EEC Reg. No 2568/91 and subsequent amendments. Oxidative stability was evaluated with the OXITEST instrument (Velp® Scientific, Usmate Velate, MB, Italy) operating under oxygen pressure (6 bar) at 100°C. Each analysis was performed in duplicate.

3. Results and Discussion

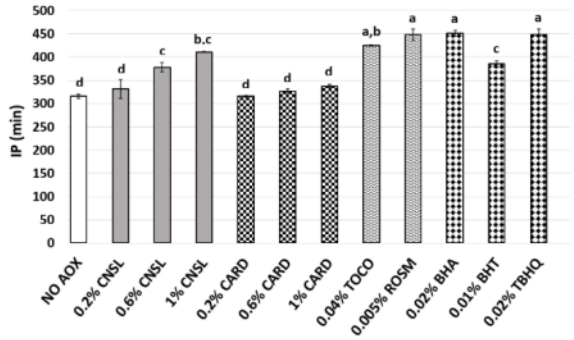
At this stage, the experimental activities have focused on the characterization and evaluation of CNSL and distilled cardanol as potential innovative antioxidants. The analysis of CNSL and cardanol composition, still ongoing, has been performed using thin-layer chromatography (TLC) and gas chromatography with flame ionization detection (GC-FID). Additional analytical methods such as high-performance liquid chromatography (HPLC) and gas chromatography coupled with mass spectrometry (GC-MS) are currently under evaluation.

The study of antioxidant activity and comparison with traditional antioxidants is also in progress. A preliminary screening was conducted to determine the minimum effective concentrations of CNSL and cardanol in model systems based on sunflower oil, using peroxide value (PV) as the primary oxidation marker after applying a thermal stress to the samples.

Once target concentrations were identified, balancing efficacy with industrial feasibility, direct comparisons were carried out using traditional antioxidants. Current tests are focused on sunflower oil systems enriched with increasing levels of CNSL and cardanol, as well as reference levels of conventional antioxidants. Oxidative stability was evaluated using the OXITEST instrument. Results showed that CNSL at 0.6% had comparable performance to BHT at 0.01%, and CNSL at 1% was similar to 0.04% tocopherol extract, and not so different from 0.005% rosemary extract, 0.02% BHA, and 0.02% TBHQ. In contrast, cardanol showed lower antioxidant activity, suggesting possible synergistic effects among CNSL's alkylphenolic compounds (Fig. 1).

To validate these results, further analyses are being conducted on thermally stressed oil samples with the different antioxidants. Specifically, PV are confirming the OXITEST trends, although some interference was observed. Future steps include optimization of the PV method and analysis of secondary oxidation products. Ongoing tests include the quantification of volatile compounds by headspace SPME-GC-MS and the detection of oxidized fatty acids (OFA) using GC-FID. Additional tests are planned to explore higher concentrations of CNSL and cardanol and expanded comparisons with conventional antioxidants.

Figure 1 *Induction Period (IP) obtained using OXITEST: comparison between sunflower oil without antioxidants and oil enriched with CNSL, cardanol and conventional antioxidants.*



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Can blueberry extract improve the oxidative stability of sunflower oil during a cooking process? (P-II-32)

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The primary objective of this study was to identify potential antioxidants capable of enhancing the oxidative stability of vegetable oils, through the research and the selection of extracts that are natural, innovative, and commercially available. The selected plant extracts - broccoli extract, blackcurrant extract, and two blueberry extract (1% anthocyanosides and 25% anthocyanosides) - were added to standard sunflower oil in order to evaluate their effectiveness in improving oxidative resistance during an oven-heating process.

L'estratto di mirtillo può migliorare la stabilità ossidativa dell'olio di girasole durante un processo di cottura?

Il primo obiettivo di questo studio è stato quello di individuare possibili antiossidanti in grado di migliorare la stabilità ossidativa di oli vegetali, attraverso la ricerca e selezione di estratti che fossero: naturali, innovativi e reperibili in commercio. Gli estratti vegetali selezionati quali estratto di broccolo, estratto di ribes nero ed estratto di mirtillo all'1% e al 25% in antocianosidi sono stati addizionati all'olio di girasole standard al fine di testare la loro efficacia nel migliorare la resistenza all'ossidazione dell'olio.

Key words: antioxidants, sunflower oil, oxidative stability, blueberry, cooking process.

1. Introduction

In accordance with the PhD thesis project, this poster reports the main results of the activities concerning:

1) the selection and characterisation of selected plant extracts from broccoli, blackcurrant and blueberry (1% and 25% anthocyanosides), by determining the total phenol content and their antioxidant activity;
2) the addition of these plant extracts to sunflower oil, whose efficacy was initially assessed under accelerated oxidation conditions. The extract that showed the best performance was then characterized by LC-MS and tested during a heat treatment in a static oven at 180 °C for 120 minutes. During cooking, the quality evolution of the oil was assessed by determining the number of peroxides and the content of total polar compounds.

2. Materials and Methods

The selected blueberry, blackcurrant, and broccoli extracts were acquired from Farmalabor Srl. The total phenolic content of the extracts was determined using the Folin–Ciocalteu method and the antioxidant activity was assessed via the ABTS assay following De Gennaro et al. (2022). Duplicate analyses were performed. A standard edible sunflower oil was used for testing. The extracts were added in oil at concentrations of 3, 10, 50, 75, and 100 mg/mL, with minimal distilled water for solubilization, and homogenized using an Ultra-Turrax T-18 (IKA-Werke GmbH & Co. KG, Staufen, Germany). Oxidative stability under accelerated conditions was assessed using a RapidOxy (Anton Paar, Blankenfelde-Mahlow, Germany) (Vurro et al. 2022). The extract showing best performance in oxitest was selected for oven-cooking trials. Oil enriched with the extract was heated in a ventilated oven at 180 °C for 120 min, with samples taken at 10, 20, 30, 60, and 120 min. A standard sunflower oil served as control. Two experimental levels were tested: 10 mg/g (0.1%) and 100 mg/g (1%) of blueberry extract. To ensure reproducibility, two independent batches were prepared and subjected to identical conditions. Samples were randomized in the oven using an algorithm to prevent positional bias. Peroxide value was determined according to Commission Delegated Regulation (EU) 2022/2104. Total polar compounds were separated via sulfuric acid–water column chromatography (IUPAC 2.507), then quantified as reported by Caponio & Gomes (2004). LC-MS analysis was performed following Noviello et al. (2024). A one-way analysis of variance (ANOVA) followed by the Tukey's HSD test were carried out using the Minitab Statistical Software (Minitab Inc., State College, PA, USA).

3. Results and Discussion

3.1 Characterisation of selected extracts

The broccoli extract exhibited a total phenolic content of 25.30 mg GAE g^{-1}, significantly higher than the 6.38 mg GAE g^{-1} reported by Borowski et al. (2008). The blackcurrant extract showed a polyphenol concentration of 21.56 ± 1.81 mg GAE g^{-1}, exceeding the 12.8 mg GAE g^{-1} found by Paunović et al. (2019), while its antioxidant activity was 19.88 ± 0.85 mg TE g^{-1}. Both blueberry extracts demonstrated good antioxidant activity, despite a marked difference in total polyphenol content. The blueberry extract (1% anthocyanins) presented a total phenolic content of 55.63 mg GAE g^{-1}, whereas the 25% anthocyanins blueberry extract showed a content of 413.91 mg GAE g^{-1}.

3.2 Formulation of fortified oil and performance assessment in cooking

Each extract was added to sunflower oil at various concentrations for accelerated oxidation testing (Chabni et al., 2024). The control sunflower oil showed an induction time of 33.86 minutes. The samples enriched with broccoli, blackcurrant, and 1% blueberry extracts had similar induction times, indicating no improvement in the oxidative stability (Table 1).

Table 2. Induction time (in minutes) of standard sunflower oil enriched with different percentages of the selected extracts.

	BROCCOLO D.E.	BLUEBERRY 1% D.E.	BLUEBERRY 25% D.E.	BLACKCURRANT D.E.
1%	34,41	34,10	48,01	34,00
0,75%	34,13	33,65	37,83	33,68
0,50%	33,88	33,23	38,76	32,91
0,10%	33,21	33,78	38,66	33,01
0,03%	32,58	33,17	33,70	33,21

D.E: dry extract.

However, the 25% blueberry extract (M25) showed promising results: at 0.1% concentration, the induction time increased to 38.66 minutes and, at 1%, it reached 48.01 minutes -14.15 minutes more than the control - suggesting an enhanced oxidative resistance. Thus, the M25 extract was selected for further testing. LC-MS analysis identified numerous phenolic compounds in M25, including anthocyanins (delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, malvidin-3-O-glucoside), flavonols (myricetin, kaempferol, kaempferol-3-O-xyloside), and caffeic acid derivatives (3-caffeoylquinic acid). The synergy among these may account for the high antioxidant activity observed. Following the cooking process, peroxide values (PV) and total polar compounds were assessed. In general, the samples containing 0.1% M25 extract (OEA) and those with 1% M25 (OEB) consistently showed lower peroxide values (PV) compared to the control oil (OC), indicating a certain antioxidant efficacy of the extract. No significant differences were observed between the 0.1% and 1% concentrations. Specifically, the OEA sample showed an initial decrease in PV within the first 10 minutes, while the OEB sample exhibited initial antioxidant activity followed by an increase in PV during prolonged cooking, likely due to a decline in the extract's effectiveness; however, after 30 minutes, PV began to decrease in this sample as well. Overall, peroxide values tended to be lower in the extract-containing samples compared to the control. After 120 minutes of cooking, total polar compounds content was similar between control and M25 oil, but the control sample showed higher oxidized triglycerides and polymers, suggesting greater oxidation (Table 2).

Table 3. Polar compounds content (g/100g) after 120 minutes of cooking.

	TAGP	ox-TAG	DAG	TOT
OC120	2.09 ± 0.03^{a}	2.2 ± 0.07^{a}	1.25 ± 0.15^{b}	5.67 ± 0.13^{a}
OEA120	0.95 ± 0.003^{b}	2.14 ± 0.02^{a}	2.27 ± 0.02^{a}	5.36 ± 0.03^{a}
OEB120	1.06 ± 0.11^{b}	1.61 ± 0.19^{b}	2.98 ± 0.28^{a}	5.64 ± 0.02^{a}

TAGP = triacylglycerol oligopolymers; ox-TAG = oxidized triacylglycerols; DAG = diacylglycerols.

Nonetheless, when the extracts were added, an increase in the hydrolytic degradation was also noted, likely linked to the water used to dissolve the extracts. These findings align with Galanakis et al. (2018). Overall, M25 was the only extract that improved the oxidative stability. A major challenge regards its use into a hydrophobic system to address which proper technologies will be tested in the next months.

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Development and optimization of sustainable protein-source-based food (P-II-33)

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This work reports the results obtained during the second year of the PhD research project, focused on the development of meat analogues using textured insect protein as a sustainable alternative to conventional protein sources. In particular, the optimization of protein extraction protocols from insect flour was carried out. Then, the physico-chemical and rheological analyses of the protein extracts were conducted to evaluate their functional properties, aiming to identify suitable formulations for the production of meat-like products. This foundational work sets the stage for further processing, formulation, and shelf-life studies of insect-based meat analogues.

Sviluppo ed ottimizzazione di cibi a base di proteine da fonti sostenibili

Questo lavoro presenta i risultati ottenuti durante il secondo anno del progetto di dottorato, incentrato sullo sviluppo di analoghi della carne mediante l'utilizzo di proteine testurizzate da insetti, proposte come alternativa sostenibile alle fonti proteiche convenzionali. In particolare, è stata condotta l'ottimizzazione dei protocolli di estrazione proteica a partire da farina di insetti. Successivamente, sono state eseguite analisi fisico-chimiche e reologiche sugli estratti proteici al fine di valutarne le proprietà funzionali, con l'obiettivo di individuare formulazioni idonee alla produzione di prodotti simil-carne. Questo lavoro preliminare costituisce la base per successivi studi relativi alla trasformazione, formulazione e shelf-life degli analoghi della carne a base di insetti.

Key words: Insect protein, protein extraction, tecno-functional properties, rheological properties, sustainable food.

1. Introduction

In accordance with the PhD thesis project previously described (Lanza, 2024), this works reports the main outcomes of the first two activities: the selection of the protein source and the optimization of the protein extraction pre-treatment and protocols, in terms of protein content and extraction yield, with the goal of identifying the most efficient and scalable protocols for protein recovery from insect flour; the assessment of texturization capabilities of the protein extract through the of analysis of their technological properties, such as water holding capacity, oil holding capacity, protein solubility, emulsifying and foaming properties, as well as their mechanical properties throughout rheological analysis and the gelling behaviour.

2. Materials and Methods

Acheta domesticus was selected as the protein source due to its high protein content, broad commercial availability and contained cost. Protein extraction from *Acheta domesticus* was carried out starting from commercial Cricket Flour, CF, (Small Giants SRL, Milan, Italy), previously subjected to defatting according to by Choi et al. (2017). Protein extraction was performed via heath alkaline treatment (0.25 M NaOH) followed by isoelectric precipitation at pH 4.5 (Zhao et al., 2016). Protein content was measured by total nitrogen analysis using the Kjeldahl method with 6.25 as nitrogen-to-protein conversion factors. The efficacy of the extraction was assessed through protein yield, calculated as the percentage of protein recovered in the extract relative to the total protein content in the Defatted Cricket Flour (DCF). Soluble proteins content and Water Holding Capacity (WHC) in Cricket Protein Extract (CPE) was determined according to Zhang et al. (2023) and Liu et al. (2020), respectively. The effect of the pH on soluble proteins content and WHC was evaluated across a pH range of 3.0 to 10.0. CPE-based gels at different concentrations (15%, 20%, 25% w/v in PBS, pH 7) were prepared via thermal treatment, using a modular compact rheometer (MCR 102, Anton Paar, Austria) with plate–plate geometry and a Peltier control system. Specifically, the thermal profile was as follows: 20–90 °C at 3 °C/min, 10 min at 90 °C, 90–20 °C,at 5 °C/min. Resulting gels were then characterized by frequency sweeps (0.1–100 Hz) at 20 °C. Yield strain was identified using amplitude sweeps (0.01–100% strain) analysed via RheoCompass® software and the strain applied in the frequency sweep was set at 85% of the yield point. All analysis was performed in triplicate. Statistical analysis of data was performed using one-way ANOVA followed by Tukey's post-hoc test, with significance set at $p<0.05$.

3. Results and Discussion

3.1 Protein extract characterization

The extraction process substantially enhanced both the protein concentration and functional attributes of the cricket protein extract (CPE). The final extract exhibited a protein content of 85.65 ± 0.72 g/100 g and an extraction yield exceeding 60%, surpassing values typically reported in the literature for insect protein extracts (Zielińska et al., 2018). Table 1 reports the values of soluble protein content and WHC for CF, DCF, and CPE. Soluble protein content in CPE markedly improved at pH 7 (59.69 ± 0.30%) compared to the untreated and defatted cricket flours, whose values remained below 1.5%. This outcome reflects the removal of lipids and insoluble components known

to interfere with protein hydration and solubilization (Zielińska et al., 2018). The solubility profile follows the expected U-shape, with a minimum at pH 4, near the isoelectric point, and an increase in alkaline conditions, likely due to protein unfolding and enhanced exposure of hydrophilic side chains. Although the soluble proteins content in CPE is lower than that of soy protein isolate (around 80% at pH 7), it compares well with that of pea protein, reported as 60% at neutral to slightly alkaline pH (Barac et al., 2015).

Table 1 *Solubility and water holding capacities (WHC) of the cricket flour (CF), defatted cricket flour (DCF) and cricket protein extract (CPE).*

pH	Solubility $g_{Soluble\ Protein}/100g_{Total\ Protein}$			Water Holding Capacity g_{H_2O}/gdm		
	CF	DCF	CPE	CF	DCF	CPE
3	1.00±0.21 ^{B}	0.87±0.23 ^{B}	51.13±1.06 ^{H}	2.07±0.02 ^{AB}	3.66±0.04 ^{A}	4.97±0.19 ^{BCD}
4	1.22±0.14 ^{AB}	1.24±0.15 ^{A}	42.19±0.98 ^{G}	1.77±0.18 ^{B}	2.29±0.06 ^{D}	4.44±0.35 ^{D}
5	1.43±0.40 ^{AB}	1.25±0.10 ^{A}	48.28±1.04 ^{F}	1.86±0.04 ^{AB}	2.46±0.06 ^{D}	4.54±0.06 ^{CD}
6	1.44±0.04 ^{A}	1.29±0.13 ^{A}	53.70±0.85 ^{E}	1.96±0.01 ^{AB}	2.79±0.10 ^{C}	4.75±0.14 ^{BCD}
7	1.44±0.32 ^{A}	1.29±0.42 ^{A}	59.69±0.30 ^{D}	1.98±0.02 ^{AB}	2.93±0.09 ^{BC}	4.91±0.17 ^{BCD}
8	1.48±0.03 ^{A}	1.40±0.06 ^{A}	79.35±0.69 ^{C}	2.01±0.01 ^{AB}	3.17±0.04 ^{B}	5.28±0.09 ^{AB}
9	1.55±0.36 ^{A}	1.44±0.05 ^{A}	90.81±0.03 ^{B}	2.08±0.03 ^{A}	3.52±0.05 ^{A}	5.52±0.15 ^{ABC}
10	1.65±0.16 ^{AB}	1.48±0.16 ^{A}	92.12±1.07 ^{A}	2.13±0.03 ^{A}	3.62±0.09 ^{A}	5.70±0.11 ^{A}

Data reported as mean ± SD. Values followed by different letters (A-H) are significantly different ($p < 0.05$).

The WHC of CPE showed a similar pH-dependent behaviour, peaking at 5.70 ± 0.11 g_{H_2O}/g_{dm} at pH 10 and reaching 4.91 ± 0.17 g_{H_2O}/g_{dm} at pH 7. These values are substantially higher than those of CF and DCF and also exceed typical WHC values reported for plant-based proteins such as chickpea (1.31 gH_2O/g_{dm}) and cowpea (1.24 g_{H_2O}/g_{dm}) (Sreerama et al., 2021). Such WHC values of CPE suggest a high degree of surface polarity and interaction potential with water, likely facilitated by the removal of lipophilic and fibrous constituents during extraction. These properties underline the suitability of the cricket protein extract for food formulations requiring water retention, texture enhancement, and structural integrity.

3.2 Rheological characterization of Cricket Protein Extract

The dependence of the storage modulus (G′) and loss modulus (G″) of CPE gels at different concentrations on frequency is shown in Figure 1. Increasing the CPE concentration from 15% to 25% resulted in a progressive rise in both G′ and G″ across the entire frequency range tested. This behaviour suggests the formation of more densely crosslinked protein networks, as higher protein content enhances molecular interactions and entanglement. For all concentrations studied, G′ remained consistently greater than G″, and no crossover was observed, confirming the solid-like nature of the gels (Monteiro et al., 2021). The moderate frequency dependence of G″, particularly at higher concentrations, reflects increased viscous energy dissipation due to greater network heterogeneity and hydration effects. These results support the classification of CPE gels as weak gels, in which concentration primarily modulates viscoelastic strength (Tunick, 2011). Although only part of the analytical framework has been presented here, the functional properties reported and the rheological behavior of CPE-based gels suggest the suitability of the cricket protein extract for advanced processing and formulation of insect-based meat analogues.

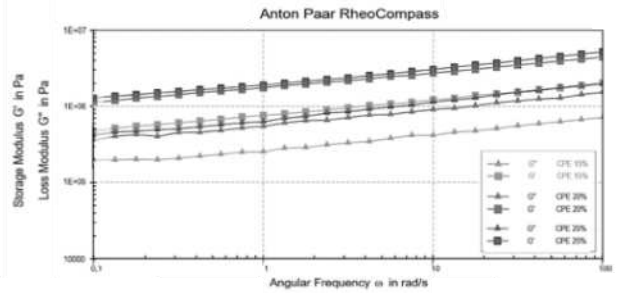


Figure 1 *Frequency sweep of cricket protein extract gel at different concentration (15, 20, 25 % w/v).*

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Improvement of the monovarietal EVOO production chain: phenolic and volatile profiling for oil authentication and by-products exploitation (P-II-34)

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Monovarietal Extra Virgin Olive Oil (MEVOO) is a growing sector, both in small-scale, high-quality productions and at an industrial level. MEVOOs from different cultivars have peculiar sensory characteristics, to date little known by the average consumer but sought after by expert consumers. A targeted characterization of cultivars is necessary to define and promote the specific attributes of high-quality MEVOOs, and to create defined commercial categories (Cecchi et al. 2022). These categories can also be used to develop chemometric models for oil classification and authentication. As for industrial production, the mechanization of agriculture has led to a widespread diffusion of intensive monovarietal cultivations, which produce oils that typically become part of blends with defined characteristics. It is therefore fundamental to understand how the cultivar of origin influences an oil's chemical and organoleptic characteristics and how these evolve over time, determining the product's shelf-life. Finally, a common problem for both commercial sectors is the economic and environmental sustainability of the supply chain. The recovery and reuse of potentially polluting byproducts, such as olive pomace and pruning residues, is a current research objective (Mallamaci et al. 2021). These waste biomasses in fact represent an underexploited resource, as they contain high concentrations of bioactive phenolic compounds with many possible applications in the food, nutraceutical, and cosmetic sectors.

Miglioramento della filiera di produzione dell'olio EVO monovarietale: studio delle componenti fenolica e volatile per l'autenticazione dell'olio e per il recupero dei co-prodotti

Il settore dell'olio EVO monovarietale (OEVOM) è in crescita, sia nell'ambito delle piccole produzioni di alta qualità che a livello industriale. OEVOM di alta qualità hanno caratteristiche sensoriali peculiari, ad oggi poco conosciute dal consumatore medio, ma sempre più ricercate dai consumatori esperti. Per promuovere i prodotti di alta qualità è necessaria una caratterizzazione delle cultivar mirata a scoprire e valorizzare le caratteristiche degli OEVOM, e a creare delle categorie commerciali definite. Queste categorie possono essere usate anche per sviluppare modelli chemometrici di classificazione ed autenticazione. Riguardo la produzione industriale invece, la meccanizzazione dell'agricoltura ha portato ad una ampia diffusione di coltivazioni intensive monovarietali; gli oli che ne derivano generalmente entrano a far parte di blend con caratteristiche definite. È quindi fondamentale capire come la cultivar di provenienza di un olio ne influenza le caratteristiche chimiche ed organolettiche, e come queste evolvono nel tempo andando a determinare la shelf-life del prodotto. Infine, un problema comune ad entrambi i settori è la sostenibilità economica ed ambientale della filiera. Il recupero e riutilizzo di coprodotti inquinanti quali le sanse e gli scarti di potatura è un obiettivo della ricerca attuale. Queste biomasse di scarto rappresentano infatti una risorsa sottoutilizzata, dal momento che contengono elevate concentrazioni di composti fenolici bioattivi con possibili applicazioni nei settori alimentare, nutraceutico e cosmetico.

1. Introduction

As outlined in the project presented at the 28th PhD workshop, the general objective of this PhD thesis to enhance the production chain of MEVOO. To this aim, we focused on three main research topics:
1) Chemical and sensory profiling of Italian MEVOO from different cultivars using chromatographic techniques and innovative tasting sheets for clustering and authentication by chemometrics.
2) Study of the shelf-life of Italian and Tunisian monovarietal oils through accelerated and non-accelerated oxidation experiments. Analysis of the evolution of sensory and chemical features over time.
3) Improvement of the management of by-products from the MEVOO supply chain, through up-cycling pruning residues in the nutraceutical field. Analysis of the phenolic profile of leaves from Tuscan cultivars, and of their seasonal variations

2. Materials and methods

2.1 Analysis of chemical and sensory data from Italian MEVOOs

Approximately 300 MEVOO samples from 13 of the main Italian varieties were chemically analysed through HPLC-DAD-MS (phenolic fraction) and HS-SPME-GC-MS (terpene and volatile fractions). The samples were also tasted by an official panel, using a detailed sensory sheet. GA-LDA-HCA was applied to the sensory data to cluster the cultivars in groups based on their similarity. Using in parallel GA-LDA and RF on chemical data, sensory clusters were 5-fold cross validated and the chemical variables that best place the samples in the groups of cultivars were identified.

2.2 Accelerated and non-accelerated storability experiments

MEVOOs from *Chemlali* and *Chetoui*, two commercially relevant Tunisian cultivars, and from *Coratina*, the most cultivated Italian variety, were used for both accelerated and non-accelerated oxidation experiments: the oils in non-accelerated conditions are being stored over a 18-months timespan, with or without exposure to light, while samples under accelerated oxidation have been stored in an oven at 90°C for ten days. Oxidated samples were sensorially tested by professional panellists and analysed with chemical and chromatographic techniques.

2.3 Study of the effects of cultivar and seasonality on the phenolic profiles of olive leaves

Olive leaves from trees of four Tuscan cultivars (i.e., *Frantoio, Moraiolo, Leccino, Leccio del Corno*) were sampled over two years and four different phenological phases. To minimize variability due to soil and climatic conditions, all samples were gathered from the same orchard and, for each sampling period, on the same day. Hydroalcoholic phenolic extracts from ultrasound-assisted extraction were analysed with HPLC-DAD-MS, and quantitative data was analysed with ANOVA and GA-LDA-HCA. Several trials for optimizing leaves drying were also performed.

3. Results

3.1 Cultivar clustering based on MEVOO sensory data and validation with chemical parameters

Three clusters of cultivars were defined after application of GA-LDA-HCA analysis on MEVOO sensory data. Using only chemical parameters to characterize the three clusters through the GA-LDA and RF methods, high and consistent degrees of classification were obtained, as highlighted by the confusion matrices (Table 1 and 2). This approach not only provided chemometric classification models but also allowed to identify the main parameters that characterize the clusters.

Table 1. *GA-LDA confusion matrix*

	C1	C2	C3	total
C1	141 (**95.9%**)	5 (3.4%)	1 (0.7%)	147
C2	2 (2.6%)	74 (**97.4%**)	0 (0%)	76
C3	2 (7.4%)	0 (0%)	25 (**92.6%**)	27
Error-Rate: **4.71 %**		Accuracy: **96 %**		

Table 2. *RF confusion matrix*

	C1	C2	C3	total
C1	142 (**96.6%**)	4 (2.7%)	1 (0.7%)	147
C2	4 (5.3%)	72 (**94.7%**)	0 (0%)	76
C3	2 (7.4%)	0 (0%)	25 (**92.6%**)	27
Error-Rate: **5.36 %**		Accuracy: **95.6 %**		

3.2 Evolution over time of Italian and Tunisian MEVOO quality parameters

EVOO production in Tunisia is rapidly increasing with 200.000 tons of oil produced in 2023, making it the third worldwide oil-producing country. Tunisian MEVOOs are particularly susceptible to oxidation due to their high polyunsaturated fatty acid content, so it is particularly important to examine their storability properties (Krichene et al. 2010). In both oxidation experiments, the evolution of legal quality parameters such as free acidity, peroxide value and UV indexes is being analyzed together with phenolic derivatives, volatile compounds and tocopherols.

3.3 Seasonal variations of phenolic profiles in olive leaves from Tuscan cultivars

HPLC-DAD-MS allowed the identification and quantification of 25 phenolic and secoiridoid derivatives. As expected, oleuropein was the most abundant phenolic compound, with a minimum average value of 4570 mg/kg and a maximum average value of 27548 mg/kg. Three-way ANOVA of quantitative data highlighted significative differences among cultivars and season of harvest, providing useful data for an efficient recovery of olive leaves bioactive compounds. GA-LDA-HCA analysis allowed the classification of olive leaf samples based on their phenolic profiles

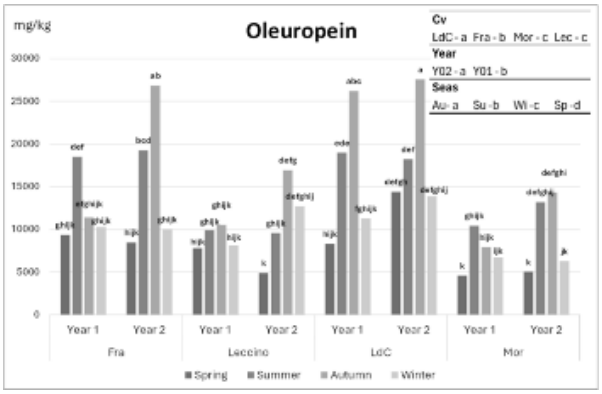


Figure 1. *Oleuropein seasonal variations in different cultivars*

4. Future perspectives

Research on high-quality MEVOOs will continue with more detailed characterization of the volatile profile of cultivars using GC-IMS-MS, in collaboration with Mannheim University. Within this project, cultivar markers or fingerprints will be searched for. The study on MEVOO shelf-life will be carried out comparing results obtained with or without accelerated oxidation; moreover, molecular oxidation markers will be searched using HR-LC-MS/MS.

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Chemical characterization of *Ocimum Basilicum* L. for industrial pesto sauce production (P-II-35)

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This work aims to support the optimisation of the basil supply chain for the industrial production of pesto, by evaluating the influence of cultivar, harvest area and harvest period on the aroma of Ocimum Basilicum L. leaves, as well as the impact of the pesto production process on the final aromatic profile. The various phases of the project were started in the first year and are repeated in subsequent years to increase the dataset and consolidate the reliability of the results.

Caratterizzazione chimica di *Ocimum Basilicum* L. destinato alla produzione di pesto per industrie

Questo lavoro mira a supportare l'ottimizzazione della filiera del basilico per la produzione industriale di pesto, valutando l'influenza della cultivar, dell'areale di coltivazione e del periodo di raccolta sull'aroma delle foglie di Ocimum Basilicum L., nonché l'impatto del processo produttivo sul profilo aromatico finale. Le varie fasi del progetto sono state avviate sin dal primo anno e, negli anni successivi, vengono ripetute per incrementare il dataset e consolidare l'affidabilità dei risultati.

Key words: *Ocimum Basilicum* L., Volatile Organic Compounds (VOC's), Pesto sauce production chain.

1. Introduction

In the first year of the PhD programme, six commercial cultivars for industrial use from three different geographic areas (Veneto, Piedmont and Emilia Romagna) were monitored during the basil harvesting season. 270 samples have been analyzed and, in six cases the pesto production process was followed at different steps (fresh leaf, blanched leaf, semi-finished product and pesto). Considering the impact of intrinsic and estrinsic factors influencing the aroma of the finish end product (Verma and Shukla 2015), the project was developed to: compare cultivars cultivated in the same area, evaluate the effect of cultivation area, understand the evolution of the aroma profile throughout the harvest season and monitor the impact of the transformation process on aromatic profile.

2. Materials and Methods

The work involved the analysis of a 270 samples of basil leaves, semi-finished products and pesto sauces.
Volatile organic compounds (VOCs) were extracted by headspace solid-phase microextraction (HS-SPME) using a MPS multipurpose sampler (Gerstel, Mülheim a/d Ruhr, Germany) installed on the GC-MS system. SPME fibers, divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (df 50/30 µm, 2 cm), were from Supelco (Bellefonte, PA). For the extraction, 0.5 ± 0.01 g of each matrix were weighed into a 20 mL vial and closed with a cap in PTFE/silicone septum. SPME device was exposed to the headspace of samples for 40 min at 50 °C. Extracted analytes were recovered by thermal desorption of the fiber into the split/splitless (S/SL) injection port of the GC system at 250 °C for 5 min. Separation of volatile compounds was performed on a HP-5ms Ultra Inert capillary column (30 m × 0.25 mm × 0.25 µm) with helium as carrier gas at a constant flow rate of 1.0 mL/min. The gas chromatograph oven was programmed to start at 50 °C, held for 1 min, followed by a temperature ramp of 3 °C/min up to 210 °C, with a final hold of 2 min. Injection was performed in splitless mode. The transfer line temperature was maintained at 280 °C and the mass spectrometer operated in scan mode over a mass range of 40 to 300 m/z. Each sample was analysed in triplicate.

3. Results and Discussion

3.1 Effect of the cultivar

As demonstrated by the principal component analysis (PCA) (figure 1), the total aromatic profile helps to discriminates varieties when they variety are compared within the same area of cultivation. The molecules most responsible for discriminating the P cultivar from the other two cultivars were 3-hexen-1-ol (cut grass notes) and 3-octanone (fruity, herbaceous notes), while those most responsible for discriminating S from the G cultivar were α-Thujene (herbaceous notes), α-Phellandrene (fruity notes) and trans-2-Hydroxy-1,8-cineole (eucalyptus, fresh, pungent notes).

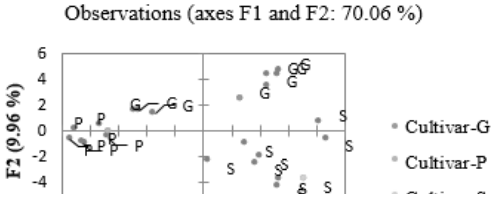


Figure 1. *PCA of 3 different O. basilicum* L.*. cultivars harvested in the same geographical area*

Then, the cultivar G demonstrates a clear discrimination based on its content of phenylethyl alcohol, which is characterised by a floral and rosy notes.

3.2 Effect of the geographical area of cultivation

Comparing cultivar G cultivated in the Veneto and Emilia-Romagna areas, no statistically significant differences in total aromatic abundance or chemical classes emerged, except for oxygenated monoterpenes, which are higher in Emilia-Romagna. Focusing on the key compounds of Genovese Basil PDO and Superbo basil variety grown in Liguria (Betuzzi et al. 2025), namely linalool, eugenol, and α -bergamotene, specific area influence were noted. Linalool is the most abundant molecule in both areas and no statistically significant differences were observed among the areas as for eugenol. Conversely, for α-bergamotene, a statistically significant difference was observed among areas, with levels 46% higher in the basil cultivated in Veneto.

3.2 Role of harvest period

Figure 2 shows the chemical classes distribution of cultivar G harvested in two different periods, at the beginning and end of the season. Most major volatile classes showed a general decrease: monoterpene hydrocarbons declined of 2.7%, sesquiterpene hydrocarbons of 2.1% and phenylpropanoids of 1.2%. However, only oxygenated monoterpenes exhibit a statistically significant difference, decreasing from 51.9% to 46.8%.

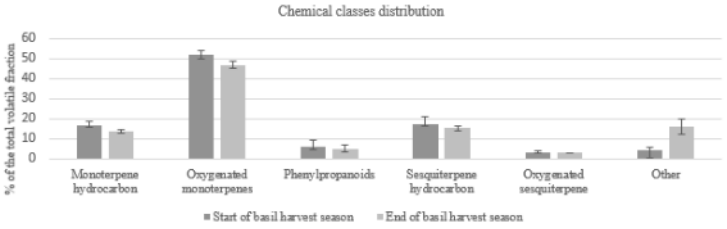


***Figure 2**. Chemical classes distribution among the same O. basilicum* L. *cultivars harvested in two different periods*

3.3 Role of processing

Processing has a significant impact on basil volatile profile due to the stabilisation procedures and the cutting process that determine oxidative reactions. Additionally, the use of other ingredients - such as oil, which is highly lipophilic can either mask or enhance the aromatic contribution of certain molecules (Xiao et al., 2017).

As shown in figure 3, a progressive decrease in total aromatic profile was observed at each processing step. Specifically, a 28% loss occurred moving from fresh leaves to blanched leaves, and a further 67% of the remaining aromatic content was lost in the subsequent steps due to the dilution with other ingredients in the semi-finish and finish end product. The overall process leads to an 82% of aroma reduction compared to the initial content.

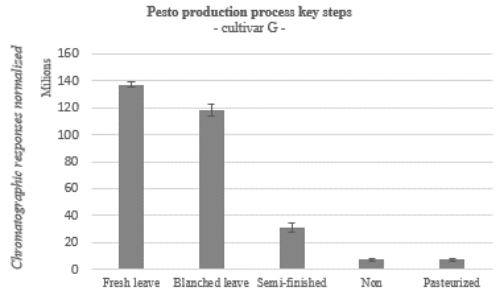


***Figure 2**. Comparison between the total aromatic abundance of the samples along the production process*

4. Conclusion

Our study highlights the crucial influence of cultivar, cultivation area and harvesting period on basil volatilome. Therefore, careful selection of the cultivar and harvesting period is essential for achieving a product with the desired aromatic profile. In addition, the blanching step of the transformation process - applied to stabilise the colour - significantly reduces the aromatic components. Therefore, reducing these processing losses is as important as optimising the primary production parameters to preserve the desired aroma of the final product.

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Central Italy Emmer wheat types: healthy foods from sustainable agriculture (P-II-36)

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Abstract
The cultivation of emmer (*Triticum turgidum* ssp. *dicoccum*) offers a sustainable solution to address climate changes, thanks to its drought resistance, and for reinforcing short food supply chains, especially in marginal areas. This research was aimed to study emmer wheat samples of Central Italy type grown in Abruzzi, which were segmented by genotype, habitus, and processing (milling and pasta-making), and were compared to durum wheat samples. Grain, flour and whole meal pasta (pilot-scale and scaled-up) were characterized by their chemical composition and physical analysis, among which rheological properties.
Significant differences were due to habitus; winter emmer flours were characterized by lower ash content than spring emmer, which had higher gluten content, and dough making quality. Discriminant analysis revealed spring emmer closer to durum than winter emmer. Resistant starch and lutein content were significantly higher in emmer compared to durum samples (especially in pasta samples). Milling by stone mill showed a detrimental effect on resistant starch and lutein contents without affecting those of β-carotene and α-tocopherol.
These findings underscore the potential of emmer in producing organic flours with peculiar functionalities and provide opportunities to develop innovative food products that meet the demands of health/environmental-conscious consumers while contributing to agricultural biodiversity and local economy in marginal areas.

Key words: *Triticum turgidum* ssp. *dicoccum*, Habitus, Milling technology, Composition, Functional compounds.

1. Introduction

Emmer wheat (*Triticum dicoccum* (Schrank.) Schuebl.) is a hulled wheat cultivated since ancient times and has been able to adapt to risen climate changes (Khmeleva, 2021). Emmer is grown in small and marginal areas with low input sustainable agriculture (Kulathunga, 2021; Sarkar, 2020). Emmer is often labeled as a "specialty grain" due to its distinctive functional characteristics (Giambanelli et al., 2010) and speculated potential health benefits, which remain unsupported by comprehensive scientific research (Lacko-Bartošová, 2015). Recently, emmer's research interest is increasing due to its potential in diversifying local food choices with "marketed patrimonies" (Akiki et al., 2025) In this perspective spring emmer with vitreous fracture is an alternative for pasta production (Giacintucci, 2014, Akiki et al., 2025). Emmer wheat samples grown in Central Italy have been collected and processed in flours and pasta. The technological properties, and content of phytochemicals (macro and micro-nutrient) of emmer products were compared to durum wheat.

2. Materials and Methods

Winter and spring emmer samples of the Central Italy type were collected in Abruzzi and processed into flours (refined, wholemeal, and stone-milled) and pasta (using lab scale and industrial plant).
Grain size and dimensions was determined according to Mandea and Săulescu (2018), 1000 kernel weight according to ISO 520:2010, test weight according to ICC Standard No. 120. Hardness and color were determined according to AACC 66-20.01, and AACC 14-22.0, respectively.
Milling was carried out with methods AACC 26-10.02 and AACC 26-95.01 using lab scale and pilot plant roller mills and stone mills. Particle size distribution of flours was determined by AACC 55-31.01 and modelled with the Weibull's distribution function.
Pasta was produced by a single screw lab scale extrusion process and by a twin-screw industrial process carried out by a pasta making company (Pastificio Artiginale Cav. Giuseppe Cocco, Fara San Martino, Italy).
The chemical composition was analysed according to AOAC methods, protein, fat, ash, water content, soluble (SDF) insoluble (IDF) and total (TDF) dietary fibers according to the 978.04, 2003.06, 930.05, 930.04, and 985.29, respectively. Rapidly digestible, semi-digestible, and resistant starch according to AOAC 996.11, unsaponifiable residues extraction according to AOAC 945.45 and sterols according to COI/T.20/Doc. no.26/Rev. 5/2020 method, carotenoids according to EN 12823-2:2000, tocopherols according to ES iso 9936:2012.
Gluten content was determined according to method AACC 38-10.01 farinographic analysis was carried out according to AACC 54-21.02 and alveographic analysis according to AACC 54-30.02.
Samples were segmented according to their habitus (winter and spring), and processing step. The study included grain samples, flours (refined roller milled flours, whole meal roller milled flours, whole meal stone-milled flours), and pasta (pilot-scale pasta and scaled-up pasta). Differences among segmented data were tested by using the ANOVA test followed by the Tukey's multiple pairwise comparisons procedure (p<0.05). Nonlinear regression was computed on particle sizes data. Principle components analysis (PCA) on correlation matrix was performed on all morphological, technological and rheological data and a discriminant analysis LDA was performed on all chemical and bioactive compounds analyses.

3. Results

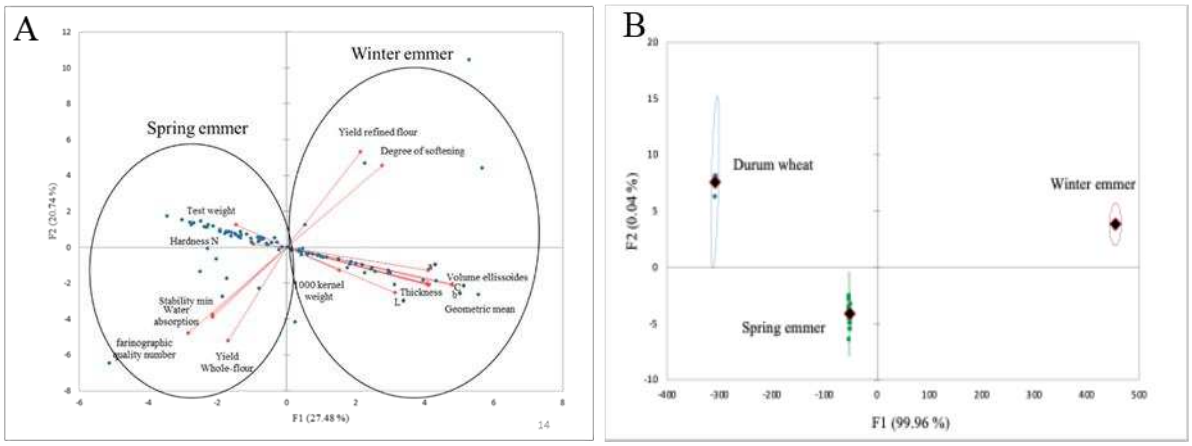


Figure 1. Principal component analysis on technological performance of emmer with loadings of emmer with different habitus (A) Discriminant analysis in spring, winter emmer, and durum on the basis of their chemical composition (B).

Winter and spring emmer exhibited different technological performances (Figure 1). Winter emmer exhibited thicker and heavier grains and greater grain volume compared to spring type one, which in turn showed higher kernel density and grain hardness. From a milling perspective, the vitreous (hard) endosperm structure of spring emmer resulted in a higher yield of whole and refined flour compared to winter types. Gluten content and farinographic quality was higher in spring emmer, contributing to its better dough making properties.

LDA analysis carried out on composition data permitted to differentiate spring and emmer wheat grains, flours and pasta products on the basis of the emmer habitus (Figure 2). One main factor was responsible for correct sample classification (100%) with spring emmer being closer to durum wheat than the winter one in terms of composition. Stigmasterol was the compound which mostly contributed to classification, followed by δ - tocotrienol, other sterols (24-methylencolesterol, Δ5,23-stigmastadienol, clerosterol, 5,24-stigmastadienol) and total fat content in pecking order. α- tocotrienol, δ- tocopherol, γ-tocoferol, TDF, total carbohydrates and ash content also contributed to correct classification. It is also interesting to stress that the content of resistant starch was higher in emmer flours than in durum ones and was increased by the milling process whilst it was dramatically decreased by the pasta making process.

Stone milled products (flours and pasta) were discriminated from roller milled ones with a 95% correct classification. Campesterol, SDF and IDF were responsible for classification with stone milled flours presenting higher values of SDF and campesterol, and lower values of IDF. Stone milled products showed also lower resistant starch respect to roller milled ones.

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Chicken Muscle Proteome as a Marker of Farming Practices: Implications for Stress response and Meat Quality (P-II-37)

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The first phase of this study focused on characterizing the proteomic profile of breast muscle from chickens reared under organic and conventional farming systems. Using label-free quantitative proteomics (LFQ) combined with nLC-ESI-MS/MS and bioinformatics, the expression of stress-responsive proteins was analyzed to investigate the impact of different rearing conditions on muscle physiology and meat quality. A total of 721 proteins were identified, showing significant abundance differences: proteins involved in oxygen transport and muscle structure were upregulated in organic chickens, while proteins related to stress-induced energy metabolism were downregulated. These findings highlight how farming practices influence muscle development and metabolic stress, providing potential biomarkers for distinguishing meat quality linked to animal welfare.

Analisi proteomica del muscolo di pollo come indicatore delle condizioni di allevamento: Implicazioni per la risposta allo stress e la qualità della carne

La prima fase di questo studio ha riguardato la caratterizzazione del profilo proteomico del muscolo di polli allevati con sistemi biologici e convenzionali. Utilizzando la proteomica quantitativa label-free (LFQ) in combinazione con nLC-ESI-MS/MS e strumenti bioinformatici, è stata analizzata l'espressione di proteine correlate allo stress per valutare l'impatto delle condizioni di allevamento sulla fisiologia muscolare e sulla qualità della carne. Sono state identificate 721 proteine, con differenze significative nella quantità: nei polli allevati con sistemi biologici si osserva un'aumentata espressione di proteine coinvolte nel trasporto di ossigeno e nella struttura muscolare, mentre le proteine correlate al metabolismo energetico associato allo stress risultano ridotte. Questi risultati evidenziano come le pratiche di allevamento influenzino lo sviluppo muscolare e lo stress metabolico, suggerendo biomarcatori potenziali per distinguere la qualità della carne in relazione al benessere animale.

Key words: label-free proteomics; muscle metabolism; stress biomarkers; animal welfare

1. Introduction

Stress plays a crucial role in shaping animal physiology and significantly influences meat quality attributes such as tenderness, flavor, and color. In poultry, stress responses alter muscle biochemistry, directly affecting these organoleptic characteristics. This study aimed to explore the proteomic profile of chicken breast muscle in chickens reared under organic and conventional farming systems, using label-free quantitative proteomics (LFQ) coupled with nano-liquid chromatography electrospray ionization tandem mass spectrometry (nLC-ESI-MS/MS) and bioinformatic tools. The goal was to identify stress-responsive proteins that could reflect the impact of different rearing environments on muscle physiology and meat quality. The findings reveal potential proteomic biomarkers capable of distinguishing organic chicken meat and provide insight into how farming practices influence biological processes related to animal welfare and product quality.

2. Material and Methods

Protein extraction was performed on 100 mg of chicken breast tissue using ammonium bicarbonate buffer supplemented with protease inhibitors. After homogenization and centrifugation, samples were treated with SDS buffer, heated, and subjected to high-intensity focused ultrasound (HIFU). For MS analysis, 30 µg of protein per sample were reduced, alkylated, and digested with trypsin. Peptides were analyzed using nLC-ESI-MS/MS on an Orbitrap Q-Exactive mass spectrometer. Label-free quantification (LFQ) intensities were used to assess protein abundance, with spectral data matched to the *Gallus gallus* reference proteome. Protein-protein interaction networks, and functional pathways were explored using STRING.

3. Results and Discussion

A total of 721 proteins were identified, with 164 upregulated and 161 downregulated in organic chickens compared to those from conventional systems. STRING analysis indicated that the upregulated proteins in organic chickens were primarily involved in oxygen transport, oxygen binding, and structural muscle components. These functions suggest an improved oxidative metabolism and muscle development,

likely due to better welfare conditions (Fig. 1A). Conversely, the downregulated proteins were mainly linked to ribosomal structure, rRNA binding, and energy metabolism (Fig. 1B), which are commonly associated with cellular stress responses.
KEGG pathway enrichment revealed that upregulated proteins were enriched in carbon metabolism, while downregulated proteins were tied to ribosomal function, glycolysis, and gluconeogenesis—pathways often influenced by stress in animals. AMPD1, PGM1, PKLR, and LDHA were downregulated, while COL1A1, COL1A2, TTN, TPM2, CA3, MB, HSPB1, ACO2, and TF were upregulated in organic chickens (Alessandroni et al., 2024; Kong et al., 2024; Hazard et al., 2011).
These molecular signatures suggest that organic chickens develop muscle tissue more effectively and experience less metabolic stress, supporting the idea that welfare-friendly environments enhance physiological resilience. In contrast, the proteomic profile of conventionally reared chickens reflects greater metabolic activation associated with environmental and handling stress. The proteomic differences observed provide valuable insights into how husbandry practices influence muscle composition and meat quality traits, offering a potential method for distinguishing meat derived from organic production systems.

In conclusion, this study demonstrates distinct proteomic profiles between chickens reared under organic and conventional farming systems. Organic chickens showed an upregulation of proteins involved in oxygen transport and muscle structure, while stress-related energy metabolism proteins were downregulated. These patterns suggest enhanced muscle development and reduce metabolic stress in organically reared chickens, likely driven by improved animal welfare. The identified proteomic markers offer promising tools for distinguishing meat quality linked to production systems and provide a molecular basis for promoting welfare-based differentiation in poultry products.

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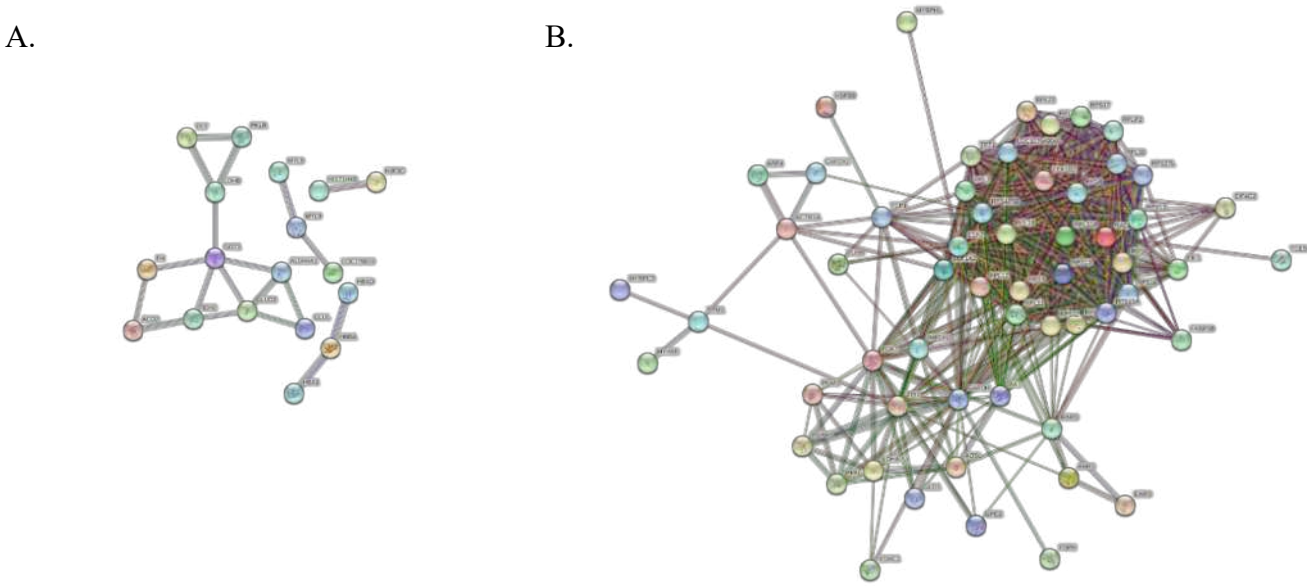


Figure 1.
High-resolution STRING network visualization of biological processes associated with proteins upregulated (A) and downregulated (B) in organic chickens compared to those reared conventionally

Application of chitosan coating and gaseous ozone to improve quality of fresh-cut apples (P-II-38)

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The first activities of the PhD thesis project are described. Firstly, the application of a chitosan coating on fresh-cut apples was evaluated on microbial and quality parameters at a laboratory scale. Then, fresh-cut apples were treated with gaseous ozone in industrial plant and microbial and quality parameters were evaluated as well.

Applicazione di rivestimento a base di chitosano e di ozono gassoso per migliorare la qualità delle mele di IV gamma

Le prime attività del progetto di tesi di dottorato sono descritte. In primo luogo, è stata valutata l'applicazione di un rivestimento edibile a base di chitosano su mele di IV gamma, analizzando i parametri microbiologici e qualitativi su scala di laboratorio. Successivamente, le mele di IV gamma sono state trattate con ozono gassoso in impianto industriale e sono stati valutati i parametri microbiologici e qualitativi.

Key words: Fresh-cut, apple, gaseous ozone, chitosan, edible coating

1. Introduction

In accordance with the PhD thesis project previously described (Giannini, 2024), this poster reports the main results of the first two activities concerning:
(A1) Effect of chitosan coating on colorimetric and sensorial properties of fresh-cut apple slices
(A2) Effect of gaseous ozone on the metabolic response of fresh-cut apple slices

2. Materials and Methods

(A1) The apples were processed at a laboratory scale. They were sanitized in hypochlorite and sliced with a cutting board in 16 slices. Then, they were subjected to 3 treatments: ascorbic acid (ctrl) (2 %), commercial chitosan (sigma) (1 %) and laboratory extracted chitosan from Squilla mantis by-product (mantis) (1 %). Both chitosan powders were dissolved in 2 % ascorbic acid solution. The treatments were applied by immersion for 1 minute. The effect of the coating was assessed by the means of colorimetric indexes, in particular browning development (% browning area) through a Computer Vision System (CVS) (Romani et al., 2009) and sensory analysis.

(A2) The apples were processed in a fresh-cut company with their equipment. They were sanitized in peracetic acid solution and then sliced with a wedging machine. Then, they were subjected to 4 treatments: distilled water (ctrl), anti-browning dipping (aa), ozone (oz), anti-browning dipping + ozone (ozaa). The dipping was composed of ascorbic acid (3 %) and calcium carbonate (0.8 %) and was applied by complete immersion for 1 minute. The ozone treatment was applied by placing the slices on a moving tray in a tunnel full of a gaseous mist for 90 seconds. The ozone generator was producing 60 mg/L of ozone. The effect of the treatments was assessed by measuring respiration rates (O_2 and CO_2) with a gas analyzer (Tappi et al., 2014) and metabolic heat release with an isothermal calorimeter (TAM) (Rocculi et al., 2007).

3. Results and Discussion

3.1 Chitosan Coating

Chitosan has demonstrated antimicrobial properties; however, its application to fresh-cut produce remains limited, and its effects on other quality parameters are still not fully understood. The computer vision system (CVS) analysis of browning area (BA) (fig.1) showed consistent results in color preservation up to 11 days for all samples in accord to Qui et al. (2011). Mantis began to develop random brown spots by day 15 and became completely unacceptable by day 20. Sigma showed better performance in maintaining color; however, the ascorbic acid control remained the most effective solution. Sensory analysis data (fig. 2) indicated similar levels of acceptance for mantis and sigma compared to the control, although the latter received the highest overall acceptance scores from the nine untrained panelists. The antimicrobial efficacy of chitosan on this product was also assessed, however, after 20 days the samples were still below the acceptability threshold, and chitosan did not appear to enhance microbial parameters. Further research is needed to explore the potential of chitosan applications, although these initial findings are not particularly promising, as ascorbic acid is an affordable, solid treatment for industries.

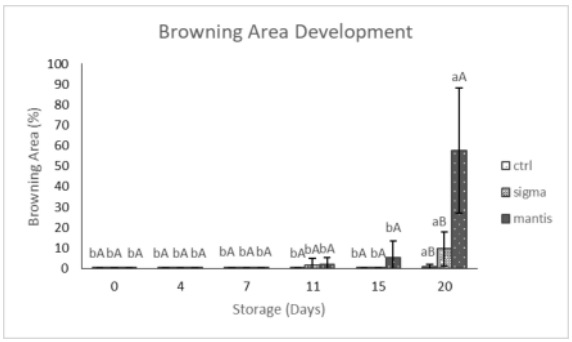


Figure 1. Color development during storage. Lowercase letters refer to the same sample during storage, uppercase letters refer to different sample in the same day

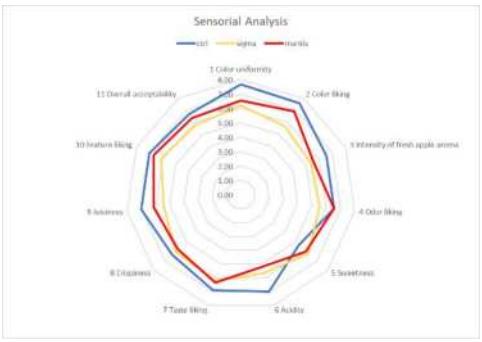


Figure 2. Radar chart of the sensorial analysis one day after treatment

3.2 Gaseous Ozone

Measuring the metabolic response of fresh-cut fruit to a treatment is crucial to anticipate its stability during storage. In general, the vegetal tissue responds to a stress with increased respiration, ethylene production and heat release. Ozone is a potent oxidizing agent; therefore, it would be expected to see an increase in the metabolic activity as a result of a major stress. The production rate of CO_2 (fig. 3) had a clear trend, in which the oz treatment was similar to control but higher, which was validated by the O_2 rate (fig. 4). This trend was reversed between the aa and ozaa treatments. In this case, the addition of ozone after the dipping treatment appeared to have a synergistic effect, contributing to a reduction in metabolic activity. In fact, although similar, the trend of ozaa showed the lowest in both CO_2 production and O_2 consumption. These results were in accord with Li et al. (2016). This trend was further validated by heat production data. TAM analysis (fig. 5) did not reveal differences between control and oz, although both showed higher heat release compared to aa and ozaa. Interestingly, ozaa showed the lowest value in heat release between all samples, indicating to be the most effective treatment in slowing down fruit metabolism.

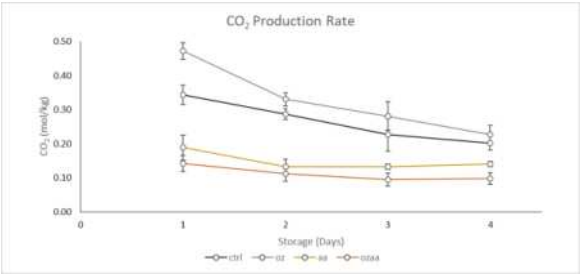


Figure 3. Carbon dioxide release rate of fresh-cut apples

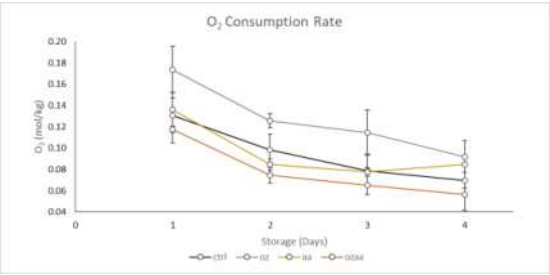


Figure 4. Oxygen consumption rate of fresh-cut apples

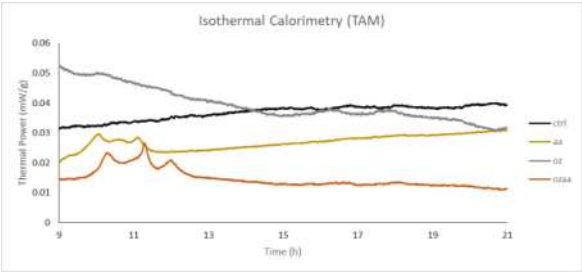


Figure 5. Heat production profile of fresh-cut apples

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Sustainable Production of Novel Natural Food Colorants by Filamentous Fungi and Yeasts through Carbon and Nitrogen Source Diversification and C/N Ratio Optimization (P-II-39)

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This research will investigate the application of microbial pigments to food systems with consideration for overall quality and food safety compliance. Several microorganisms that produce pigments, such as *Monascus ruber* MR1, *Metschnikowia pulcherrima* MRS 5.5 e *Rhodotorula mucilaginosa* TM1, were investigated in a controlled laboratory environment. A Central Composite Design (CCD) to optimize the fermentation parameters for maximum pigment production, establishing optimal conditions for pigment biosynthesis was used.

Recupero di alcuni metabolici microbici da soluzioni modello per osmosi inversa

Questa ricerca esamina l'applicazione di pigmenti microbici nei sistemi alimentari, con particolare attenzione agli attributi di qualità e conformità alla sicurezza alimentare. Sono stati studiati diversi microrganismi produttori di pigmenti, come *Monascus ruber* MR1, *Metschnikowia pulcherrima* MRS 5.5 e *Rhodotorula mucilaginosa* TM1, in condizioni controllate di laboratorio. In precedenza, è stato utilizzato un disegno composito centrale (CCD) per ottimizzare i parametri di fermentazione al fine di massimizzare la produzione di pigmenti, individuando così le condizioni ottimali per la biosintesi pigmentaria.

Key words: Microbial pigments, Natural food colorants, Fermentation optimization, Pigment-producing microorganisms, Central Composite Design

1. Introduction

In accordance with the PhD thesis project previously described (Sona, 2024), this poster reports the main results of the first two activities concerning:

A1) Microorganism Selection for Biotechnological Food Coloring. A Central Composite Design (CCD) to identify microorganisms and the most suitable conditions for pigment production was used. Temperature, pH, carbon, and nitrogen source were key parameters investigated in searching for the ideal conditions for food colorant production.

A2) Pigment Production and Extraction: This study determined the extraction method according to each bacterium's cellular structure and pigment production capacity. The applied extraction methods were suitable for these microorganisms, and additional studies may have improved them further.

2. Materials and Methods

Based on an initial series of pigment synthesis attempts, three strains (*Rhodotorula mucilaginosa* TM1, *Monascus ruber* MR1, and *Metschnikowia pulcherrima* MRS 5.5) were selected for further optimization studies, testing various carbon and nitrogen sources and culture conditions to enhance pigment yield.

R. mucilaginosa was grown in 25 media combinations, which supplied glucose, fructose, lactose, malt extract, or sucrose as carbon sources, and peptone, yeast extract, urea, NH_4Cl, or $NaNO_3$ as nitrogen sources. Cultures were incubated statically for 10 days at 30 °C. Pigments were extracted with 0.05 g dried biomass using 2 mL DMSO and 6 mL acetone, and pigments were purified using a two-phase NaCl-hexane system.

Monascus ruber was grown in modified PDB using 25 formulations of glucose, fructose, sucrose, maltose, or glycerol and peptone, yeast extract, NH_4Cl, or MSG. Fermentation was performed at 30 °C, for 10 days, and pigments were extracted using 70 % ethanol.

M. pulcherrima was tested in 21 media combinations that were grouped as minimal, medium, and maximal compositions, which provided glucose (20 g/L) as the carbon source while a range of nitrogen and mineral components of peptone, yeast extract, $(NH_4)_2SO_4$, K_2HPO_4, $MgSO_4$, $FeCl_3$, sodium citrate, $CaCl_2 \cdot 2H_2O$, and Tween-80. Cultures were incubated at 30 °C for 2, 5, and 8 days, respectively. Pigments were extracted from 0.5 g wet biomass in 5 mL methanol, followed by NaOH treatment.

A Central Composite Design (CCD) was used to optimize pigment production, testing 20 C/N-based media formulations in triplicate. The resulting data supported predictive modeling of optimal large-scale fermentation conditions.

3. Results and Discussion

3.1 Determination of the main physical properties

This method is expected to provide a nuanced understanding of these interactions secondary to the localization of

maximal yields of microbial pigment production within nutrient and environmental conditions.
For *Monascus ruber*, the most excellent pigment production of red pigment was achieved with glycerol as the carbon source and peptone as the nitrogen source (GL-P) at a total yield of 152.75 g·L (Figure 1).
The best carotenoid concentration, 40µg/g, was achieved for *Rhodotorula mucilaginosa* in a medium composed of malt extract and yeast extract (M-Y) (Figure 2).
The greatest pigment concentration produced by *Metschnikowia pulcherrima* was 0.487 mg/mL. This is measured under moderate cultivation conditions with a medium made of glucose (20 g/L), peptone (3 g/L), yeast extract (2 g/L), $(NH_4)_2SO_4$ (3.2 g/L), $K_2HPO_4 \cdot 3H_2O$ (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), and $FeCl_3$ (0.5 g/L) (in Figure 3).
These findings demonstrate that carbon, nitrogen, and mineral sources react differently on a strain-by-strain basis, showing the need for tailored optimization plans. The use of a Central Composite Design provided an opportunity to carefully develop specific fermentation conditions that maximized pigment production per fermentation while also ensuring repeatable processes. Overall, this study demonstrates environmentally friendly and sustainable alternatives to using artificial colours in food products with microbial pigments.

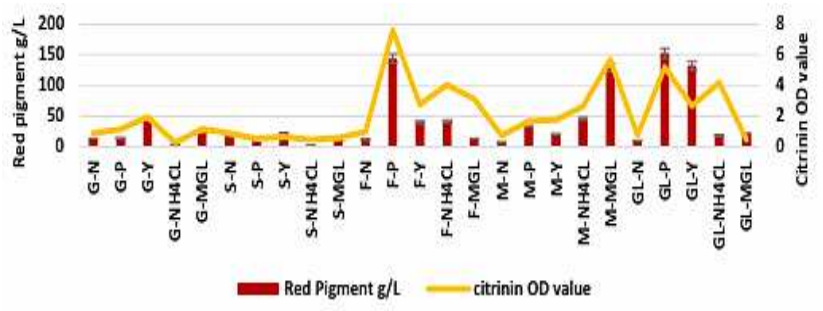


Figure 1. Red pigment production of *Monascus ruber* under different C/N source combinations. *The highest pigment yield was obtained glycerol, and peptone 152.75 g/L red pigment and citrinin OD value 5.188 (calculated based on dilution factor)*

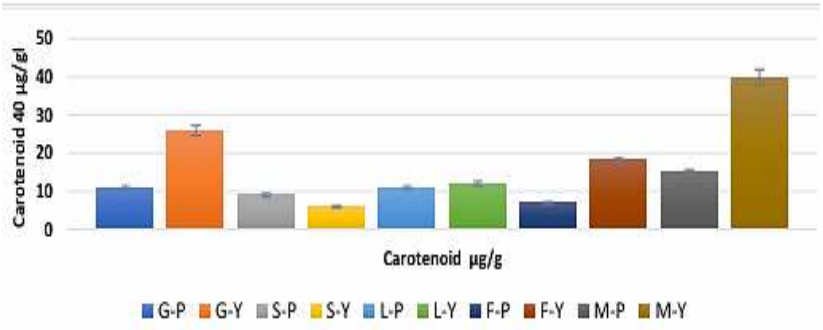


Figure 2. Carotenoid concentration of *Rhodotorula mucilaginosa* in tested in media. *The highest pigment yield was obtained Malt extract + yeast extract (M-Y) yielded the highest carotenoid level (40 µg/g).*

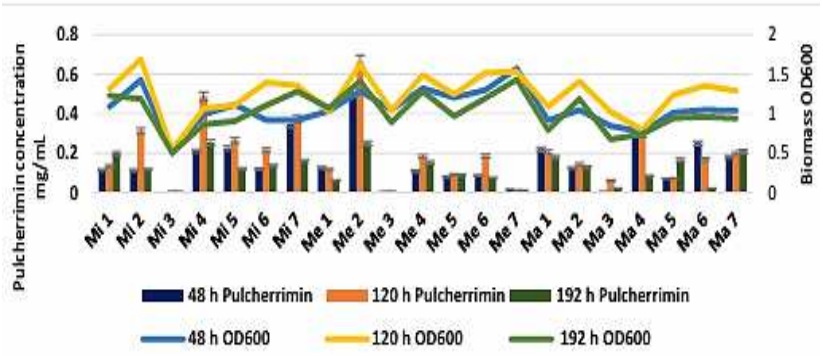


Figure 3. *The highest pigment yield was obtained glucose, yeast extract, $K_2HPO_4 \cdot 3H_2O$, $MgSO_4 \cdot 7H_2O$, and $FeCl_3$ at a concentration of crude extract 0.634 mg/mL*

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From Waste to Taste: Formulation of Fiber-Rich Bakery Products from Coffee Silver Skin (P-II-40)

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The processing of coffee beans generates a substantial amount of waste materials. The objective of our research was to evaluate the feasibility of substituting white flour with 5% and 8% of coffee silver skin (CSS) flour to develop bread. The formulated breads were examined for microbial, total polyphenols, and total dietary fiber analysis. According to the findings of our research, breads with 5% and 8% of CSS had a high percentage of total dietary fiber and polyphenols as compared to the control. There was no microbial count in baked breads.

Dagli scarti al gusto: Formulazione di prodotti da forno ricchi di fibre da silverskin di caffè

La lavorazione dei chicchi di caffè genera una notevole quantità di materiali di scarto. L'obiettivo della nostra ricerca è stato valutare la fattibilità della sostituzione della farina di grano tenero con il 5% e l'8% di farina di silverskin di caffè (CSS) per la produzione di pane. I pani ottenuti sono stati analizzati dal punto di vista microbiologico, del contenuto di polifenoli totali e di fibra alimentare totale. Secondo i risultati, i pani con il 5% e l'8% di CSS mostravano un contenuto più elevato di fibra alimentare totale e polifenoli rispetto al controllo. Nei pani cotti non è stata riscontrata presenza microbica.

Key words: Functional foods, food waste valorisation, bakery products, coffee silver skin flour, bread.

1. Introduction

The processing of the coffee bean generates tons of waste materials annually within the coffee supply chain. Since roasting is a crucial step in producing the coffee beverage, coffee roasting companies collect and discard tons of coffee silver skin (CSS), the single waste product from the coffee bean roasting processes (Nolasco et al., 2022). CSS has a low-fat content, a good quantity of protein, and total dietary fiber, making it suitable for use in the food industry (Iriondo-DeHond et al., 2019; Klingel et al., 2020). Therefore, the objective of our research was:

A) To evaluate the feasibility of substituting white flour with 5% and 8% of CSS flour as a source of total dietary fiber (TDF) to develop breads.
B) Determine the total phenolic content (TPC) in the control and breads treated with CSS.
C) To evaluate the microbial load in the final baked breads.

2. Materials and Methods

Raw CSS was initially subjected to at least six washings and then dried to 80°C in a Binder vacuum dryer/oven until the dryer attains a pressure below 5. The dried CSS was converted into flour form using a homogenizer. Three different breads were baked with 5%, 8%, and 0% of CSS flour. Breads were evaluated for TDF, TPC, and microbiological analysis. TDF was quantified using the Megazyme Integrated Total Dietary Fiber Assay kit, which aligns with the CODEX Alimentarius definition of dietary fiber and has been validated as an AOAC method 2017.16 since 2018 (Martuscelli et al., 2021). The analytical procedure involved enzymatic digestion employing heat-stable α-amylase, protease, and amyloglucosidase to effectively remove starch and protein components. After enzymatic hydrolysis, the sample underwent ethanol precipitation, followed by filtration and drying of the residue. TDF content was determined gravimetrically by subtracting the residual protein and ash from the final dried mass. Total phenolic content (TPC) was determined using a method described by Kupina et al. (2018), with some modifications. 1 g of the bread sample (3 replications each) was used in 5 mL of 70% ethanol for TPC extraction using ultrasound-assisted extraction for 60 minutes at 40 kHz, and after that, centrifugation and ultracentrifugation were performed at 6000 rpm for 15 minutes and 15000 rpm for 5 minutes, respectively. Only 50 µl of sample or standard was used in a 24-well plate. Absorbance was measured at 765 nm after 1 hour at 37°C.
The microbial safety of CSS flour and breads was assessed by using plate count agar (PCA) and dichloran rose bengal chloramphenicol (DRBC) for the enumeration of total aerobic mesophilic bacteria and yeast and mold detection, respectively. 10g of sample from each bread was aseptically weighed and homogenized in 90 ml of Ringer solution using a stomacher. PCA plates were incubated at 30°C±1 and DRBC plates were incubated at 28°C±1 for 72 hours.

3. Results and Discussion

3.1 Determination of total dietary fiber

CSS is recognized for its high dietary fiber content, especially insoluble fiber. The TDF content of bread samples showed a marked increase with the addition of CSS, as shown in Figure 3.1. The TDF of bread samples increased

from 2.70% in the control to 4.30% in 5% CSS bread and 5.26% in 8% CSS bread. This significant improvement in TDF is in agreement with findings from Rizkaprilisa et al. (2023), who reported that CSS contains 60% TDF, making it a sustainable source for fiber enrichment in food systems. The incorporation of CSS thus enhances the nutritional value of bread, particularly in terms of fiber-related benefits, such as improved digestion and potential glycemic regulation.

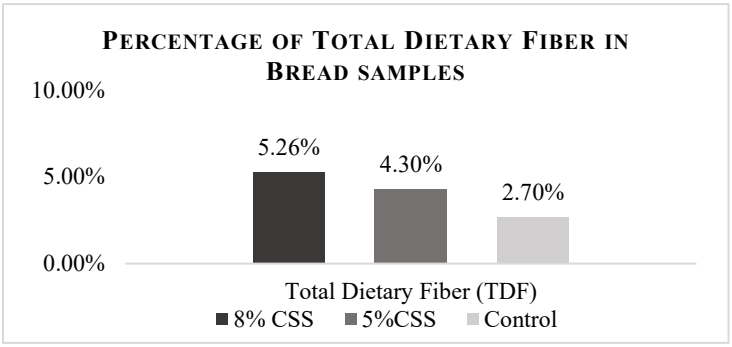


Figure 3.1 *TDF increases in bread samples due to the addition of CSS flour*

3.2 Determination of total phenolic content

TPC of bread also increased due to the addition of CSS, represented in Figure 3.2. The bread fortified with 8% CSS exhibited the highest TPC (0.845mg GAE/g), followed by 5% CSS (0.805 mg GAE/g), whereas the control showed the lowest TPC (0.505mg GAE/g). This increment in TPC is consistent with the known composition of CSS, which contains various bioactive phenolic compounds such as chlorogenic acid, caffeic acid, and other antioxidants retained during the roasting process. According to Iriondo-DeHond et al. (2019), CSS exhibits considerable antioxidant activity and is a promising functional ingredient due to its high content of phenolics. The increased phenolic content in CSS-enriched bread enhances its antioxidant potential, which may play a role in mitigating oxidative stress.

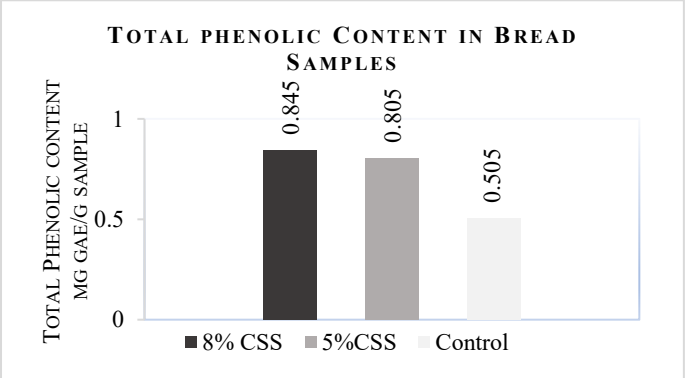


Figure 3.2 *TPC (mg GAE/g sample) increases in bread samples due to the incorporation of CSS flour*

3.3. Microbiological Analysis

PCA and DRBC media were used for total aerobic bacteria and yeast, and mold count, respectively. Some colonies of PCA were observed in CSS flour treated at 80°C in a vacuum oven, but these colonies were within the acceptable limit. There were no yeast and mold counts in CSS flour. No microbial count was observed in any of the baked bread samples.

In conclusion, we have analyzed the total dietary fiber (TDF), total phenolic content (TPC), and microbial load of the breads developed with CSS and the raw CSS flour. The results regarding the phenolic composition and amino acid profiles of the breads are currently being processed.

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Effect of closure system on the evolution of Pinot gris and Pinot noir wines (P-II-41)

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The overarching aim of this PhD thesis project is to identify the characteristics of cork and understand the underlying mechanisms that influence the evolution of bottled wines, both from a positive and negative point of view. A part of this research is focused on the comparison, in terms of long-term performance, of cork stoppers with alternative closure systems, such as screw caps or glass stoppers. A white wine and a red wine bottled with five different types of closure were assessed, considering the evolution of their analytical and sensory profile during bottle ageing.

Effetto del sistema di chiusura sull'evoluzione di vini Pinot grigio e Pinot nero

L'obiettivo generale di questo progetto di tesi di dottorato consiste nell'individuare le caratteristiche del sughero e comprendere i meccanismi che condizionano l'evoluzione dei vini in bottiglia, sia da un punto di vista positivo che negativo. Una parte di questa ricerca è focalizzata sulla comparazione, in termini di performance a lungo termine, dei tappi in sughero con sistemi di chiusura alternativi, come i tappi a vite o in vetro. Sono stati presi in esame un vino bianco e un vino rosso, imbottigliati utilizzando cinque diverse tipologie di chiusura, considerando l'evoluzione del loro profilo analitico e sensoriale nel corso dell'invecchiamento in bottiglia.

Keywords: Wine evolution, white wine, red wine, cork stoppers, alternative closures, bottle ageing.

1. Introduction

Several studies have shown that the features of the closure system that is chosen to seal a wine bottle play a non-negligible role in influencing the post-bottling ageing process and wine quality evolution over time, preventing the occurrence of organoleptic deviations during the expected shelf-life of a particular wine style (Echave *et al*., 2021). This poster reports the main results concerning the comparative assessment of the potential impact of five different types of closures – including conventional cork stoppers as well as alternative materials – on the evolution of the analytical composition and sensory profile of both white and red wine up to 18 months post-bottling.

2. Materials and Methods

A white (Pinot gris) and a red wine (Pinot noir), bottled with five different closure types (two microagglomerate corks, natural cork, screw cap and glass stopper), were supplied by a local winery in Northeast Italy. Both wines were sampled at 4, 12 and 18 months post-bottling, considering several analytical parameters. Free and total sulphur dioxide content, wine chromatic characteristics (CIELab coordinates, Abs 420 nm, 520 nm, 620 nm, color intensity and hue), total polyphenol index (Abs 280 nm), flavanols and total tannins (by spectrophotometry), reduced glutathione and monomeric anthocyanins (by RP-HPLC) were evaluated as described by Voce *et al*. (2024) and Comuzzo *et al*. (2020). Ascorbic acid and acetaldehyde were determined enzymatically with a BioSystems Y15 multiparametric analyzer (BioSystems S.A., Barcelona, Spain). The volatile profile of the wines was analyzed by HS-SPME/GC-MS, considering both the aroma compounds (Voce *et al*., 2024) and carbonyl compounds (Moreira *et al*., 2019). The wine samples were also analyzed by cyclic voltammetry using disposable screen-printed carbon electrodes (DRP-110, Metrohm DropSens, Oviedo, Spain). Sensory analysis of the wines was performed at 12 months post-bottling. Statistical analysis was carried out using the software Statistica for Windows Version 8.0 (StatSoft, Tulsa, OK, USA).

3. Results and Discussion

3.1 White wine evolution

The results of the Principal Component Analysis (PCA) considering the main chromatic and enological parameters as well as the volatile carbonyl compounds detected in the Pinot gris samples are shown in Figure 1. While no substantial differences could be noticed between 4 and 12 months post-bottling, it is possible to observe that, at 18 months of storage, all the wine samples – regardless of the closure system used – were generally characterized by an increasingly deeper yellow color (higher Abs 420 nm and b^*), decreasing levels of free and total SO_2 and increasing concentrations of volatile aldehydes and ketones, which are regarded as oxidation markers.

As for the aroma profile of the wines (data not shown), acetate esters generally decreased, while ageing esters increased with storage time. In terms of semi-quantitative concentrations, ethyl esters were found to increase over time, most notably from 4 to 12 months, with a slight decrease at 18 months of bottle ageing. At this sampling point, the wine samples bottled with natural cork were generally characterized by higher mean concentrations of ethyl esters (in particular ethyl hexanoate, octanoate and decanoate), compared to the other closures.

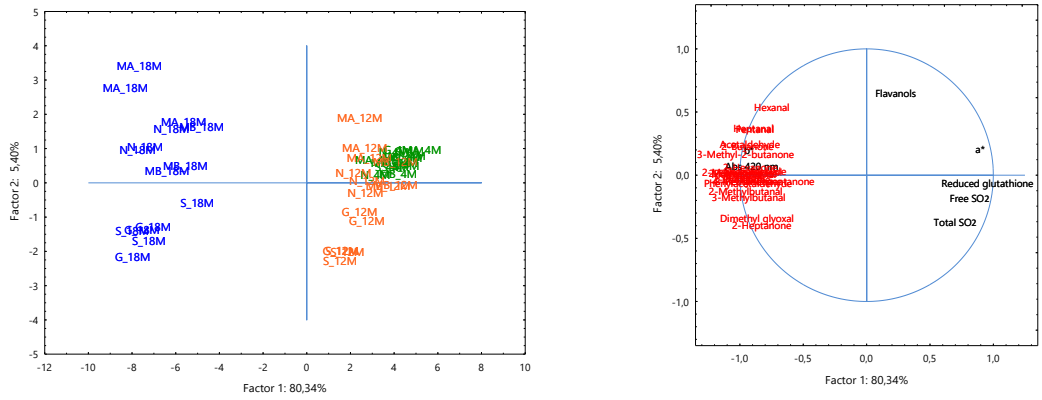


Figure 1 *PCA results considering the main chromatic and enological parameters (black) and volatile carbonyl compounds (red) detected in the Pinot gris samples at 4 (green), 12 (orange) and 18 (blue) months post-bottling. MA: microagglomerate cork A; MB: microagglomerate cork B; N: natural cork; S: screw cap; G: glass stopper.*

However, at the present moment, the effect of the passing of time was found to be apparently stronger than that of the different closures themselves in determining considerable changes in terms of white wine evolution, especially taking into account the fact that the free SO_2 level in the white wines was still above the 10 mg/L threshold, which is usually considered the minimum concentration of this additive required to protect wine against oxidation (Liu *et al*., 2015).

3.2 Red wine evolution

The results of the PCA considering the main chemical parameters, monomeric anthocyanins and volatile carbonyl compounds detected in the Pinot noir samples are depicted in Figure 2. The red wine samples are clearly separated according to ageing time, with little effect of the closure system used. In addition to a decrease in free/total SO_2 and a general increase in carbonyl compounds, a sharp decline in monomeric anthocyanin content can be noticed as the storage time progresses. In particular, vitisins, which contribute to the red color (Comuzzo *et al*., 2020), slightly increased in concentration from 4 to 12 months of storage, but afterwards tended to decrease over time, especially vitisin A. This trend could be also linked to the observed changes in wine color during ageing, including a shift towards orange-brownish hues.

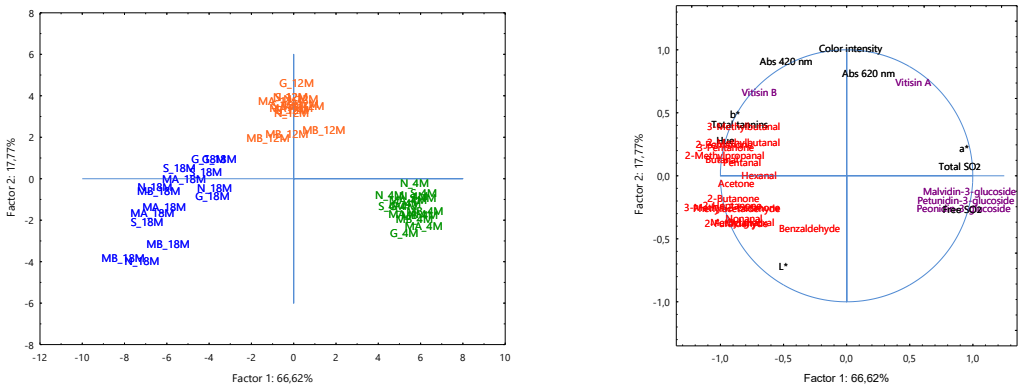


Figure 2 *PCA results considering the main chromatic and enological parameters (black), monomeric anthocyanins (purple) and volatile carbonyl compounds (red) detected in the Pinot noir samples at 4 (green), 12 (orange) and 18 (blue) months post-bottling. MA: microagglomerate cork A; MB: microagglomerate cork B; N: natural cork; S: screw cap; G: glass stopper.*

This part of the PhD research project is still on-going. Therefore, the next sampling points will likely shed further light on the potentially broader effects associated with the overall performance of the closure systems investigated and the resulting differences in terms of wine evolution that might progressively arise during prolonged storage periods.

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Development of a Synthetic Microbial Community for Producing A Sardinian Functional Acid-Alcoholic Dairy Beverage (P-II-42)

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This project develops a multi-kingdom microbial starter for an acid-alcoholic dairy functional beverage. From 10 sheep's milk samples, 300 isolates were obtained and 100 strains were identificated (43 yeasts, 57 bacteria). Yeasts were identified by ITS sequencing and selected based on safety (QPS/GRAS). Of 8 strains, 4 showed proteolytic activity, 3 produced biogenic amines. *P. kudriavzevii* and one strain of *C. jadinii* grew at 45 °C. *P. fermentans*, *K. marxianus*, and *D. hansenii* efficiently metabolized lactose. Selected strains will undergo further technological/safety characterization and co-fermentation trials with a commercial bacterial starter.

Sviluppo di una comunità microbica sintetica per la produzione di una bevanda lattiero-casearia acido-alcolica funzionale sarda

Il progetto mira a sviluppare uno starter microbico multi-regno per una bevanda lattiero-casearia funzionale acido-alcolica. Da 10 campioni di latte ovino crudo sono stati isolati 300 ceppi e identificati 100 (43 lieviti, 57 batteri). I lieviti, identificati mediante sequenziamento ITS, sono stati selezionati in base alla sicurezza (QPS/GRAS). Degli 8 ceppi scelti, 4 hanno mostrato attività proteolitica, 3 produzione di ammine biogene. *P. kudriavzevii* e un ceppo di *C. jadinii* sono cresciuti a 45 °C. *P. fermentans*, *K. marxianus* e *D. hansenii* metabolizzano bene il lattosio. Seguiranno prove di co-fermentazione con uno starter batterico commerciale.

Key words: yeasts, dairy beverage, characterization, safety assessment, multi-kingdom microbial interactions.

1. Introduction

In accordance with the PhD thesis project previously described, this poster reports the main results of the first two activities concerning:

(A2-A3-A4) sampling of raw sheep's and goat's milk, followed by the isolation and identification of lactic acid bacteria and yeasts using classical and molecular techniques (sequencing of 16S and ITS regions; RAPD/Rep-PCR), and subsequent inclusion of the isolated strains in the microbial culture collection of the University of Sassari (MBDS-UNISSCC);

(A5-A6) Safety assessment of the isolated strains, focusing on the production of harmful metabolites such as biogenic amines and antibiotic resistance (ongoing). Technological characterization includes evaluating fermentation efficiency under different temperatures and assessing enzymatic activities, such as protease and lipase production and the ability to ferment lactose.

2. Materials and Methods

A total of 300 colonies, isolated from 10 samples of raw milk obtained from traditional, small-scale sheep farms in Sardinia, were selected based on their distinct morphology on Wallerstein Laboratory Nutrient Medium agar (WLN, VWR Chemicals, Leuven, Belgium), YPG agar (1% yeast extract, 2% peptone, 2% galactose, 2% agar; all VWR Chemicals, Leuven, Belgium),YPL agar (1% yeast extract, 2% peptone, 2% lactose, 2% agar; all VWR Chemicals, Leuven, Belgium), and M17 agar (Merck, Darmstadt, Germany). WL, YPG and YPL agar were supplemented with 0.001% chloramphenicol to avoid/limit bacterial growth. Genomic DNA was extracted from the 300 colonies using the phenol-chloroform method. The extracted DNA was subjected to molecular fingerprinting to detect strain-level similarities using RAPD-PCR with the M13 primer. The resulting banding patterns on 1.5% agarose gels were analyzed using GelJ software (Heras *et al.*, 2015), and isolates with more than 90% similarity were considered as replicates of the same strain. Taxonomic identification of yeast strains was performed *via* PCR amplification and sequencing of the ITS rDNA region. The identified yeast strains were preserved in cryotubes at –80 °C in YPD medium with 40% glycerol and deposited in the microbial culture collection of the University of Sassari (MBDS-UNISSCC). The food safety potential of 8 selected yeast strains was assessed through evaluation of biogenic amine production on YPD agar supplemented with 1% (w/v) of the amino acid under investigation and 0.006% (w/v) bromocresol purple (Sigma-Aldrich). Amino acids tested included L-ornithine, L-lysine, L-histidine, and L-arginine (Merck, Panreac, Sigma-Aldrich). After pH adjustment to 6.0, plates were inoculated and incubated at 25 °C for 7–14 days. Medium color changes indicated activity: yellow for acidification, and purple around colonies for decarboxylase activity. Proteolytic activity was evaluated as described by López-Enríquez *et al.* (2022). Briefly, single colonies were spread on plates containing 1% (w/v) yeast extract (Biolife), 2% (w/v) peptone, 2% (w/v) dextrose (VWR), 2% (w/v) skim milk powder (Sigma-Aldrich), and 2% (w/v) agar, and incubated at 25 °C for 5–7 days. Proteolytic halos were highlighted by flooding

plates with 5 mL of staining solution containing 0.25 g Coomassie Brilliant Blue R-250, 400 mL water, 500 mL methanol, and 100 mL acetic acid. Lipolytic activity was qualitatively assessed using tributyrin agar plates (peptone 5 g/L, yeast extract 3 g/L, tributyrin 10 mL/L). After inoculation with 0.1 mL of yeast suspension, plates were incubated at 25–30 °C for 48–72 h. Clear halos around the colonies indicated tributyrin hydrolysis. Thermotolerance was assessed by culturing the strains at 30 °C, 37 °C, 42 °C, and 45 °C in YPL broth (1% yeast extract, 2% peptone, 2% lactose). Cultures were inoculated at a standardized OD600 of 0.1 and incubated for 48 h. Yeast growth and viability was evaluated by serial dilution and plating on YPL agar plates. Finally, growth kinetics on lactose were assessed by spectrophotometric assays in a Corning™ Costar™ 96-well plate. Each well was inoculated with 0.1 mL of a 24 h culture in YPL broth (OD 600 of 0.05) and incubated at 25°C for 72h. Growth was monitored every hour using a Multiskan GO plate reader.

3. Results and Discussion

3.1 Isolation and Identification

Molecular analyses of 300 isolates obtained from 10 samples of sheep's raw milk resulted in the identification of 100 main clusters (>90% similarity), indicating the presence of 100 unique strains, among which 43 yeasts and 57 bacteria. Subsequent molecular identification of the yeast isolates resulted in the identification of 14 dominant yeast species: *Candida parapsilosis, Wickerhamiella pararugosa, Magnusiomyces capitatus, Apiotrichum domesticum, Geotrichum silvicola, Galactomyces pseudocandidus, Cystobasidium slooffiae, Cutaneotrichosporon curvatum, Cyberlindnera jadinii, Cutaneotrichosporon cutaneum, Pichia fermentans, Pichia kudriavzevii, Debaryomyces hansenii,* and *Kluyveromyces marxianus*. Among these, 8 yeast strains (*Pichia fermentans, Pichia kudriavzevii, Cyberlindnera jadinii* (2 strains), *Debaryomyces hansenii* (3 strains), *Kluyveromyces marxianus*) were selected based on literature information regarding the pathogenicity, QPS or GRAS status of their reference strains.

3.2 Determination of technological properties and Lactose Fermentation Assay

Clear halos were observed on the tested substrates, indicating the presence of detectable proteolytic activity in four strains: UNISSCC Y94 *(K. marxianus),* UNISSCC Y46 *(D. hansenii),* UNISSCC Y 23 *(D. hansenii)* and UNISSCC Y24 *(D. hansenii)*. Morever, the same last three strains showed a purple color shift in the bromocresol purple-containing medium, suggesting the presence of biogenic amine production. None of the strains exhibited lipolytic activity compared to the control. Regarding the ability of the selected strains to grow at different temperatures, strain UNISSCC Y6*(C. jadinii)* showed good growth at all four tested temperatures. Strains UNISSCC Y10 *(C. jadinii),* UNISSCC Y6 *(C. jadinii)* and UNISSCC Y94 *(K. marxianus)* exhibited good growth up to 42 °C. In contrast, strains UNISSCC Y46 *(D. hansenii),* UNISSCC Y23 *(D. hansenii)* and UNISSCC Y24 (*D. hansenii*) showed growth only at 30 °C (Table 1).

Table 1. *Yeast growth at different temperatures.*

Sample ID (Species)	30°C	37°C	42°C	45°C
UNISSCC Y34 *(P. fermentans)*	+	+	-	-
UNISSCC Y10*(C. jadinii)*	+	+	+	-
UNISSCC Y5*(P. kudriavzevii)*	+	+	*	+
UNISSCC Y6*(C. jadinii)*	+	+	+	+
UNISSCC Y46 *(D. hansenii)*	+	-	-	-
UNISSCC Y 23 *(D. hansenii)*	+	-	-	-
UNISSCC Y24 *(D. hansenii)*	+	-	-	-
UNISSCC Y94 *(K. marxianus)*	+	+	+	-

3.3 Lactose Fermentation Assay

The lactose fermentation assay revealed that strains UNISSCC Y34 (*P. fermentans*) and UNISSCC Y94 (*K. marxianus*) exhibited a strong ability to ferment lactose. In contrast, the remaining strains showed poor or no lactose fermentation capacity under the tested conditions. These results highlight the potential of UNISSCC Y34 and UNISSCC Y94 for applications involving lactose-rich substrates, such as dairy fermentation processes.

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Development of biological and monitoring tools for improving energy efficiency and sustainability in winemaking (P-II-43)

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The PhD project focuses on the setting up of tools for improving the sustainability of the wine-making process. The first activity involves the selection of *Saccharomyces cerevisiae* strains capable of maintaining good fermentative performance even at non-optimal temperatures for this yeast. The aim is to reduce energy consumption associated with strict temperature thermal control during fermentation, thus contributing to energy savings in the winemaking process.

Sviluppo di strumenti biologici e di monitoraggio per migliorare l'efficienza energetica e la sostenibilità della vinificazione

Il progetto di dottorato è indirizzato alla messa a punto di strumenti utili ad incrementare la sostenibilità del processo di vinificazione. La prima attività prevede la selezione di ceppi di *Saccharomyces cerevisiae* capaci di mantenere buone performance fermentative anche a temperature non ottimali per questo lievito. L'obiettivo è quello di ridurre il consumo energetico associato allo stretto controllo termico durante la fermentazione, contribuendo così al risparmio energetico nel processo di vinificazione.

Key words: sustainability, alcoholic fermentation, temperature, energy saving.

1. Introduction

In accordance with the PhD thesis project previously described (La Rocca, 2024), this poster reports the main results of the first two activities concerning:

1a) Growth tests at different temperatures in order to evaluate the strain ability to develop at higher and lower temperatures than optimal value (26°C).
1b) Laboratory-scale fermentations at different temperatures with yeast strains selected in the first step.

2. Materials and Methods

In the preliminary phase of the project, indigenous *S. cerevisiae* strains, previously isolated from various sources, including spontaneously fermented grapes, fruits, winery environments, and equipment, were tested. The yeast strains were cultivated in 96-well microtiter plates containing YPD medium (1% yeast extract, 2% peptone, 2% glucose). Each well was inoculated with a standardized cell suspension at a concentration of 1×10^7 cells/mL. Uninoculated medium was used as a negative control. The plates were incubated at 15°C, 20°C, 25°C, and 30°C for 24–48 hours. Yeast growth was assessed spectrophotometrically by measuring optical density (OD) at 600 nm. For each strain, the growth level was evaluated as ratio between OD values at each temperature and OD at 25°C. On the basis of these results, strains growing better at low and high temperatures were selected for fermentations performed at low and high temperatures, respectively. Fermentations were carried out by inoculating 100 mL of grape juice—previously heat-treated to inactivate the native microflora—with each yeast strain. The fermentations were conducted at 15°C, 20°C, 25°C, and 30°C. Fermentation progress was monitored by measuring: **weight loss**, due to CO_2 production, **decrease in °Brix**, detected by refractometer, and **sugar and ethanol content**, determined using a Fourier-transform infrared (FTIR) WineScan instrument (OenoFoss). At the end of fermentation, the experimental wines were analyzed for main aromatic compounds, such as acetaldehyde, ethyl acetate, acetoin, and higher alcohols, by gas chromatography following the method described by Capece et al. (2013).

3. Results and Discussion

3.1 Growth tests at different temperatures

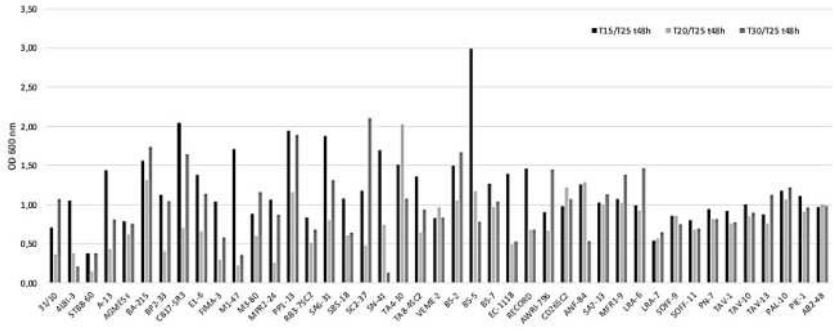
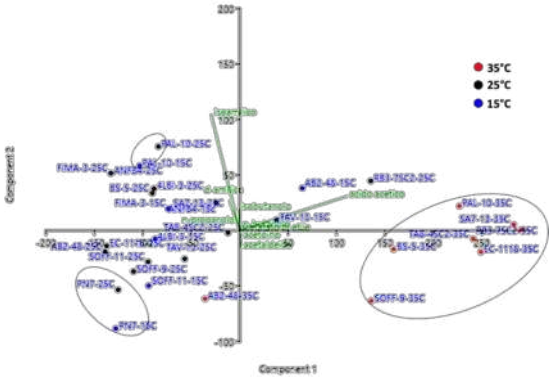


Figure 1 *Growth (OD 600) after 48 h under three thermal conditions (T15/T25, T20/T25, T30/T25)*

This figure 1 shows the growth performance of *Saccharomyces cerevisiae* strains under different temperature conditions, evaluated after 48 hours of incubation. There is a wide variability in OD600 values between the various strains/conditions. Some strains show very low values (<0.5), while others exceed 2.0, indicating substantial differences in growth. In many cases, the T30/T25 treatment shows higher growth than the other two conditions, suggesting that an increase in temperature from 25°C to 30°C may stimulate growth in different strains. However, in some cases, the highest growth level was observed at T15/T25, suggesting that some strains may prefer lower temperatures. Some strains show little or no significant variation between conditions, indicating a wide temperature tolerance or adaptation. Sample BS-5 shows a very high T30/T25 ratio (~3.0), much higher than the others, indicating a very high growth level at 30°C.



3.2 Laboratory-scale fermentations with the selected yeast strains.

The selected yeast strains were tested in fermentations performed in 100 ml of Aglianico del Vulture must at different temperatures (15-20°C, 30-35°C), compared to the control temperature (25°C). The Principal Component Analysis (PCA) of data related to the content of main secondary compounds detected in the experimental wines (Figure 2) shows that temperature has a significant effect on the production of aromatic compounds, although this effect also depends on the strain. For someones, such as PN7 and PAL-10, no significant differences were found between the aromatic profile obtained at 15 and 25°C. Samples obtained at 35°C clustered separately, suggesting a significant variation in volatile composition compared to those fermented at lower temperatures.

Figure 2 *Principal component analysis (PCA) of the main aromatic compounds detected in wines fermented at different temperatures (15°C in blue, 25°C in black and 35°C in red)*

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Modelling of time and temperature effects for the identification of ageing trends in volatiles composition of Malvasia di Candia Aromatica wines (P-II-44)

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This study examined aroma evolution in Malvasia di Candia Aromatica wines produced from vines of differing vigour and stored at various temperatures for one year. GC×GC-qMS analysis identified 112 volatile compounds, with terpenes being more abundant in low-vigour wines. However, storage time and temperature exerted a greater influence on aroma composition than vine vigour, with marked oxidative transformations in terpenes and significant modulation of norisoprenoids. These results confirm known shelf-life dynamics for aromatic wines and highlight the central role of storage conditions over viticultural origin.

Modellazione degli effetti di temperatura e tempo per l'identificazione delle tendenze d'invecchiamento nei composti volatili in vini Malvasia di Candia Aromatica

Questo studio ha analizzato l'evoluzione dell'aroma in vini di Malvasia di Candia Aromatica, prodotti da vigne a diverso vigore e conservati a diverse temperature per un anno. L'analisi GC×GC-qMS ha identificato 112 composti volatili, in particolare terpeni, che sono risultati più abbondanti nei vini da piante a basso vigore. Tuttavia, l'invecchiamento e la temperatura di conservazione hanno mostrato un impatto maggiore sulla composizione aromatica rispetto al vigore, con evidenti trasformazioni ossidative nei terpeni e una significativa modulazione dei norisoprenoidi.

Key words: Shelf-life, wine, aroma compounds, GCxGC-MS, modelling.

1. Introduction

In accordance with the doctoral project, an experimental plan was chosen, the results of which could be modelled in different ways, such as discriminating/classifying and mathematical models. In this poster, the results of the first type of modelling will be shown, while mathematical modelling is still in progress and will not be presented. For this experimental plan wines coming from different vigour plants were stored at different temperatures for different wines, simulating different shelf-life conditions. The wines were produced from Malvasia di Candia Aromatica grapes, a typical variety from Piacenza's province, characterized a huge accumulation of terpenes in free and especially bound forms.

2. Materials and Methods

In this experimental plan, different wines were produced according to their vigour, determined by means of a proxymal sensor (MecsVINE) throughout all the growing season. At harvest time, the grapes were picked using an automatic grape harvester (New Holland Braud) capable of separating the harvest of high (HV) and low vigour (LV) plants. A control (C) was also picked up, without any regard to the vigour. Each thesis was collected in triplicate according to a random blocks design. After the harvest, the grapes were vinified and a total of 9 wines were obtained (3 HV, 3 LV and 3 C). At the end of alcoholic fermentation wines were bottled in dark 0,33 L glass bottles, in absence of oxygen and with crown corks. After that 4 bottles for each wine were put in different storage condition to start the shelf life. The conditions selected for shelf life were:

- Time: 0, 90, 180, 270 and 360 days, describing the usual time of shelf-life of a young aromatic wine.
- Temperature: 5, 15 and 25°C, describing a home fridge, cellar and room temperature storage.

At each time, the wines volatiles were analysed by a GCxGC-qMS system. Briefly 4 ml of diluted wine were poured in a 20 ml HS glass vial. The 2D-GC system (Nexis GC-2030 GC×GC, Shimadzu) consisted of a polar (SLB-5ms)-apolar (SupelcoWax) columns setup. The temperature ramp ranged from 40 to 220°C and the physical flux modulator operated at 20°C. The MS detector (TQ8040 NX, Shimadzu) operated at 250°C and the m/z range was 41-360 amu with a scanning speed of 20,000 amu/s.
The obtained chromatograms were analysed by ChromSquare 2.1 (Chromaleont s.r.l.) and the identification of the compounds was performed by comparison against pure standard or by comparison on NIST20 and Adam's Library. Only compounds with similarity>80% and ΔLRI<10 were putatively identified. All the compounds were semi-quantified against an internal standard (2-Octanol).
The data harvesting was performed by Excel (Microsoft), while data normalization and handling was performed on Metaboanalyst.ca 6.0 and R-Studio.

3. Results and Discussion

3.1 Screening of volatiles

A total of 112 volatile compounds were identified and semi-quantified, comprising 63 terpenes, 5 norisoprenoids, 34 esters, and 5 higher alcohols. At the initial time point (T0), only minor differences were observed in the concentrations of esters; however, significant differences were detected among terpenes. Specifically, wines derived from low-vigor vines exhibited notably higher concentrations of terpenes compared to those from high-vigor vines. Among the terpenes, linalool and L-α-terpineol were the most abundant and thus the most relevant in terms of concentration. In contrast, no statistically significant differences were found in the levels of norisoprenoids across the samples.

3.2 Modelization of Temperature and time effect

The effects of storage time and temperature were investigated using a Partial Least Squares Discriminant Analysis (PLS-DA) model, which incorporated Variable Importance in Projection (VIP) scores coupled with Fold Change (FC) analysis to clarify trends in volatile compound dynamics. To assess the influence of these two factors, the complete dataset was analyzed through the PLS-DA model, and the behavior of VIP-identified compounds was subsequently examined using FC calculations.

Regarding storage time, the compounds most representative of temporal changes were primarily terpenes, which exhibited a general trend of down-accumulation over time. Notable examples include trans-Geranylacetone, trans-Nerolidol, α-Pinene, trans-2-Pinanol, and Linalool, all showing significant decreases in concentration.

In contrast, the effect of storage temperature highlighted more heterogeneous results, involving both terpenes and esters, and showing instances of both up- and down-regulation. Specifically, a reduction was observed in key terpenes that initially characterized the T0 samples, such as Linalool and Geraniol. At the same time, there was an increase in oxidized derivatives of these compounds, including Linalool 3,7-oxide and Nerol oxide, indicating possible oxidative transformations induced by elevated temperatures. Additionally, significant up-regulation was detected for TDN and TPB, both of these norisoprenoids are known to contribute to aged wine aroma, due to their slow degradation from carotenoids.

These preliminary findings support the expected variation in aroma composition associated with wines at different times and temperature of storage. As expected, terpenes played a central role in differentiating samples. Overall, the observed changes in aroma compounds under varying storage conditions align well with patterns previously reported in the literature, particularly those related to long-term storage at higher temperatures. In conclusion, but unexpectedly, the effect of the vigor showed to be less relevant respect to the storage conditions along the shelf life.

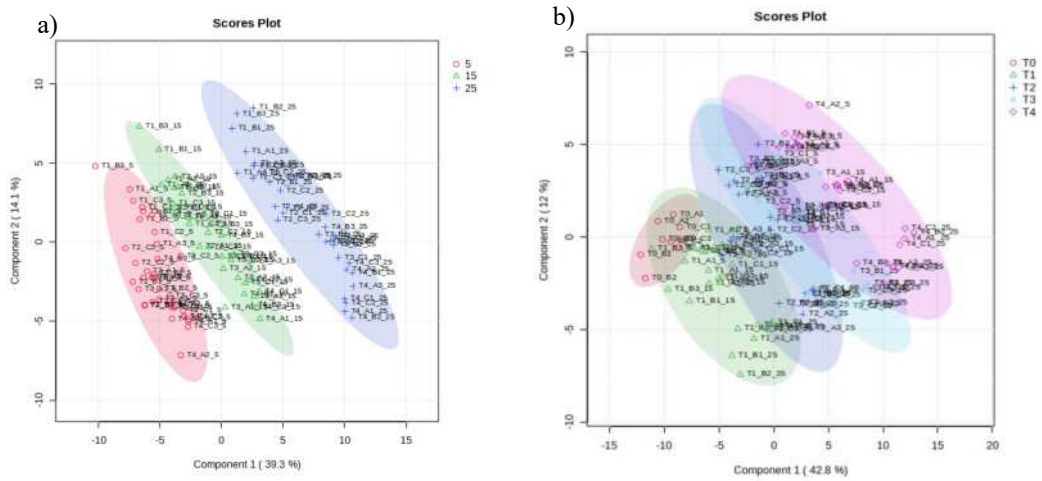


Figure 6 *PLS-DA graphical output for* ***(a)*** *temperature (T0 samples not included) and* ***(b)****time.*

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High Value Food Products from Microalgae (P-II-45)

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The paper reports the results obtained during the second year of the PhD research project, regarding the extraction and characterization of the proteins from *Arthrospira platensis* (Spirulina). Two extraction methods were employed: an alkaline treatment (CH) and a non-thermal mechanical method based on high-pressure homogenisation (HPH), applied at different pressure levels (200, 500, 800 and 1000 bar). The obtained protein extracts were compared in terms of solubility, foaming and emulsifying properties, water- and oil- holding capacities as well as viscoelastic behaviour assessed through rheological analysis.

Prodotti alimentari ad alto valore dalle microalghe

Il presente lavoro riporta i risultati ottenuti nel secondo anno del progetto di ricerca di dottorato inerente all'estrazione e la caratterizzazione delle proprietà funzionali e tecnologiche delle proteine da *Arthrospira platensis* (Spirulina). Le proteine sono state estratte mediante due metodi differenti: estrazione in ambiente alcalino (CH) e metodo meccanico non termico basato sull'omogenizzazione ad alta pressione (HPH), con diversi livelli di pressione (200,500,800 e 1000 bar). Gli estratti proteici sono stati confrontati in termini di solubilità, capacità schiumogena ed emulsionante, capacità di trattenere acqua ed olio e comportamento viscoelastico tramite analisi reologica.

Key words: Spirulina, alternative proteins, HPH, techno-functional properties, rheology, sustainability

1. Introduction

In response to the growing demand for sustainable protein ingredients, the study aimed to optimize an extraction method by investigating HPH as a green alternative to conventional alkaline methods. The two methods were compared in terms of protein yield and techno-functional properties of the resulting protein fractions. Spirulina is particularly rich in proteins, among which phycobiliproteins, especially C-phycocyanin (C-PC), being the major components. These water-soluble pigment-protein complexes are known for their antioxidant and health-promoting effects. C-PC remains soluble at neutral pH due to its isoelectric point facilitating its recovery in the soluble phase (Zhang et al., 2023).

2. Materials and Methods

Arthrospira platensis (Spirulina) biomass (SB) was supplied by (Sevenhills Wholefoods, UK). Protein extraction from SB was carried out by alkaline (pH 10) and HPH using PANDA Plus 2000 (GEA Niro Soavi, IT) according to modified methods of Shkolnikov Lozober et al. (2021) and Magpusao et al. (2021), respectively. Both methods were applied to destroy the Spirulina cell wall, and the protein extracts were recovered by isoelectric precipitation (pH 3). After the isoelectric precipitation, the supernatant (-S) and precipitate (-P) were freeze dried to obtain two different protein extracts. Protein content was determined using the Kjeldahl method (NTP factor 6.25). Protein yield was calculated as the percentage of protein recovered in each fraction relative to the protein content in SB. The pellets were characterized by soluble protein content using the Bradford Assay (Zhang et al., 2023), Water- and oil-holding capacities (WHC and OHC), foaming capacity and stability (FC and FS), emulsifying activity and stability (EAI and ESI) according to Purdi et al. (2023) at pH 7. The elastic (G') and viscous (G'') moduli were determined by frequency sweep using a rheometer (MCR 102e, Anton Paar GmbH, IT) at a T=20°C, with an angular frequency ω ranging from 0.1 to 100 rad/s (Shkolnikov Lozober et al., 2021). The supernatants were characterized by their protein soluble content beside their phycobiliprotein and C-PC content, as well as extract purity (EP). Data were statistically analysed by one-way ANOVA and Tukey's test ($p < 0.05$).

3. Results and Discussion

3.1 Effect of extraction method on Spirulina protein recovery

Comparing the two extraction methods, all HPH precipitates showed a significantly higher protein yield (55% at 200 bar, 68% at 500 bar, 60% at 800 bar, and 57% at 1000 bar) compared to CH (30%). In contrast, the CH-P had the highest protein content (77.32%), while HPH-P showed decreasing value as pressure increased, from 69% at 500 bar to 49% at 1000 bar. Thus, based on the combined evaluation of protein yield and content, 500 bar was identified as the optimal condition for protein recovery in the precipitate fraction. At higher pressures, both parameters decreased, likely due to increased solubilization of proteins into the supernatants. This finding aligns with the increasing protein yield in the corresponding supernatant fractions (data not shown), which reached a maximum at 1000 bar.

3.2 Techno-functional and rheological characterization of Spirulina protein extracts

The technological properties of the pellet extracts are reported in Table 1. H500-P led to the highest solubility, FC, FS, EAI and ESI, suggesting that moderate pressure enhances protein surface functionality by promoting partial unfolding without aggregation (Ma et al., 2023). In contrast, all these properties decreased at 800 and 1000 bar, probably due to protein denaturation and aggregation. WHC and OHC followed a similar trend, reaching their highest value at 500 bar and then decreasing, suggesting that excessive pressure reduces the proteins' capacity to retain water and oil. HPH supernatants showed significantly higher phycobiliproteins (PBP), C-PC and EP than the CH-S, with the highest values observed at 1000 bar (Table 2). However, solubility reached a maximum at 800 bar and decreased at 1000 bar, probably due to protein aggregation.

Table 1. *Solubility, foaming capacity (FC) and stability (FS), emulsifying activity (EAI) and stability index (ESI), water-and oil-holding capacities (WHC and OHC) of pellet fraction of SPE. All results are expressed relative to protein content.*

Precipitate	Solubility	Foaming		Emulsion		WHC	OHC
		FC	FS	EAI	ESI		
	$g_{PSol}/100g_{Ptot}$	%	%	m^2/g_{Ptot}	min	g_{H2O}/g_{Ptot}	g_{oil}/g_{Ptot}
CH-P	42.88±0.25^{C}	92.5±3.5^{C}	87.5±3.5^{C}	4.39±0.19^{D}	113.49±15.44^{C}	5.78±0.04^{A}	1.16±0.03^{D}
H200-P	39.05±0.39^{D}	107.5±3.5^{B}	102.5±3.5^{B}	25.41±0.45^{C}	205.06±13.75^{A}	4.80±0.06^{B}	1.95±0.05^{C}
H500-P	50.74±0.95^{A}	127.5±3.5^{A}	122.5±3.5^{A}	34.47±0.34^{A}	183.84±6.35^{B}	4.68±0.05^{B}	2.14±0.07^{BC}
H800-P	47.73±0.60^{B}	90.0±0.0^{CD}	82.5±3.5^{CD}	29.87±0.25^{B}	154.06±2.94^{B}	3.39±0.09^{C}	2.44±0.15^{B}
H1000-P	31.60±0.54^{E}	79.5±0.7^{D}	72.5±3.5^{D}	28.41±0.89^{BC}	142.09±3.37^{B}	3.60±0.20^{C}	2.75±0.06^{A}

Values are expressed as means ± SD. Different superscript letters in each column indicate significant differences ($p < 0.05$).

Table 2. *Solubility, phycobiliproteins (PBP), C-phycocyanin (C-PC), and extract purity (EP) of supernatant fraction of SPE. All results are expressed relative to protein content.*

Supernatant	Solubility	PBP	C-PC	EP
	$g_{PSol}/100g_{Ptot}$	mg_{PBP}/g_{Ptot}	$mg_{C\text{-}PC}/g_{Ptot}$	-
CH-S	6.67±0.09^{D}	33.65±0.89^{E}	23.42±0.31^{E}	0.27±0.10^{B}
H200-S	13.69±0.13^{B}	26.74±0.02^{D}	26.35±0.01^{D}	0.53±0.00^{B}
H500-S	15.22±0.80^{B}	95.85±0.04^{C}	89.34±0.01^{C}	0.91±0.14^{A}
H800-S	17.10±0.28^{A}	51.59±0.01^{B}	44.84±0.00^{B}	0.96±0.02^{A}
H1000-S	11.85±0.02^{C}	117.82±0.28^{A}	117.54±0.30^{A}	1.04±0.00^{A}

Values are expressed as means ± SD. Different superscript letters in each column indicate significant differences ($p < 0.05$).

As regards the viscoelastic behaviour (Figure 1a-b), all the pellets showed G′ > G″, indicating an elastic-dominant response (Shkolnikov Lozober et al., 2021). CH-P had the highest moduli, which can be attributed to its higher protein content, resulting in a denser network. Increasing HPH pressure led to reduced G′ and G″, reflecting decreased protein content in the pellet, probably due to solubilization into the supernatant. However, at moderate pressure (200 and 500 bar) the pellets retained more protein, favouring the formation of more structured networks.

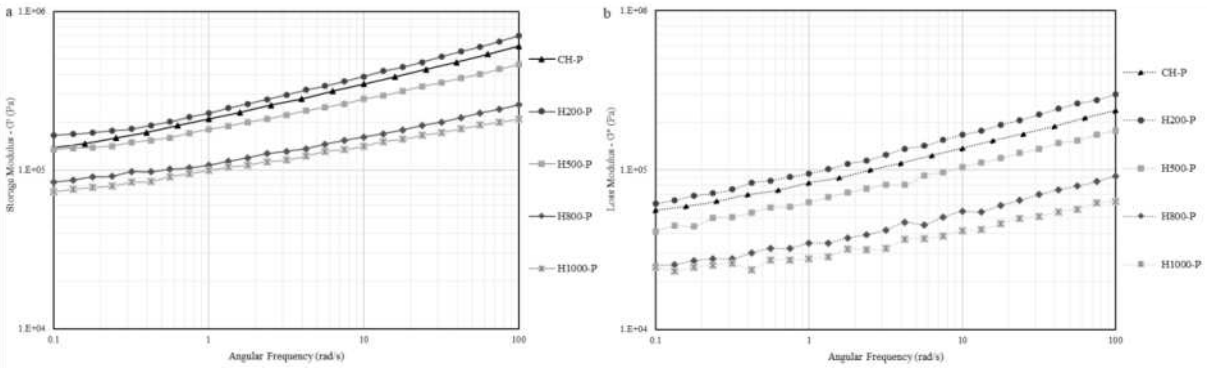


Figure 7. *Frequency sweep of chemical (CH) and HPH precipitates. (a) Storage modulus, G' (b) Loss modulus, G''.*

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Application of high pressure treatment (HPP) to extend shelf life of liquid artisanal ice cream bases (P-II-46)

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Research activities were carried out on samples of artisanal ice cream, with the aim to extend shelf life, maintaining the high-quality characteristics of the product. For this reason, we tested high pressure treatment (HPP) as a pasteurization method at low temperatures, without having, therefore, negative effects on the sensorial and nutritional values of the treated product. Liquid ice cream bases were subjected to HPP treatment, then stored at positive temperature (2-4°C) for different times, then whipped to obtain the defined ice cream product, analysed from microbiological, aromatic-sensory and chemical-physical points of view.

Applicazione di Trattamenti con alte pressioni (HPP) per estendere la shelf life di basi liquide per gelato artigianale

Le attività di ricerca sono state svolte per estendere la shelf life di gelati artigianali, mantenendo le elevate caratteristiche qualitative del prodotto. Per tale motivo, si sono utilizzate le alte pressioni (HPP) per la pastorizzazione a basse temperature, limitando gli effetti negativi sulle caratteristiche sensoriali e nutrizionali del prodotto trattato. Le basi liquide di gelato sono state sottoposte al trattamento HPP, conservate poi a temperatura positiva (2-4°C) per tempi diversi, successivamente mantecate per ottenere il prodotto gelato finito, analizzato sotto il profilo microbiologico, aromatico-sensoriale e chimico-fisico.

Key words: Ice cream, High-Pressure Processing HPP, Shelf life, quality, microbiology, structure.

1. Introduction

In accordance with the PhD thesis project, this poster reports the main results of the first two activities concerning:
(A1) Determination of target products: artisanal ice cream;
(A2) Evaluation of the innovative technological solution appliable: high-pressure processing (HPP);
(A3) Preparation of samples: liquid ice cream bases that after HPP were stored in the fridge and whipped;
(A4) Assessments: 1) DSC, texture, rheology, melting; 2) VOCs, colour, sensory analysis; 3) microbial growth

2. Materials and Methods

Three ice cream flavours with different fruits were chosen: mango, lemon and pistachio produced in an artisan ice cream company (Cappadonia gelati s.r.l., Cerda-Pa). Therefore, the three liquid bases, previously pasteurized as per normal process, were vacuum-packed and divided into bags that would undergo HPP treatment versus control bags that would not undergo any treatment. All bags were stored at a temperature (2–4°C). Hydrostatic HPP treatment was carried out with an AV-50X machine (Avure Technologies, Ohio, USA) with increasing pressure up to 6000 bar held constant for 300 seconds. Following the treatment, one set of ice cream bases (HP-T0 and noHP-T0) was immediately whipped to obtain the finished ice creams and analysed. The other sets of bags (T7, T15 and T30) were kept sealed in cold storage for various storage times (7, 15 and 30 days) until they were to be whipped and then analysed. Coding of samples is based on the flavour (P: pistachio, L: lemon or M: mango), if treated with HPP or not (HP/noHP) and on the period of storage (0, 7, 15 or 30 days) before whipping and analyses. The following analyses were carried out at the end of each storage period and following whipping: overrun, using the weight of the mixture and the final ice cream; melting rate, evaluated with weight loss every 15 minutes for 1 hour at 20°C; texture analysis with a penetration test using a 5mm cylindrical probe; colour measurements using Konika Minolta CR300 spectrophotometer; two sensory tests (discriminant Duo-trio test and Sensory Descriptive Analysis); lastly, evaluation of microbiological growth for yeasts, moulds, pseudomonads, Enterobacteriaceae family, coagulase-positive staphylococci, total coliforms, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp, total psychrophils (TPM) and mesophils microrganisms (TMM), according to the specific guidelines of the ISO standards. Only the microbiological analyses were performed on the liquid bases, following the predetermined cold storage period, to avoid microbiological contamination during whipping operations, up to 1 month of storage. All other analyses were performed on finished ice cream samples following whipping and evaluated up to 15 days of storage.

3. Results and Discussion

3.1 Microbiological Evolution

Microbiologically, the effectiveness of HPP treatment is evident. Of the 11 microbial populations previously mentioned, all showed a significantly lower growth trend in the treated samples (HP) than that shown by the

untreated samples (noHP). The reduction in the microbial load at the different times of analysis results in an average of 3 logarithmic cycles on each of microbial population evaluated. In general, the lemon ice cream samples appear to be those in which microorganisms are most unlikely to develop due to the low pH of the raw material used, which inhibits their growth, but where microbial development is shown, the L-HP-treated sample always shows lower growth values than the L-noHP. As for *Salmonella* spp, *L. monocytogenes*, *S. aureus* and *E. coli* were not found in any sample and at any testing time, thus ensuring their food safety according to EU Regulation (EC) No. 2073/2005. The following Table 1 reports values for microbial counts for pistachio samples as an example of the efficiency of the HPP treatment.

Table 1 *Microbial counts for pistachio ice cream samples (P-noHP and P-HP), assessed after 0, 7, 15 and 30 days of storage at 2-4°C. Values are expressed in Log CFU/mL.*

Microbial population	Samples	Time 0	Time 7	Time 15	Time 30
TMM	P-noHP	3.09 ± 0.3	5.44 ± 0.23	6.12 ± 0.02	8.2 ± 0.03
	P-HP	2.62 ± 0.11	3.59 ± 0.16	3.96 ± 0.14	4.13 ± 0.07
TPM	P-noHP	0 ± 0	2.59 ± 0.16	5.04 ± 0.06	6.91 ± 0.05
	P-HP	0 ± 0	1.5 ± 0.28	2.41 ± 0.17	3.85 ± 0.1
Enterobacteriaceae	P-noHP	0 ± 0	0 ± 0	4.91 ± 0.03	7.03 ± 0.05
	P-HP	0 ± 0	0 ± 0	0 ± 0	2.85 ± 0.21
Total Coliforms	P-noHP	0 ± 0	0 ± 0	4.24 ± 0.09	5.96 ± 0.02
	P-HP	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Pseudomonas	P-noHP	0 ± 0	0 ± 0	5.27 ± 0.05	7.13 ± 0.07
	P-HP	0 ± 0	0 ± 0	0 ± 0	2.91 ± 0.29
Yeasts	P-noHP	0 ± 0	0 ± 0	0 ± 0	3.8 ± 0.28
	P-HP	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Molds	P-noHP	0 ± 0	1.89 ± 0.27	2.85 ± 0.21	3.02 ± 0.32
	P-HP	0 ± 0	0 ± 0	0 ± 0	0 ± 0

3.2 Sensory analysis and Colour measurement.

Differences in colour between control (noHPP) and experimental samples (HPP) were determined as follows:

$$\Delta E = \sqrt{(L_c - L_s)^2 + (a_c - a_s)^2 + (b_c - b_s)^2} \quad (1)$$

Samples with ΔE higher than 3 are considered with difference perceivable by human eyes (Giannoutsos et al., 2023). Based on the results, pistachio samples do not show any colour perceivable difference at any time of assessment, while lemon shows difference at T0 and T15 (ΔE= 3.69 and 4.72, respectively), and mango just at T7 (ΔE= 5.53). The Discriminant Sensory test shows that only pistachio samples are distinguishable due to the high melting rate of the untreated P-noHP compared to the P-HP. This is confirmed by the Descriptive analysis in which the melting rate reported values of 4.4 and 7.4 for P-noHP and P-HP respectively, valuating the P-HP more appreciable. For lemon and mango samples, there is no perceivable distinction between experimental and control.

3.3 Texture Analysis, Overrun and Melting Rate

For pistachio and lemon ice creams, the HP samples show lower rate of melting compared to the noHP samples, showing higher resistance to melt down (Nath et al., 2023). P-HP showed minimal melting just after 30 minutes (4.4g), while P-noHP lost 58.1g. Consistently, P-HP showed higher hardness than P-noHP in texture analysis. This is related to the presence of proteins (caseins and whey proteins of milk) and fats (from milk and pistachio) that can be influenced, with a formation of protein network induced by HPP treatment (Huppertz et al., 2011). Furthermore, after HPP, smaller fat globules are obtained, that are able to interact better within the system and influencing chemical-physical properties (Huppertz et al., 2011). Interesting it is also that, for lemon and mango, the hardness is higher at T0 for noHP samples, but after 7 days this higher hardness of control samples falls, showing the HP more stable during storage. It is evident that for mango and pistachio ice creams the longer is the storage time, the higher is the melting rate, showing that the storage surely influences the capability of the system to retain the water. While for lemon this trend is in reverse, probably due to the different composition. For the same reasons, the overrun results show P-HP has lower air volume compared to the P-noHP at all times of analysis, due the higher viscosity of the mixture during whipping (Nath et al., 2023). Mango and lemon ice cream, at the contrary, show a percentage of overrun higher for HP samples compared to the controls noHP, suggesting that the fibres contained in the mango mixtures are not affected by HP treatment by a rheological point of view.

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Optimization of Instant Fresh Egg Pasta Enhancing Rehydration Efficiency, Structural Integrity, and Nutraceutical Potential (P-II-47)

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The growing demand for convenient and healthy food products has accelerated research into nutraceutical-rich instant pasta. This study focused on developing instant fresh egg pasta, specifically tortellini and quadrucci. Freeze-drying and rehydration parameters were optimized to achieve suitable water activity levels, preserve structural integrity, and maintain sensory quality. Future directions include scaling industrial production of these new products.

"Ottimizzazione della pasta fresca istantanea all'uovo per migliorare l'efficienza di reidratazione, l'integrità strutturale e il potenziale nutraceutico"

La crescente domanda di prodotti alimentari pratici e salutari ha accelerato la ricerca sulla pasta istantanea ricca di nutraceutici. Questa ricerca si è concentrata sullo sviluppo di pasta fresca all'uovo istantanea, in particolare nei formati tortellini e quadrucci. I parametri di liofilizzazione e reidratazione sono stati ottimizzati per garantire livelli ottimali di attività dell'acqua, preservare l'integrità strutturale e mantenere proprietà sensoriali accettabili. Le direzioni future si concentrano sulla scalabilità industriale di questi nuovi prodotti.

Keywords: *pasta istantanea; pasta fresca all'uovo; liofilizzazione; reidratazione; attività dell'acqua; valutazione sensoriale.*

1. Introduction

As consumer preferences shift towards more convenient, health-conscious food choices, the transformation of traditional fresh egg pasta into instant ready formats has gained momentum (Sun *et al.,* 2020) Freeze-drying was employed to preserve quality and extend shelf life (Al Faruq *et al.,* 2025), while rehydration efficiency was evaluated with the goal of ensure fast rehydration, desirable texture, and moisture content similar to conventionally cooked pasta.

2. Materials and Methods:

Fresh meat-filled *tortellini* and *quadrucci* were supplied by **Toscanità S.r.l.** (Monteriggioni, SI, Italy). Samples were frozen and stored at **-18 °C** for at least 24 hours before freezing. These freeze-drying experiments were conducted using a **Modulyo 4k freeze dryer** (Edward High Vacuum International, UK). *Tortellini* samples were processed at 6, 12, 18, and 24 hours, while *quadrucci* were freeze-dried for 6, 8, 10, and 12 hours. Pressure conditions gradually decreased from 2.0 mbar to 0.3 mbar to enhance optimal sublimation after 2 hours of freezedrying. To improve water absorption and heat dispersion, especially for the bread-meat filling, surface and deep perforations (6 holes per sample) were introduced equally in batches with a small, 1 mm stainless steel pin. **Water activity (a_w)** was assessed using an AquaLab device (Decagon Devices, Inc., WA, USA). Separate measurements were taken for pasta and meat filling portions. Rehydration tests were performed using 70 g of freeze-dried pasta rehydrated in 180 mL of boiling water (100 °C) inside a thermostable paper cup for up to 4 minutes. Microwave rehydration tests were also conducted for comparison. Water-to-pasta ratios of 1:1.5, 1:2.5, 1:3, and 1:4 were tested. The structural performance and rehydration levels were tracked over time. Textural quality and visual appearance were assessed by a sensory panel for *tortellini*, focusing on rehydration completeness, filling softness, and product resemblance to freshly cooked pasta. Samples were compared across freeze-drying durations and perforation treatments.

3. Results and Discussion:

Among the *tortellini* samples, the 6-hour freeze drying time provided optimal results when combined with perforation. Water activity was 0.335 ± 0.047 for pasta and 0.353 ± 0.103 for filling. Rehydration with boiling water achieved moisture content values of 56.19 % for pasta (Fig.1,2) and 41.23 % for meat, (Fig.3,4) comparable to traditionally boiled fresh samples 57% Pasta and 49% for meat. Untreated samples took longer freeze-drying times (12 hours) and exhibited lower humidity, especially for meat 34% and tougher textures. Microwave rehydration with a 1:2.5 water to pasta ratio also yielded effective results. Building on the insights from the

tortellini trials, *quadrucci* was subjected to shorter freeze-drying durations (6–12 hours). Similar to *tortellini*, the 6-hour treatment again performed best, producing an a_w of 0.34 ± 0.02 for pasta and 0.36 ± 0.09 for filling. Structural evaluation confirmed that the compact shape of *quadrucci* allowed for faster sublimation and efficient rehydration.

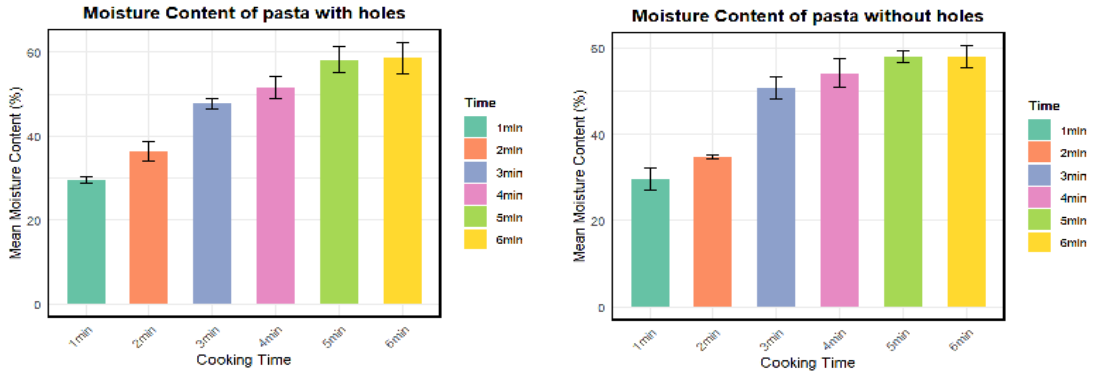


Fig. 8,2. Rehydration of tortellini pasta after 6 hours of freeze drying with 1:2.5 water to pasta ratio.

The perforation method further enhanced internal water dispersion during rehydration. Both pastas demonstrated that reducing freeze-drying time (6-8 hours) while applying structural perforation (6 holes per sample) enhanced rehydration performance, product quality, and time efficiency. The optimal water-to-pasta ratio (1:2.5) was consistent across *tortellini* and *quadrucci*, indicating the potential for process standardization. Sensory tests (*tortellini*) confirmed consumer acceptability in samples freeze-dried for shorter times with perforations.

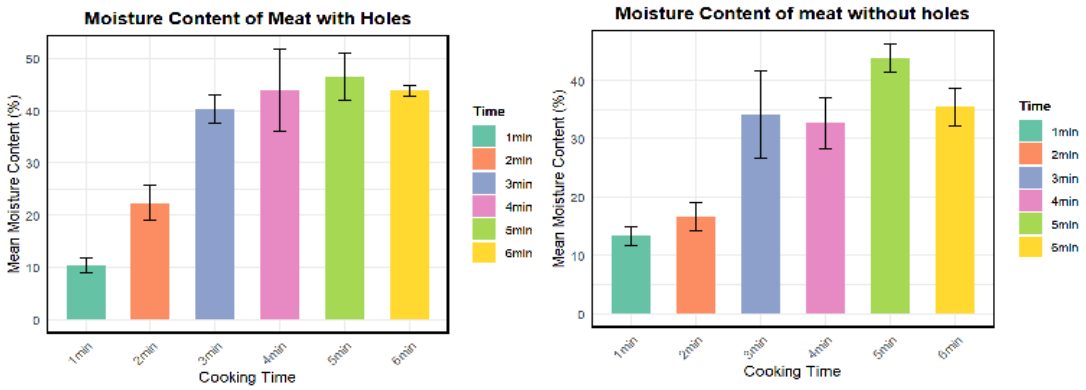


Fig. 3,4, Rehydration of tortellini filling after 6 hours of freeze drying with 1:2.5 water to pasta ratio.

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Salt Reduction Strategies In Breadmaking: Exploring Food-Grade Seawater (P-II-48)

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This work, representing the initial phase of my PhD thesis, investigates the technological, nutritional, and sensory impacts of using food-grade seawater as a low-sodium alternative in bread making. In this phase, we evaluated the rheological and pasting properties of doughs, along with the texture, crumb quality, and color of finished breads, in which table salt was replaced with seawater at various ratios (0%, 50%, and 100%).

Keywords: seawater, bread, low-sodium, rheology, texture

Strategie di riduzione del sodio in panificazione: l'utilizzo di acqua di mare ad uso alimentare

Questo lavoro, che rappresenta la fase iniziale della mia tesi di dottorato, analizza gli impatti tecnologici, nutrizionali e sensoriali dell'utilizzo di acqua di mare ad uso alimentare come alternativa a basso contenuto di sodio in panificazione. In questa fase, abbiamo valutato le proprietà reologiche e di *pasting* degli impasti, insieme alla texture, alla qualità della mollica e al colore dei pani finiti, in cui il sale da cucina è stato sostituito con acqua di mare a diversi livelli (0%, 50% e 100%).

1. Introduction

In accordance with the PhD thesis project previously described (Tomassini, 2024), this work reports the main results of the first three activities concerning:
A1) Set up the experimental design: choice of the seawater substitution levels, optimization of the process variables and determination of optimal water absorption.
A2) Characterization of experimental doughs through viscoelastic properties, extensibility, resistance to extension, and pasting profile analysis.
A3) Characterization of laboratory-scale bread for specific volume, texture, and crumb color using selected salt substitution levels.

2. Materials and Methods

Three dough formulations were prepared using commercial type 00 wheat flour, baker's yeast, sodium chloride, and food-grade seawater. The control dough (SALT) contained distilled water and 1.8% sodium chloride, while seawater partially (50%, MIX) or fully (100%, SEA) replaced the salt. Water content was adjusted using a farinograph to achieve a consistency of 500 Brabender Units. Dough underwent a two-step fermentation with initial proofing at 30°C and 85% relative humidity, manual moulding, a second proofing until volume doubled, and baking at 200 °C for 40 minutes. Dough properties were characterized by their extensional properties by using Kieffer Dough and Gluten Extensibility Rig, and rheological measurements with a rotational rheometer. Bread quality was evaluated in terms of specific volume, textural attributes using a Texture Analyzer TA. XT Plus (Stable Micro Systems), and crust color was measured with a Minolta CR-300 colorimeter. All analyses were performed in duplicate.

3. Results and Discussion

The Kieffer dough extensibility test showed that the SALT dough had significantly higher extensibility ($p < 0.05$) (Table 1). While NaCl is known to enhance gluten network development and dough extensibility (Tanaka et al., 1967), chaotropic salts in seawater may have an opposing effect (Preston, 1989). Seawater incorporation, however, increased the dough's viscoelastic properties (Figure 1), likely due to the presence of specific ions such as K^+, Mg^{2+}, and Ca^{2+}, which can act as electrostatic shields and strengthen protein–protein interactions within the gluten network (Zhang & Cremer, 2006). RVA analysis confirmed that wheat starch exhibited a lower tendency to gelatinize in the presence of seawater, indicated by significantly lower peak and final viscosity value, as it is clear by their pasting profile curves (Figure 2). The improved viscoelastic properties of dough may have a positive influence on several quality characteristics of bread, such as its volume (Khatkar & David Schofield, 2002). In fact, bread produced with total seawater substitution exhibited the highest ($p < 0.05$) specific volume. As can be observed in Table 2, high bread volumes correspond to values comparable to control of several texture profile analysis attributes, including hardness, chewiness and resilience. Similar values to the control were also found

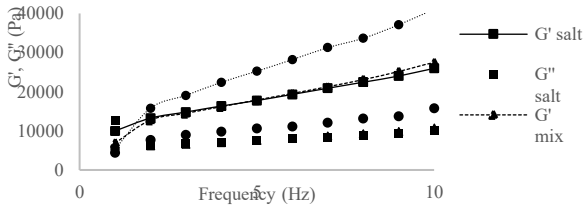
regarding the colour of the bread crust. These results are incosistent with those reported by Simsek & Martinez (2016), who observed a darkening effect on the bread crust with increasing amounts of sea salts added.

Table 1 Effect of seawater substitution on extensive properties of doughs.

Samples	Resistance to extension (N)	Extensibility (mm)
SALT	0.23±0.02a	81±4a
MIX	0.24±0.02a	73±2b
SEA	0.23±0.04a	72±2b

Means not sharing a common letter are significantly different ($p<0.05$), according to Tukey's HSD post-hoc test.

Figure 1 Fundamental rheological properties of dough samples.



Means not sharing a common letter are significantly different ($p<0.05$), according to Tukey's HSD post-hoc test.

Figure 2 Effect of seawater substitution on pasting properties of doughs.

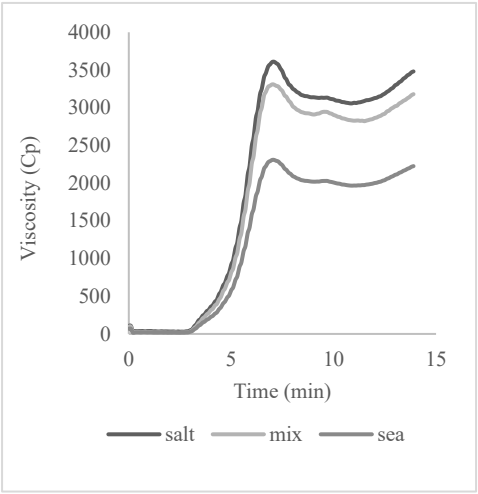


Table 3 Specific volume, textural properties, and crust color coordinates of experimental breads.

Volume	SALT	MIX	SEA
Specific volume (ml/g)	2.71±0.05b	2.77±0.06ab	2.82±0.08a
Textural Properties			
Hardness (N)	9.42±0.25b	10.5±0.63a	9.17±0.52b
Springiness	0.97±0.02ab	0.98±0.01a	0.96±0.01b
Cohesiveness	0.88±0.01a	0.87±0.01b	0.88±0.00a
Chewiness	8.05±0.28b	8.66±0.53a	7.75±0.40b
Resilience	0.57±0.01a	0.57±0.01a	0.56±0.01a
Crust color			
*L**	68.24±2.69a	67.33±3.3a	68.25±2.72a
*a**	8.14±1.73a	9.66±2.24a	9.37±1.56a
*b**	31.92±1.83b	34.41±1.59a	33.73±1.84a

Means not sharing a common letter are significantly different ($p<0.05$), according to Tukey's HSD post-hoc test.

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Valorization of Second Cheese Whey through microalgal cultivation for high-value biomass production (P-II-49)

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The first two activities of the PhD project are presented. Firstly, the mixotrophic growth of *Tetraselmis chuii* was evaluated in media supplemented with lactose, glucose, and galactose at different concentrations. Secondly, microalga was cultivated in second cheese whey (SCW) at various concentrations using both preadapted and non-preadapted cultures to assess the suitability of SCW as a sustainable substrate.

Valorizzazione della scotta attraverso la coltivazione di microalghe per la produzione di biomassa ad alto valore aggiunto

Le prime due attività del progetto di dottorato hanno riguardato, rispettivamente: la valutazione della crescita mixotrofica di *Tetraselmis chuii* in terreni di coltura arricchiti con lattosio, glucosio e galattosio a diverse concentrazioni e, successivamente, la coltivazione della microalga in scotta a differenti concentrazioni, impiegando sia inoculi preadattati che non, con l'obiettivo di valutare l'idoneità della scotta come substrato sostenibile e la capacità di adattamento di *T. chuii* alla crescita in sottoprodotti dell'industria lattiero-casearia.

Key words: *Tetraselmis chuii*, second cheese whey valorization, mixotrophic cultivation, microalgal bioprocesses.

1. Introduction

Following the PhD thesis project previously described, this poster reports the main results of the first two years of research activities concerning:

(A1) The evaluation of the mixotrophic growth of *T. chuii* in culture media supplemented with lactose, glucose/galactose mixture at the concentration of 2.0%, 3.5%, and 5.0%, to assess whether the microalga grows more efficiently on lactose or its hydrolyzed form (glucose/galactose mixture).

(A2) The cultivation of *T. chuii* in second cheese whey (SCW) at different concentrations (50%, 75%, and 100%), using both preadapted and non-preadapted inocula, to assess the suitability of SCW as a sustainable substrate and the adaptability of *T. chuii* to complex dairy by-products.

2. Materials and Methods

2.1 Mixotrophic growth of *T. chuii*

To assess the mixotrophic potential of *T. chuii*, cultures were grown in N-ALGAL medium supplemented with different concentrations (2.0%, 3.5% and 5.0%) of lactose (L2.0, L3.5, L5.0) or a glucose/galactose mixture (G/G2.0, G/G3.5, G/G5.0%) to simulate lactose hydrolysis. Cultures of 100 mL were incubated in 250 mL flasks at 24 ± 1 °C, 130 ± 10 µmol photons m^{-2} s^{-1}, under a 12:12 h light/dark cycle for 10 days. A photoautotrophic culture was used as a control (PC+). Every two days, Optical Density at 540 nm (OD_{540}), cell density, photosynthetic efficiency, and pH were monitored. On days 0 and 10, supernatants and biomass were analysed for nutrients and biochemical composition.

2.2 Cultivation of *T. chuii* in SCW

A second experimental phase was conducted using SCW as the growth medium, tested at 100%, 75%, and 50% (diluted with N-ALGAL) with both pre-adapted (P100, P75, P50) and non-pre-adapted (N100, N75, N50) cultures. Pre-adaptation involved culturing *T. chuii* for 7 days in N-ALGAL + lactose before inoculation. SCW was pretreated by sonication (20 kHz, 150 kW, 10 min), centrifugation, and filtration (0.25 µm), then adjusted to ±35 ppt salinity (NaCl), pH ± 8.0 (NaOH), and ± 1.13 g/L nitrate (KNO_3). Photoautotrophic culture and mixotrophic culture were used as controls (PC+ and MC+, respectively). Growth conditions and analyses were the same as in the first experimental step.

3. Results and Discussion

3.1 Mixotrophic growth of *T. chuii*

T. chuii exhibited higher growth under mixotrophic conditions compared to the autotrophic control. Cultures supplemented with 3.5% and 5.0% lactose reached approximately 1,580 and 1,530 cells/µL, respectively, after 10 days, outperforming those supplemented with glucose or galactose. This enhanced growth may result from the

slower rate of lactose utilization, possibly mediated by microalgae-associated indigenous bacteria, which helps maintain a stable and favorable pH. In contrast, glucose and galactose were rapidly consumed by bacteria, often leading to medium acidification. High bacterial activity in glucose-rich environments has been shown to significantly impair microalgal growth, for example, in co-culture with *Ochromonas*; bacterial overgrowth in glucose-enriched media reduced algal growth rates by up to 74% (Zhang et al., 2022). Since *T. chuii* thrives optimally at neutral to slightly alkaline pH (7–8), such acidification likely hinders its proliferation while favoring bacterial competition for space and nutrients. Moreover, contrary to the findings of Lu et al. (2017), which reported lower protein content under mixotrophic conditions, our results demonstrated notably higher protein productivity. Specifically, cultures with 3.5% and 5.0% lactose achieved a protein productivity of up to 0.21 $g\ L^{-1}\ day^{-1}$, indicating efficient biomass and protein synthesis under these conditions. Although *T. chuii* is not known to directly assimilate lactose, its presence appears to effectively support algal growth and protein accumulation. A deeper investigation into the underlying metabolic interactions among microbial communities could help elucidate the mechanisms driving the enhanced protein yield observed under these conditions.

3.2 Cultivation of *T. chuii* in second cheese whey (SCW)

Compared to both mixotrophic and autotrophic control groups, *T. chuii* exhibited higher growth performance. Similar observations were reported by Ribeiro et al. (2023), who demonstrated the potential of green microalgae to valorize dairy waste. In the present study, the pre-adapted cultures (P75 and P100) achieved significantly higher cell densities, reaching approximately 2,600 and 2,300 cells/µL, respectively, while non-pre-adapted groups remained below 600 cells/µL. Protein productivity followed a similar trend, with P75 reaching a maximum of 0.20 g/L/day, followed by 0.18 g/L/day in P100 and 0.13 g/L/day in P50, whereas non-pre-adapted groups remained below 0.08 g/L/day. These findings strongly suggest that pre-adaptation plays a critical role in modulating microbial dynamics and enhancing algal fitness under suboptimal conditions, such as undiluted cheese whey. In fact, non-pre-adapted cultures showed daily pH fluctuations and signs of indigenous bacterial proliferation, which likely competed with *T. chuii* for space and nutrients, ultimately impairing algal growth. Conversely, the pre-adaptation phase carried out in N-Algal medium supplemented with lactose appeared to stabilize the inoculum, allowing *T. chuii* to better cope with the challenging environmental conditions of the cheese whey matrix. Pre-adaptation is increasingly recognized as a strategic tool in microalgal biotechnology to enhance stress tolerance and biomass production in unconventional media (Bhatnagar et al., 2011; Zhou et al., 2019). This is particularly relevant in dairy wastewater valorization, where high organic loads and microbial competition pose substantial barriers to microalgal establishment. Future research should focus on elucidating the metabolic interactions between microalgae and bacteria in lactose-rich environments, optimizing pre-adaptation protocols, and evaluating the scalability of *T. chuii* cultivation for sustainable SWC valorization.

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Understanding the effects of heat treatment conditions on the physicochemical and technological properties of mycelium-based meat substitute (P-II-59)

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The initial research activities of this PhD thesis project focused on studying the effects of different heat treatment conditions on a semi-industrial mycoprotein formulation obtained by solid-state fermentation of *Rhizopus oligosporus,*to be used as a meat substitute. Heat treatments are traditionally used to reduce the RNA content to levels safe for human consumption (<2%). In particular, activities focused on studying the effects on physico-chemical (moisture content, water activity, pH) and sensory properties (color and texture). In addition, the microbiological risk and purine content were evaluated for each experimental condition.

Comprendere gli effetti delle condizioni di trattamento termico sulle proprietà chimico-fisiche e tecnologiche di un sostituto della carne a base di micelio

Le attività sperimentali iniziali del progetto di tesi di dottorato si sono focalizzate sullo studio degli effetti di diverse condizioni di trattamenti termici su un formulato semi-industriale a base di micoproteine, ottenuto da fermentazione su strato solido da *Rhizopus oligosporus*, da usare come sostituto della carne. I trattamenti termici sono tradizionalmente impiegati per ridurre il contenuto di RNA a livelli di sicurezza per il consumo umano (<2%). In particolare, le attività hanno riguardato lo studio degli effetti sulle proprietà chimico-fisiche (contenuto di umidità, attività dell'acqua, pH) e su quelle sensoriali (colore e texture). Inoltre, per ogni condizione sperimentale sono stati valutati il rischio microbiologico e il contenuto di purine.

Keywords: Mycoprotein, solid-state fermentation, purine content, sensory properties, microbiological safety.

1. Introduction

Mycoproteins represent a promising alternative protein source due to their high nutritional profile, including the high content in proteins with all essential amino acids and high biological value, the low fats content, primarily mono- and polyunsaturated, and the high content in dietary fibers. Fungal biomass used for mycoprotein production is obtained through a fermentation process carried out by filamentous fungi belonging to the *phyla Ascomycota* and *Zygomycota*. This process can be performed using two main approaches, such as solid-state fermentation (SSF) and submerged fermentation (SmF). The key difference lies in the moisture content of the culture medium, which is near zero for SSF and above 95% for SmF. Due to their fungal origin, mycoproteins are characterized by a relatively high content of nucleic acid (6–11%). The consumption of foods rich in RNA, particularly purines, can lead to increased levels of uric acid in the blood, which is considered a risk factor for the development of gout and kidney stones. For example, in Japan, the daily intake of dietary purines is recommended to be less than 400 mg (Kaneko et al., 2014). Although thermal treatment is the conventional method used to reduce the RNA content in fungal biomass, detailed and validated protocols are still lacking. Moreover, in SmF, the fungal biomass is physically separated from the culture medium after heat treatments, allowing a large part of the purine content to be removed through the liquid phase. This separation does not occur in SSF, where the absence of a liquid medium limits purine removal. In this context, the initial research activities of the PhD project aimed to:
WP 1. Investigate the effects of different thermal treatment conditions on the physicochemical properties (moisture content, water activity, pH) and sensory attributes (color and texture) of a mycelium-based meat substitute.
WP 2. Evaluate the impact of the heat treatment conditions on the purine content of the mycelium-based meat substitute obtained via SSF.
WP 3. Explore the influence of thermal treatment on the product's microbiological risk profile.

2. Materials and Methods

The mycelium-based meat substitute was supplied by the company Millow AB, Sweden. It is a food product obtained through SSF operated by *R. oligosporus* using oat okara as substrate. The product, packaged in 400g batches, was exposed to different steam pasteurization conditions (**Table 1**). For each sample, the microbiological investigation for *Enterobacteriacea*, *Lactobacillus sp*, *Sporigen sp*, *S. aureus*, *E. coli*, *Salmonella*, *Clostridium sp*, was performed.
The texture profile analysis (TPA) was conducted using 10 g sample for each experimental condition. The remaining portion was used to determine the moisture content (MC), water activity (a_w), pH, and color (L*, a*, b*). The total color difference (ΔE) was calculated as follows:

Temperature (°C)	Time (min)
NT	/
70*	45*
60	7- 14- 21
75	7- 11- 14
90	3- 6- 9

***Table 1**. Heat treatment conditions applied to the mycelium-based meat substitute. NT: not treated; * traditional heat treatment performed by Millow AB.*

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (1)$$

Purine quantification was performed by HPLC analysis of the extract, following the method described by Fan et al., (2007).

3. Results and Discussion

Compared to the NT products, samples subjected to mild thermal treatment (60 °C) - across all treatments durations exhibited lower values for L*, a*, b* (data not shown), and significant color changes with ΔE values ranging from 3.68 to 4.23, in agreement with the threshold scale reported by Pasqualone et al. (2020). MC and pH were not significantly affected by the thermal treatments, with values within the ranges of 54–56% and 4.94–5.22, respectively.

Water activity was found to be more influenced by the temperature than by the time of thermal exposure. Specifically, the NT sample showed an a_w value of 0,977± 0,003, while treated samples exhibited a_w values ranging from 0,966 to 0,987. TPA showed, for each tested temperature (60, 75 and 90 °C), a progressive and statistically significant reduction in hardness over time ($p<0,05$) (***Fig. 1***). Furthermore, as the treatment temperature increased, an overall increase in hardness was observed, with higher values in samples treated at 90 °C, and lower values at 60 °C (***Fig. 1***). Comparison with the NT sample (120.47 ± 2.96 N) showed significant hardening of the product treated at 90 °C; in contrast, samples treated at 60 °C showed general softening. At 75 °C, intermediate behavior was observed: a slight initial hardening (at 7 minutes), followed by a subsequent softening. Atypically, the sample treated at 70 °C for 45 minutes showed a slight increase in hardness (133.3 ± 2.52 N). Overall, the results suggest that temperature and heat treatment time significantly affect the texture of the product. Therefore, with the purpose of obtaining the desired characteristics of the final product, the precise modulation of these variables should be adequately considered. Microbiological analysis revealed an overall safety of the mycelium-based product even in the absence of thermal treatment, except for *Enterobacteriaceae* that were detected in the NT samples at a mean concentration of 2,72 log CFU/g, while they were absent under all thermal treatment conditions. Regarding the total purine content (***Fig. 2***), none of the heat treatment conditions were effective in reducing purines. Comparison between the NT and treated samples revealed no statistically significant differences ($p>0.05$), although slight variations were observed among the different heat treatment conditions. This result could be attributed to the difficulty of physically removing the purines from the biomass obtained through the SSF process. This limitation suggests the need for further studies aimed at identifying alternative technological strategies, such as sonication or thermosonication, able to hydrolyse RNA and facilitate the physical removal of purines.

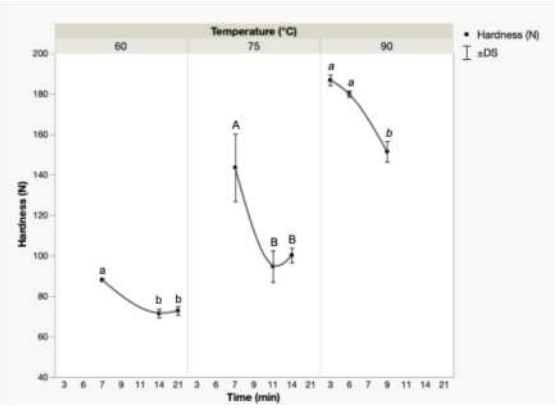


Fig.1 *Variation in hardness (N) of thee mycoprotein-based product as a function of heat treatment time for different temperatures (60 °C, 75 °C and 90°C). Different letters and font style indicate statistically significant differences among treatment times within the same temperature.*

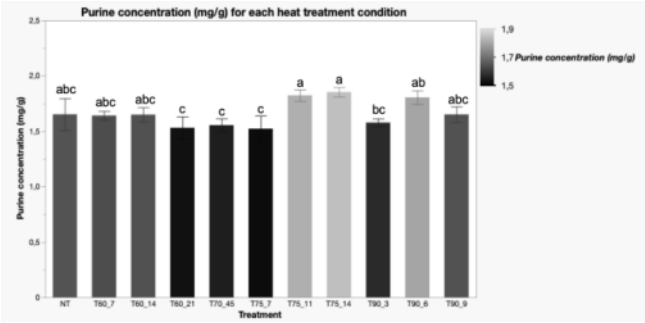


Fig.2 *Total purine content (mg/g) at each heat treatment condition. NT: not treated; T60_7: Treated at 60 °C for 7 min; T60_14: Treated at 60 °C for 14 min; T60_21: Treated at 60 °C for 21 min; T70_45: Treated at 70 °C for 45 min; T75_7: Treated at 75 °C for 7 min; T75_11: Treated at 75 °C for 11 min; T75_14: Treated at 75 °C for 14 min; T90_3: Treated at 90 °C for 3 min; T90_6: Treated at 90 °C for 6 min; T90_9: Treated at 90 °C for 9 min. Different letters indicate statistically significant differences ($p < 0.05$).*

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Optimizing Taggiasca table olives fermentation: development of autochthonous microbial starter culture (P-II-51)

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This PhD project, part of the European DOMINO project, aimed to characterize yeast and lactic acid bacteria (LAB) communities from the Taggiasca olive orchard system to develop an autochthonous microbial starter for better management of the olive fermentation. Strain selection was performed using an integrated approach combining Whole Genome Sequencing (WGS), Genome Scale Modelling (GSM), and physiological screening. Pilot-scale fermentations of Taggiasca table olives were carried out using selected strains in three combinations. Salinity (6% and 10% NaCl) was tested as an additional variable. The inoculated strains were monitored throughout the fermentation process to evaluate their performance.

Ottimizzazione della fermentazione delle olive Taggiasche da tavola: sviluppo di colture starter microbiche autoctone

Questo progetto di dottorato, parte del progetto Europeo DOMINO, ha avuto l'obiettivo di caratterizzare le comunità di lieviti e batteri lattici (LAB) del sistema olivicolo della Taggiasca per sviluppare uno starter microbico autoctono per un'innovativa fermentazione delle olive. La selezione dei ceppi è stata condotta con un approccio integrato che ha combinato Whole Genome Sequencing (WGS), Genome Scale Modelling (GSM) e screening fisiologico. Sono state avviate fermentazioni in scala pilota delle olive da mensa Taggiasca con ceppi autoctoni selezionati, in tre consorzi microbici. È stata testata la salinità (6% e 10% NaCl) e monitorata la dinamica dei ceppi durante la fermentazione.

Key words: Taggiasca table olives, WGS, GSM, strains selection, pilot-scale fermentation, microbial consortia.

1. Introduction

In line with the PhD project described by Vanzetti (2024), this poster presents the main results from the first two activities:
(A1) Selection of strains for fermentation of Taggiasca table olives using *in silico* and *in vitro* techniques.
(A2) Pilot-scale fermentation and strains monitoring

2. Materials and Methods

A1) Physiological screening for starter culture selection
Fifty-three yeast strains, isolated in 2021 and sequenced via Whole Genome Sequencing (WGS), were screened for growth under stress. Strains were tested at 10, 25, and 30 °C, NaCl concentrations of 0, 8, 10, and 12% (w/v), and pH 4.5 and 5.5. Growth (OD600) was monitored at 24, 48, 72 hours and 7 days to assess kinetics and stress tolerance. Enzymatic screening for β-glucosidase and pectinase activity was also performed (Bevilacqua *et al.*, 2013).
A2) Natural Pilot-scale fermentation with selected consortia
Taggiasca table olives (Olea europaea L., cv. Taggiasca) were harvested in October 2024 by Fratelli Merano (Chiusavecchia, Liguria, Italy). For pilot-scale fermentation, 24 buckets (~20 kg each) were prepared, each with 15 kg of olives and 10 L brine. Twelve buckets were filled with 10% (w/v) brine (traditional method); twelve with 6% (w/v) brine to assess reduced salt. Three selected autochthonous microbial consortia were inoculated in triplicate (10^6 CFU/ml); six buckets served as non-inoculated controls.

3. Results and Discussion

2.1 Selection of the consortia
The microbial strains selected for the consortium were identified using an integrated approach combining Genome Scale Modelling (GSM), growth capacity assays, and enzymatic screening. In-silico analysis, based on genome-scale models, enabled the prediction of yeast contributions to vitamin production and the identification of potential metabolic interactions within the community. GSM results indicated that *Wickerhamomyces anomalus* was essential for effective fermentation, requiring its presence in all consortia. The growth capacity assay confirmed the relevance of *W. anomalus*, which exhibited remarkable tolerance to salinity (up to 10–12% NaCl) at 25–30 °C, and of *Pichia membranifaciens*, which showed strong adaptability under multiple stress conditions, including high salinity and elevated temperatures. Enzymatic screening of all 53 strains identified an alternative *P.*

membranifaciens strain with positive β-glucosidase activity and negative pectinase activity. Additionally, GSM highlighted the functional importance of *Candida diddensiae* within the microbial community. These findings support the selection of strains with desirable technological traits for fermentation under challenging environmental conditions.
Based on the results, three consortia were selected (Table 1), all containing yeast strains. A lactic acid bacterium (LAB), *Lactiplantibacillus plantarum O2*, previously isolated from olive matrix (Botta *et al.*, 2014), was added to one consortium. Two different salinity conditions were tested.

***Table 1**: Selected microbial consortia and brine salt concentrations applied in pilot fermentation trials.*

Consortium	Code	Consortia composition	Salt concentration	Replicates
C1	WPCL	*Wickerhamomyces anomalus* Y53, *Pichia membranifaciens* Y36, *Candida diddensiae* Y45 + *L. plantarum* O2	6% and 10% NaCl	Triplicate
C2	WPC	*Wickerhamomyces anomalus* Y53, *Pichia membranifaciens* Y36, *Candida diddensiae* Y45	6% and 10% NaCl	Triplicate
C3	CW	*Wickerhamomyces anomalus* Y53, *Candida diddensiae* Y45	6% and 10% NaCl	Triplicate
Control	C	Not inoculated	6% and 10% NaCl	Triplicate
Total fermentations				24

3.2 Monitoring of the inocula

Microbial counts and pH measurements were carried out monthly to monitor the progress of fermentation, from 0 to 160 days of fermentation. Unexpectedly, in the second month of fermentation, no LABs were detected on plate, while pH reached above 6.5. As Vaccalluzzo et al., 2020 showed, low temperatures serve as an inhibitor for the growth of LABs, and this would partially explain the high pH and the absence of viable LABs from 30 days onward. These preliminary results show that fermentation has been guided by yeast strains as shown in figures 1 and 2.

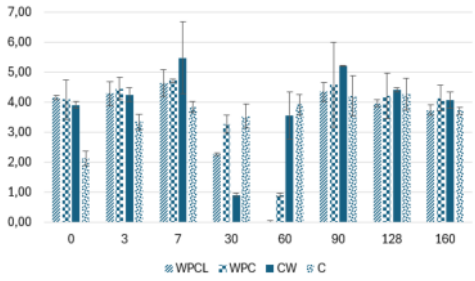


Figure 9: *Microbial loads of yeasts under NaCl 6%*

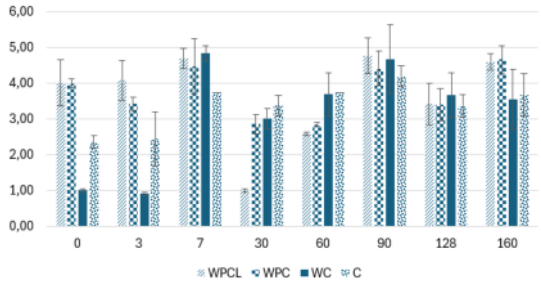


Figure 10: *Microbial loads of yeasts under NaCl 10%*

Fermentation yielded 804 yeast isolates. Preliminary MALDI-ToF MS classification showed that many belong to target consortia species, which appear to predominate the microbiota. Strain-level resolution is ongoing, and MALDI-IR Biotyper is being validated to enhance taxonomic accuracy. Metagenomic and metabolomic analyses on the final 24 samples are underway to further characterise community dynamics and metabolic profiles.

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Interspecies interactions in cheese microbiota: metabolic synergies among lactic acid bacteria species (P-II-52)

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The first two activities of the PhD thesis project are described. Metabolic profiling of strains was performed, along with genome sequencing by Illumina and Nanopore sequencing. Long and short reads were then used to obtain hybrid assemblies, allowing for high-precision genome reconstruction, plasmid typing, and gene annotation. Inter and intra-specific metabolic diversity could be linked to genomic information, allowing for full characterization of metabolic properties of these strains. This workflow provides a useful method to facilitate the selection of strains for precision fermentations and production of biomolecules of interest

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Interazioni interspecie nel microbiota del formaggio: sinergie metaboliche tra specie di batteri lattici

L'elaborato riguarda le prime due attività del progetto di tesi di dottorato. È stato effettuato il profiling metabolico dei ceppi, insieme al sequenziamento del genoma tramite tecnologie Illumina e Nanopore. Le sequenze lunghe e corte sono state successivamente utilizzate per ottenere assemblaggi ibridi, permettendo una ricostruzione ad alta precisione del genoma, la tipizzazione dei plasmidi e l'annotazione genica. La diversità metabolica inter- e intra-specifica è stata correlata con le informazioni genomiche, consentendo una caratterizzazione completa delle proprietà metaboliche di questi ceppi. Questo flusso di lavoro rappresenta un metodo utile per facilitare la selezione di ceppi destinati a fermentazioni di precisione e alla produzione di biomolecole di interesse.

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Key words: lactic acid bacteria, hybrid assemblies, phenotypic microarrays, metabolic characterization.

1. Introduction

In accordance with the PhD thesis project previously decribed (28th Workshop on the Developments in the Italian PhD research on Food Science, Technology and Biotechnology), this poster reports the main results of two activities concerning:
(A1) Metabolic Profiling of strains of *Lacticaseibacillus* (*L.rhamnosus* 1019 and 1473, *L.casei* 2306, *L.paracasei* 2333 and 4186) genus to characterize the metabolic activities of the strains over several sets of substrates by using Phenotype Microarrays (PM) provided by Biolog, focusing on PM2A plates.
(A3) Sequencing of genomes of strains and annotations of genes involved in metabolic pathways.

2. Materials and Methods

2.1 Phenotypic profiling of strains

Phenotypic assays using Biolog Phenotype Microarrays PM2A assessed substrate utilization, providing insights into metabolic capabilities for carbon sources. Cell cultures were incubated at 37°C up to their late exponential phase. Microarrays were inoculated at a transmittance level of 65% and incubated at their optimum growh temperature, and absorbance at 590 nm was measured for 72 hours every 10 minutes. Kinetic data were then analyzed by using growthcurver R package.

2.2 Genome Sequencing and annotations of hybrid assemblies

After DNA extraction, sequencing reads were generated using both Illumina and Nanopore platforms. A suitable pipeline was employed for data trimming and quality filtering. Hybrid genome assemblies were reconstructed using Unicycler v0.4.8, and genome completeness and contamination were assessed with CheckM2 v1.2.1. Plasmid typing was performed using MOB-suite v3.0.5 (mob_recon). Genome features were evaluated with QUAST v5.0.2.
Functional annotation of microbial genes was carried out using Clusters of Orthologous Genes (COG), which were classified and analyzed through the COG database and processed with COGclassifier v1.0.5. CAZyme gene clusters (CGCs) and substrate predictions were identified using run_dbcan v4.1.4, in combination with the dbCAN3 database.

3. Results and Discussion

As shown in **Figure 1**, both intra- and inter-specific diversity in substrate utilization was observed. In particular, *Lacticaseibacillus* strains shared a core set of substrates utilized by all isolates. However, certain substrates were metabolized in a strain-specific manner, indicating a potential metabolic specialization of *L. casei* 2306 for specific pathways. Principal Component Analysis (PCA), based on kinetic data, revealed a clusterization of one *L.paracasei* strain (2333) with two *L.rhamnosus* (1019 and 1473) strains, with a distinct separation of *L.casei* 2306 and *L.paracasei* 4186 strains.

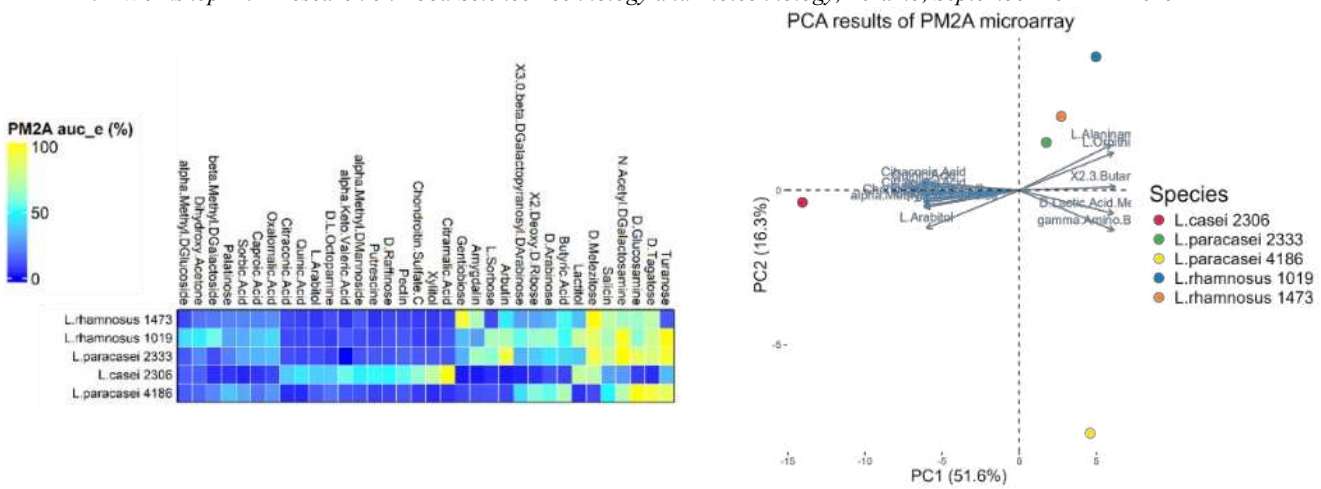


Figure 1 *Metabolic profiling based on Phenotype Microarrays. On the left, a heatmap representation of metabolic activity of each strain across the most utilized carbon sources. The color scale represents the empirical relative Area Under the Curve (AUC_e), calculated using the growthcurver R package. On the right, PCA graph obtained by using the FactoMineR and factoextra R packages to visualize variability in metabolic profiles among strains.*

Table 1 summarizes genomic features of hybrid assemblies. For all assemblies, completeness was above 99.97 %, while contamination was under 0.5 %. COG functional analysis revealed a general specialization in carbohydrate utilization, with notable variations in the number of genes associated with carbohydrate transport and metabolism. *L. rhamnosus* 1019 exhibited the highest gene count, whereas *L. paracasei* 4186 displayed the lowest.

Sample Species	Sample ID	Plasmid number	Genome Size (bp)	GC (%)	CDS	rRNA	tRNA	Depth
Lacticaseibacillus rhamnosus	1019	2	3004620	46.7	2793	15	60	253
Lacticaseibacillus rhamnosus	1473	7	2944387	46.8	2759	15	61	166
Lacticaseibacillus casei	2306	6	3080113	46.3	2920	15	59	140
Lacticaseibacillus paracasei	2333	1	2987373	46.8	2781	15	60	116
Lacticaseibacillus paracasei	4186	2	2778761	46.6	2670	15	60	109

Table 1 *Genomic features of hybrid assemblies for each strain, ranging from number of identified plasmids, genome size, GC content, number of coding sequences (CDS), rRNA and tRNA genes, and sequencing depth.*

Hybrid sequencing enables precise genome reconstruction, supporting detailed functional analyses of LAB strains and their roles in food fermentation, including cheese ripening. The results displayed in this abstract suggest a potential correlation between phenotypic variations and differences in the gene content of each strain, although further investigation is needed to clarify the functional roles of these genes. Additional metabolic profiling, genome sequencing, and annotation will be conducted on strains belonging to the *Lacticaseibacillus* genus, due to their importance in curd acidification and the early stages of cheese ripening[1]. Integration of genomic and phenotypic data facilitates a comprehensive understanding of metabolic pathways, providing insights into strain-specific adaptations and metabolic interactions that influence ripening processes such as flavor and texture development[2-3]. Correlating genomic features with functional phenotypes aids the rational selection of LAB strains for industrial applications. Optimizing bacterial consortia based on metabolic profiles may enhance fermentation efficiency, improve consistency in cheese production, and support the development of dairy products with tailored sensory attributes.

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Fermented foods: from raw material fermentation to microbiota and related post-digestion bioactive compounds (P-II-53)

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The outset activities of the PhD thesis project are described. The first part focused on fermenting raw matrices of both hedonic indulgence and sustainability relevance. We investigated the microbiological safety of spontaneous versus controlled fermentation of *Boletus edulis*, a wild edible mushroom, by monitoring the evolution of microbial communities and their impact on the volatile profile. Additionally, guided fermentation was investigated on blue crab (*Callinectes sapidus*), an invasive species in the Mediterranean, targeting the edible pulp to produce "*garum*"-like sauces. The second activity centred on starters selection for the development of new fermented foods. In this context, we explored the potential of fermentation to deodorize and valorise several microalgal matrices.

Alimenti fermentati: dalla fermentazione delle materie prime al microbiota e ai relativi composti bioattivi post-digestione

Vengono descritte le prime due attività del progetto di tesi di dottorato. La prima attività si è concentrata sulla fermentazione di matrici grezze di indulgenza edonica e rilevanza per la sostenibilità ambientale. Abbiamo studiato la sicurezza microbiologica della fermentazione spontanea rispetto a quella guidata di *Boletus edulis*, un fungo commestibile selvatico commestibile, monitorando l'evoluzione delle comunità microbiche ed il loro impatto sul profilo aromatico. Inoltre, è stata studiata la fermentazione guidata del granchio blu (*Callinectes sapidus*), una specie invasiva del Mediterraneo, usando la polpa commestibile per la produzione di salse simili al *garum*. La seconda attività è stata incentrata sulla selezione di starter per lo sviluppo di nuovi alimenti fermentati. In questo contesto, abbiamo esplorato il potenziale della fermentazione per deodorare e valorizzare diverse matrici microalgali.

Key words: fermentation, microbiological safety, starter selection, valorisation, aromatic profile

1. Introduction

In accordance with the PhD thesis project previously described (28th Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, 2024), this poster reports the main results of the first two activities concerning:

A1) **Fermentation of raw matrices** - Characterization of fermented foods (microbiological, chemical and bioactive potential)
A2) **Starter selection for new fermented foods** – screening of microbial strains (bacteria and yeasts), isolating strains from natural sources, such as raw ingredients, traditional fermented foods, or specific environments. Small-scale fermentation trials to evaluate the performance of each starter culture.

2. Materials and Methods

All the guided fermentations involved strains belonging to the University of Parma Culture Collection (UPCCO, Parma, Italy). Spontaneous and guided anaerobic fermentation with *Lactiplantibacillus plantarum UPCCO 4932 (8 Log UFC/g)* of *B. edulis* lasted 6 days with plate counts and pH measurement every two days to monitor the microbial and acidification kinetics. Both, spontaneous (SPO) and guided (LPB) fermentations were conducted under controlled conditions at the food microbiology laboratories of the University of Parma (PR) and at "Rifugio La Roda", a professional kitchen and restaurant on Monte Paganella (TN). Genomic DNA was extracted from the fermented *B. edulis* samples using the Maxwell® RSC PureFood Pathogen Kit (Catalog No. AS1660; Promega Corporation, USA). Shotgun sequencing was performed on the Illumina NovaSeq 6000 platform by Novogene Co., Ltd. Two guided fermentations of blue crab were performed using *Lactobacillus helveticus* UPCCO Lh43 and *Lpb. plantarum UPCCO 4932 (6 Log/UFC).* The fermentation lasted 90 days during which the kinetic of microorganisms were monitored using different agar media. Solid-state fermentation of four different *Chlorella vulgaris* biomasses and *Arthrospira platensis* biomass were conducted using different UPCCO lactic acid bacteria (LAB) and yeast strains. Volatile compounds (VOCs) determination was performed on all the aforementioned fermented matrices by means of HS-SPME/GC-MS technique, as described by Martelli et al., in 2020 [1].

3. Results and Discussion

3.1 Novel Fermented Food

Four different samples of fermented *Boletus edulis*, produced with a different type of fermentation (Guided: LPB or Spontaneous SPO) and in two different places (Kitchen TN and Laboratory PR) were produced and the microbial concentration was monitored using a culture dependent approach. The total microbial count (PCA), presence of yeast and moulds (YEDC) and of LAB (MRS) were monitored during the 6 days of fermentation. Both fermentations were successful with the spontaneous one being slower, as confirmed by the acidification kinetics, showing the pH in the guided fermentation decreasing by 48 hours below 4.5, a critical factor in inhibiting the growth of most pathogens in food [2]. Taxonomic analysis performed with MetaPhlAn [3] on short-read sequences from each sample revealed a broad diversity of genera in spontaneous fermentations, while only *Lpb. plantarum* UPCCO 4932 was detected in guided fermentations. Pathogenic species such as *Clostridium perfingens* and *Bacillus cereus,* as well as horizontally transmissible antibiotic resistance genes, were found within the spontaneously fermented matrices. The volatile profile characterization showed no significant differences between spontaneous and guided fermentation in the key volatile compounds 1-Octen-3-ol, 1-Octanol, and 3-Octanone, respectively (Figure 2). Thereby, spontaneous fermentation of *Boletus edulis* showed strong microbial variability and occasional presence of potentially pathogenic microorganisms. Guided fermentation, successfully implemented even in culinary settings, ensured faster acidification while preserving the key volatile profile of the product.

The production of blue crab garum-like sauce was designed through the use of LAB in order to allow for proper and safe management of microbial transformation [4]. However, an inoculum concentration of 6 Log CFU/g was insufficient to effectively drive the fermentation as intended. As seen by culture-dependent method, the selected starters (*Lpb. plantarum UPCCO 4932* and *L. helveticus UPCCO Lh43*) were no longer present in the matrices after 7 days of fermentation. Moreover, the fermentation temperatures used (30°C and 42°C) favored the development of other microorganisms leading to the prevalence of staphylococci and, on the other hand, disadvantaged the growth of inoculated LAB. DNA shotgun metagenomics analyses are currently underway and will allow for the characterization of both the taxonomic composition and functional profiles of the microbial community.

3.2 Starter selection for the deodorization of microalgae

It is known that fermentation can be used as a tool to improve the flavor profile of various matrices. In this context, microalgae fermentation is a field of increasing scientific interest since these matrices have great nutritional value but with sensory limitations. Five different commercial microalgae, namely *Chlorella vulgaris* “Smooth” (CHL1), *Chlorella vulgaris* “Premium” (CHL2), *Chlorella vulgaris* “Honey” (CHL3) and *Chlorella vulgaris* “White” (CHL4), and *Arthrospira platensis,* were utilized as fermentation substrates with different microbial strains belonging to UPCCO.

The solid-state fermentation applied to both *Chlorella vulgaris* and *Arthrospira platensis* was successful, as most of the selected strains were able to grow efficiently in the matrices, maintaining cell density ≥8 Log UFC/mL for over 72 hours. The volatile characterization showed a general decrease in aldehydes levels across fermented samples, particularly hexanal. Linear aldehydes, saturated and unsaturated, containing from 6 to 9 carbon atoms as hexanal, have been associated to the typical “seaweed” flavor [5,6]. This reduction indicates a positive modulation of the aroma profile. The fermentation with certain strains had led to an increase of alcohols and esters, generally associated with pleasant scent [7]. Thus, several LAB strains emerged as promising starter cultures for deodorizing algal biomasses. Furthermore, *A. platensis* fermented with selected yeast strains also showed a marked decrease in aldehyde content, accompanied by a notable increase in alcohols, highlighting the complementary potential of yeast in improving the aromatic profile of this matrix.

These findings contribute valuable insights to the emerging field of fermented algal-based foods, supporting the potential of fermentation and microbial strain selection as effective strategies to improve sensory qualities of microalgal biomasses.

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Bio-valorization of Pâtè Olive Cake (POC) for food applications (P-II-54)

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The first activities of the doctoral thesis project focused on the bio-valorisation of Olive Cake Paté (POC), a by-product of the two-phase extraction of olive oil, through controlled fermentation using different microbial strains isolated from fermented foods. Six different fermentation tests were conducted, including single and mixed cultures in a sequential inoculum, under controlled conditions. During the fermentation, microbiological, physico-chemical parameters were monitored up to 14 days. The fermented matrix will subsequently be used as a functional ingredient in bakery products, to enhance their bioactive compound content and, in turn, their antioxidant properties.

Bio-valorizzazione del Pâtè Olive Cake (POC) per potenziali applicazioni nell'industria alimentare

Le prime attività del progetto di tesi di dottorato hanno riguardato la valorizzazione del Patè Olive Cake (POC), un sottoprodotto dell'estrazione a due fasi dell'olio di oliva, attraverso fermentazioni controllate. In particolare, campioni di POC sono stati fermentati con differenti ceppi microbici, precedentemente isolati da matrici alimentari. Sono state condotte sei differenti prove fermentative, che hanno incluso fermentazioni singole e fermentazioni miste e sequenziali, in condizioni controllate. Durante il processo, sono stati monitorati i parametri microbiologici, chimico-fisici e colorimetrici per 14 giorni. La matrice ottenuta, opportunamente essiccata, sarà successivamente impiegata come ingrediente nella produzione di prodotti da forno, con l'obiettivo di arricchirne il contenuto in composti bioattivi ad azione antiossidante.

Key words: Biovalorization; by-products; controlled fermentation; lactic acid bacteria; yeasts; bioactive compounds.

1. Introduction

In line with the objectives of the PhD research project, this section outlines the main findings from the following two experimental activities:

- (A1) The biovalorization of pâtè olive cake (POC), a phenolic-rich olive oil by-product, was achieved through controlled fermentation with selected lactic acid bacteria and yeasts. Six fermentation conditions, including mono- and co-cultures, were tested.
- (A2) Microbiological and physico-chemical parameters were monitored over 14 days to evaluate the success of fermentation. The obtained fermented POCs were chemically characterized for pH, total soluble solids (TSS), color (L*, a*, b*), and total phenolic content (TPC). Moreover, the HUPLC/DAD analysis was applied to obtain the phenolic profiles.

2. Materials and Methods

In the present study, the Pâtè Olive Cake (POC), a by-product of the olive oil industry, was used. POC was diluted with olive mill wastewater (OMWW) and distilled water in a 3:2 ratio (530 mL OMWW + 190 mL H_2O per kg of POC) and supplemented with 2% (w/w) glucose to promote microbial growth. The homogenised matrix was used to set up six fermentation trials, carried out at 25 ± 2 °C, under constant stirring for 14 days, using strains previously isolated from fermented foods. Experimental conditions, performed in duplicate, included a non-inoculated sample (control), four samples inoculated with single culture strains (*Lactiplantibacillus plantarum* C11C8, *Lactiplantibacillus pentosus* L7, *Wickerhamomyces anomalus* F5.60.05, and *Wickerhamomyces anomalus* F5.60.01), and a sample inoculated with a sequential culture, by inoculating the yeast blend and the LAB blend, after 72 hours. Strains were inoculated at 8 Log CFU/mL (LAB) and 7 Log CFU/mL (yeasts). Sampling was performed at 0, 8, and 14 days, and microbiological, physico-chemical (pH, total soluble solids; L*, a*, b*), and compositional analyses were performed. Conventional microbiological analyses were performed using selective media. Total phenolic content (TPC) was quantified spectrophotometrically as mg GAE/L. The antioxidant activity was performed as DPPH, and the phenolic profiles (including hydroxytyrosol and oleuropein) were obtained by HUPLC/DAD analysis.

3. Results and Discussion

3.1 Microbiological traits

During the fermentation process, a clear evolution of the microbial population was observed, with effective competition of the inoculated microorganisms against the native microbiota. In particular, in samples inoculated with *L. plantarum* and *L. pentosus*) the LAB counts increased, reaching 7.81± 0.02 and 6.84 ± 0.04 Log CFU/mL at the 8th day, respectively, followed by a physiological decline to 6.55 ± 0.04 and 5.27 ± 0.03 Log CFU/mL after 14 days. Regarding samples inoculated with single cultures of yeasts, *W. anomalus* F5.60.01 and *W. anomalus* F5.60.05, a good performance was observed, with yeast values of 6.03 ± 0.02 and 6.52 ± 0.03 Log CFU/mL at the 8th day, with a slight reduction after 14 days. In co-inoculated samples (yeasts + LAB) a constant increase of LAB counts was observed with values of approximately 5.00 Log CFU/mL until the end of fermentation. The total mesophilic counts showed a general decrease of about 1 Log CFU/mL in all inoculated samples, confirming the antimicrobial effect of the selected strains. In particular, Enterobacteriaceae and *Escherichia coli*, initially present in the matrix (at 4.79 and 5.04 Log CFU/mL, respectively), were not detected in any inoculated samples after 14 days, whereas in the control samples, *E. coli*, was detected at the end of fermentation at a cell density of 1.04 ±0.05 Log CFU/mL. No sulphite-reducing clostridia or staphylococci were detected in any samples. These results confirm the ability of the used microbial consortia to effectively colonise the matrix, inhibiting the growth of undesirable microorganisms.

3.2 Physico-chemical traits

The pH parameter showed a progressive decrease throughout the fermentation process, with final values ranging from 3.76 ± 0.01 to 3.97± 0.06. The pH decrease was more pronounced in samples inoculated with *L. pentosus* and *L. plantarum*. The TSS values decreased in all samples during fermentation, indicating an active microbial metabolism. The decrease was more evident in the inoculated samples, with values ranging from the initial value of 9.1 °Brix to 6.8–7.2 °Brix after 14 days. Colorimetric analyses (L*, a*, b* parameters) revealed colour changes in the fermented matrix. The L* parameter, indicative of brightness, decreased more markedly in samples inoculated with yeasts and in samples co-inoculated, suggesting a greater degradation of the original colour. The a* and b* coordinates increased in the inoculated samples, especially in samples with LAB, indicating a shift towards redder and yellower values, consistent with the release and the modification of pigments and phenolic compounds. The TPC, expressed in mg GAE/L, increased in all inoculated samples, with the highest value observed in the sample inoculated with *L. pentosus*, which reached 3041.79 mg GAE/L. The increase in TPC is attributable to the enzymatic bioconversion of oleuropein into hydroxytyrosol and other derivatives, as confirmed by UPLC/DAD analysis. These data suggest that driven fermentation using selected strains improves the microbiological safety and enhances the nutritional value of the matrix, making it potentially suitable as an ingredient in the food industry.

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Valorization of Agrifood Waste for Bioplastic Production via Microbial Fermentation Using *Cupriavidus necator* (P-II-55)

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The first activities of the PhD thesis project are described. Firstly, the various waste biomasses were subjected to a series of pre-treatment processes, prior to their utilization as growth media, with the aim of releasing ready-to-use organic carbon. A high-yielding microorganism, *Cupriavidus necator* DSM 428, was selected due to its substantial intracellular accumulation of polyhydroxybutyrate (PHB). Secondly, following laboratory-scale optimization and characterization of the extracted biopolymer, process conditions were transferred to a pilot-scale bioreactor.

Key words: Polyhydroxybutyrate (PHB), *Cupriavidus necator*, Agrifood waste, Extracted oil coffee.

1. Introduction

In accordance with the PhD thesis project previously described, this poster reports the main results of the first two activities concerning:

(A1) **Biotechnological process development**

A.1.1) Set-up of the substrates using enzymatic and chemical-physical treatments to maximize the growth of PHB-producing microorganisms.

A.1.2) Selection of the most efficient growth conditions using the microbial starter *Cupriavidus necator*

A.1.3) Characterization and analysis of the extracted polymer products to obtain information on the biodegradability and technological properties (purity, molecular mass and physicochemical properties).

(A2) **Scale-up**

Transferring all the process on pilot scale (3-10 liters bioreactor) monitoring the yields in terms of microbial biomass and PHB recovered.

2. Materials and Methods

Three by-products of the food industry were used: bread scraps consisting of the crusts and outer layers of sandwich bread, discarded during industrial preparations; potato processing by-products for commercial preparations, including peel and unsuitable parts and artichoke cultivation and processing by-products, including outer bracts, stems, and fibrous central parts rich in inulin. Finally, ground coffee powder, by-products from industrial processes, was considered as an additional element in the formulation of substrates. Bread scraps were finely ground using a laboratory mill and mixed with deionized water. α-Amylglucosidase from *Aspergillus niger* (120 U/mg, Sigma-Aldrich, USA) was added at 0.05 g/100 g solid to hydrolyze 1,4-α-D-glycosidic starch bonds (Montemurro *et al*., 2022), and samples were incubated at 55 °C for 24 h. Potato waste was washed, diced (~1 cm), blended in deionized water, and mixed 1:1 with 1 M HCl in a glass flask. Acid hydrolysis was performed at 97 °C for ~60 min (Guerra-Rodríguez *et al*., 2012). Freeze-dried artichoke bracts and stems were ground and hydrolyzed with 0.1 M H_2SO_4 (1:20 ratio) at 97 °C for ~60 min (Stökle *et al*., 2020). Powdered coffee waste was placed in a cellulose thimble (3.5 × 9.5 cm) inside a Soxhlet extractor. Oil was extracted with 300 ml n-hexane over ~4 h (Hanif *et al*., 2019), and the solvent was recovered by rotary evaporation. Saccharides recovered from bread, potato, and artichoke food-wastes, containing initial concentrations of glucose and fructose at 25 g/L and 15 g/L, respectively; were used for the cultivation of *C. necator*. Furthermore, Extracted Oil Coffee (EOC) obtained via Soxhlet extraction from coffee waste was supplemented at a specific concentration (i.e., 1.5 %, v/v) in order to boost PHB storage capacity (Ingram & Winterburn, 2021). Hydrolysates derived from agri-food by-products, with an initial sugar concentration of 5 g/L, were utilized as substrates in combination with EOC. The determination of the PHB purity and composition in all extracted samples has been performed by means of gas chromatography (GC) analysis. The thermal stability of the obtained PHB was investigated by thermogravimetric analysis (TGA), using a Mettler TG 50 thermobalance equipped with a Mettler TC 10 A processor. The most effective conditions were achieved by scaling up to a 3-litre pilot bioreactor with accurate control of oxygenation and pH.

3. Results and Discussion

3.1 Determination of the fermentable sugars released

The amount of glucose/fructose released was determined using the enzyme kit D-GLUCOSE (GOPOD format) KIT © and D-FRUCTOSE / D-GLUCOSE KIT © (Megazyme International Ireland, Ireland) following the manufacturer's instructions. The efficiencies of enzymatic hydrolysis of bread waste and acid hydrolysis of potato and artichoke wastes were assessed on glucose (Y_G) and fructose yield (Y_F). Results on hydrolysis treatments pointed out that Y_G accounted for 65 g/100g dry-bread and 32 g/100g dry-potatoes, while Y_F accounted for 33 g/100g dry-artichoke.

3.2 Bioplastic production

The protocol reported by Jacquel *et al*. (2008) was used for the extraction and purification of PHB from the dry biomass of *C. necator*. The method involved complete cell lysis as the first step, followed by separation of the biopolymer from the rest of the cellular components and finally recovery of the PHB with chloroform. The polymer yield (%) was calculated as [PHA (mg/L) / CDM (mg/L)] × 100 (Table 1). Saccharides recovered from bread, potato, and artichoke food-wastes, containing initial concentrations of glucose and fructose at 25 g/L and 15 g/L, respectively; were used for the cultivation of *C. necator*. Under these conditions, the polyhydroxybutyrate (PHB) intracellular content was 24 (%, wt/wt), 32 (%, wt/wt), and 26 (%, wt/wt), respectively. Hydrolysates derived from agri-food by-products, with an initial sugar concentration of 5 g/L, were utilised as substrates in combination with EOC. These strategies highlighted that the PHB intracellular content reached a value of 37 (%, wt/wt) from bread waste, 47 (%, wt/wt) from potato waste, and 45 (%, wt/wt) from artichoke waste. The same conditions were scaled up to verify the production of PHB by *C. necator* in a bioreactor. The growth data and polymer purity were comparable to those obtained in flask tests.

Table 4. *Yield of recovered biopolymer (PHB/CDM) by C. necator, obtained using different high-sugar substrates derived from food wastes with and without supplementation of extracted oil from coffee waste (EOC).*

	PHB/CDM (%)
Bw	24.14 ± 0.17
Pw	32.71 ± 0.15
Aw	25.61 ± 0.18
Bw-EOC	37.51 ± 0.19
Pw-EOC	46.75 ± 0.21
Aw-EOC	45.45 ± 0.33

Notably, the presence of EOC in the mixture of fermentable sugars obtained from organic wastes did not significantly affect the molecular weight (Mw). Indeed, Mw of polymer stored at 25 g/L of sugar derived from potato waste hydrolysis accounted for 104 KDa, while when EOC was supplemented, the Mw accounted for 226 KDa (Table 2). Generally, the Mw and PDI values herein detected are fully comparable with data collected by previous studies. However, EOC significantly enhanced microorganism growth and PHB accumulation (Bathia *et al*. 2018). These preliminary results demonstrated the feasibility of employing various food wastes as substrates or biopolymer production through the fermentation process of *C. necator*. Ongoing research aims to optimize the process using EOC as sole nutrient source. The next step in the project will be to develop an active packaging using commercially available polyhydroxybutyrate (PHB), which can be functionalised with natural antimicrobial and antioxidant biomolecules and proceed with shelf-life tests directly on packaged foods.

Table 2. *Weight average molecular weight (Mw), number average molecular weight (Mn) and polydispersity index (PDI = Mw/Mn) of PHB samples analysed by gel permeation chromatography (GPC).*

	Mn (kDa)	Mw (kDa)	PDI
Bw	54.16	153.9	3.6
Pw	50.66	103.8	1.7
Bw-EOC	140.5	213.7	2.2
Pw-EOC	112.2	225.9	2
Aw-EOC	125.5	277.2	2.2

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Evaluation of the ability to induce well-being and health by plant extracts and their constituents (P-II-56)

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The project evaluates *in vitro* the beneficial properties of polyphenol-rich plant extracts, such as apple "Mela rosa dei Monti Sibillini", pomegranate, and olive pomace, determining their bioactive characteristics - antimicrobial effect - and their potential prebiotic effect for a symbiotic formulation in which could act synergistically or complementarily with the bacterial part. Minimal Inhibition Concentration of extracts inhibiting the growth of pathogenic bacteria and probiotic, growth assessment of selected probiotic bacterial strains in the presence of the polyphenol-rich extracts, Short Chain Fatty Acid presence, and antimicrobial activity of Cell Free Supernatant of probiotic culture supplemented with extracts, were studied.

Valutazione della capacità di estratti vegetali e dei loro costituenti di indurre benessere e salute

Il presente progetto valuta *in vitro* le proprietà benefiche di estratti vegetali ricchi di polifenoli, come l'estratto di "Mela Rosa dei Monti Sibillini", di melograno e di sansa di oliva (cultivar Raggia) determinando le attività biologiche - effetto antimicrobico - e il potenziale effetto prebiotico, per una formulazione sinbiotica, sinergica o complementare verso la componente batterica. Determinata la Concentrazione Minima Inibente degli estratti sui patogeni e i probiotici in studio, valutata la crescita dei probiotici con gli estratti ed evidenziata la presenza di acidi grassi a catena corta e l'attività antimicrobica del liquido della coltura probiotica in presenza degli estratti.

Key words: Probiotics, pathogenic bacteria, plant extracts, Short Chain Fatty Acids, Cell Free Supernatant, polyphenols.

1. Introduction

The first phase of this PhD project is based on the evaluation of the beneficial properties of polyphenol-rich plant extracts through an *in vitro* study. Apple extract produced from "Mela rosa dei Monti Sibillini", pomegranate extract (cultivated in the Marche region), and olive pomace extract (from Raggia cultivar from the Marche region) were analysed for determining their bioactive characteristics related to their antimicrobial effect against some pathogenic bacteria and their potential prebiotic effect with the final aim to be a component of a hypothetical symbiotic formulation in which could act synergistically or complementarily with the bacterial part. Firstly, Minimal Inhibition Concentration tests were conducted to determine the concentrations of extracts needed to inhibit the growth of pathogenic bacteria (*Escherichia coli* ATCC13076*, Pseudomonas aeruginosa* DSM 1117*, Bacillus cereus* ATCC 9634*, Staphylococcus aureus* ATCC25923) and probiotic bacteria of choice (*Lacticaseibacillus rhamnosus* IMC501® and *Lactiplantibacillus plantarum* IMC509® - Synbiotec Srl, Camerino, Italy - and *Pediococcus acidilactici* 46A). Secondly, the growth of selected probiotic bacterial strains in the presence of the polyphenol-rich extracts was assessed. Lastly, the Short Chain Fatty Acid (SCFA) (LeBlanc et al., 2017) presence and the antimicrobial activity of Cell Free Supernatant (CFS) (Mani-López et al., 2022) of samples obtained from the growth of probiotics supplemented with extracts were studied.

In accordance with the PhD thesis project previously described, this poster reports the main results of the two branches of research.

(A1) The determination of Minimum Inhibitory Concentrations of plant extracts needed to suppress the growth of pathogenic and probiotic bacteria, and subsequent analysis of the potential of extracts to improve the growth of probiotic bacteria.

(A2) Assessment of properties of fermenting fluids produced by the extract-probiotic cultures.

2. Materials and Methods

For monitoring of inhibitory effect on probiotic strains and pathogens, the MIC test was performed. ELISA plates were used to distribute varying concentrations of extracts (ranging from 35% to 1%), 50µL of bacterial strains (OD = 0.100 for pathogens, OD=0.035 for probiotics), and de Man, Rogosa, Sharpe (MRS; VWR, Italy) or Tryptic Soy Broth (TSB; VWR, Italy) were added to reach final volume of 100µL. After 24/48 hours of incubation, the turbidity of each well was measured using a spectrophotometer at 570 nm wavelength. Both probiotic and pathogenic strains were subjected to the test to ensure the absence of inhibitory activity against probiotics and the antipathogenic effect of substances. Growth curves of probiotics in the presence of the substances of interest were constructed by monitoring 30-hour bacterial cultures. Each final probiotic culture broth was used to analyse the concentration of SCFA and produce CFS. The latter was then used to study its antimicrobial activity against

pathogenic strains through the Agar Well Diffusion Test (Huang et al., 2025).

3. Results and Discussion

3.1 Determination of Minimum Inhibitory Concentration

Firstly, the minimum inhibitory concentration was determined using the following formula

$$\%\, Inhibtion = 100 * (1 - \frac{x - min1}{max - min2})$$

where *x* is the absorbance of the well of interest, *min1* is the absorbance of the well that contains extract+broth and lacks bacteria, *max* is the absorbance of the well containing bacteria+broth, and *min2* is the absorbance of the well containing only broth. Extracts were considered to inhibit the growth of bacteria when the % inhibition was ≤50% (IC50) or above. To be noted that solvents used for the dilution of extracts have been used as controls in the MIC tests, and in most cases had no effect on the inhibition of bacterial growth. Based on the MIC results, appropriate concentrations of extract were selected to be used as supplements for probiotic bacterial cultures.

3.2 Assessment of properties of Cell Free Supernatant

One of the scopes of the study was to analyse the SCFA profile of probiotic culture fluid. It is well known that SCFAs contribute to host energy balance and are essential for host well-being (LeBlanc et al., 2017). SCFA profile of probiotic culture supplemented with selected plant extract was analysed through sample collection at different time points of probiotics growth. Interestingly, probiotic bacteria supplemented with polyphenol-rich extracts have not produced higher levels of SCFA, instead producing the same or slightly lower quantities.

CFS obtained from the bacterial culture is a complex matrix, containing fatty acids, organic acids, proteinaceous compounds, and other substances, a combination of which contributes to its antimicrobial and antifungal properties (Mani-Lopez et al., 2022). Well diffusion test results have revealed a decrease in the antimicrobial activity of the CFS. Preliminary results of CFS (*L. rhamnosus* IMC 501® supplemented with olive pomace extract) antimicrobial activity is represented in Figure 1. No evident changes in SCFA production and antimicrobial activity of CFS have stimulated further analysis to investigate the potential metabolic alterations caused by the selected extracts.

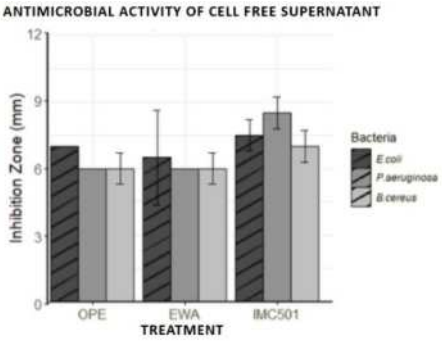


Figure 1: *Antimicrobial activity of cell-free supernatant produced from the growth of L. rhamnosus IMC 501® supplemented with olive pomace extract (OPE), EWA (mix of ethanol 20%: water 80%) used as a control of the effect of solvent. IMC501 stands for CFS of L. rhamnosus IMC 501® culture without any supplements.*

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Characterisation of plant-derived extracellular vesicles of different origin as innovative health food ingredients (P-II-58)

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In this work, the results of the research activities of first part of the PhD project are described. Plant-Derived Extracellular Vesicles (PDEVs) were obtained from three sources, namely hops, tea, and grapefruit by using optimized a standardized extraction procedure. The selection of the most promising plant matrix was driven by the results of the physical characterization (e.g. particle concentration, size distribution) of the PDEVs and scientific relevance. Then, hops-derived PDEVs, selected for the following studies, were evaluated and complemented by the analysis of their antioxidant activity and the key bioactive compounds as health and technological performance indicators.

Caratterizzazione di vescicole extracellulari da diverse fonti vegetali come ingredienti innovativi per alimenti funzionali.

In questo lavoro sono descritti i risultati delle attività di ricerca della prima parte del progetto di dottorato. Le vescicole extracellulari di origine vegetale (PDEV) sono state ottenute da tre fonti, ovvero luppolo, tè e pompelmo, utilizzando una procedura di estrazione standardizzata e ottimizzata. La selezione della matrice vegetale più promettente si è basata sui risultati della caratterizzazione fisica (es. concentrazione di particelle, distribuzione dimensionale) delle PDEV e dalla rilevanza scientifica. Successivamente, le PDEV derivate dal luppolo, matrice selezionata per gli studi seguenti, sono state sottoposte ad analisi per caratterizzare la loro attività antiossidante, e la composizione dei principali composti bioattivi come indici di proprietà salutistiche e performance tecnologiche.

Key words: Plant-derived extracellular vesicles, hop extracts, bioactivity characterisation.

1. Introduction

In accordance with the PhD thesis project previously described (Regi, 2024), this work reports the main results of the first part of the activities aimed to:

(A1) identifying the raw material for the extraction of plant-derived extracellular vesicles (PDEVs) intended for the production of natural, clean-label ingredients or additives for foods and non-food purposes. A standardised centrifugation-based methodology was applied on 3 raw plant sources, i.e. hops, tea leaves, and grapefruit produced according to organic procedures; extraction yield based on the physical parameters of the vesicles, including particle concentration and size distribution was taken as key criterium for the selection.

(A2) identifying raw material quality properties of the selected plant material that could affect the physical, health, and techno-functional properties of the PDEVs. Based on A1 results, hops were selected; the characterisation included physical measurements, bioactivity (ABTS and FRAP antioxidant assays), and quantification of bioactive compounds by HPLC of PDEVs extracted from cones or pellets from 2 different varieties.

2. Materials and Methods

For this study three plant matrices were selected, i.e. fresh grapefruit (*Citrus paradisi*), dried green tea leaves (*Camellia sinensis*) hop (*Humulus lupulus L.; cv. Hercules* and *Cascade),* in the form of dried cones and pellet. All plants were purchased from local suppliers. For the first step of selection, experiments were carried out on hops cones cv. Cascade, while in the second one, cones and pellets of cv. *Hercules* and *Cascade* were then used. Initial sample preparation was adapted to the specific matrix. Prior to extraction, dried hop cones and green tea leaves were ground into a fine powder and subsequently dispersed in water and kept overnight at 20°C. Grapefruit juice was obtained from the fresh fruits using a cold-press juicer (Hurom, Italy). Subsequently, all resulting liquids extract were processed to isolate nanovesicles according to the method proposed by Logozzi et al. (2021), with slightly modifications. Briefly, the juice and the leaves or cones dispersions were centrifuged at 2000 × g for 30 min and then filtered through 100 μm filters to eliminate cells and large debris. The clarified supernatant was further centrifuged at 15,000 × g for 30 min to remove and collect the microvesicle (MV) fraction. Then, the resulting supernatant was subjected to ultracentrifugation at 110,000 × g for 90 min (Sorvall WX Ultracentrifuge Series, Thermo Fisher Scientific). The final pellet, corresponding to the nanovesicles fraction (EXO), was resuspended in distilled water and then freeze-dried to a low moisture stable powder for further analyses. The particle size distribution and concentration of PDEVs were determined via Nanoparticle Tracking Analysis (NTA) using a NanoSight NS300 instrument (Malvern Panalytical, UK). To this aim fifty mg of the lyophilized PDEV material was diluted in particle-free phosphate-buffered saline (PBS) to reach the optimal particle concentration for analysis. Zeta potential was determined with a Zetasizer Nano ZS (Malvern Panalytical, UK); samples were appropriately diluted in a distilled water and analysis carried out at 25 °C.
The bioactive properties of the hops PDEVs were evaluated following the methodologies described by Tatasciore

et al. (2023) after dissolution of the freeze-dried extract in methanol Total Phenolic Content (TPC) was determined by the Folin-Ciocalteu method and results expressed as mg gallic acid equivalents (mg GAE) per g dry extract. The in vitro antioxidant capacity was assessed through the Ferric Reducing Antioxidant Power (FRAP) assay and the ABTS radical scavenging. For hop-PDEVs the analysis of the main bioactive compounds such as xanthohumol, α-acids, and β-acids by HPLC-UV applying the standard protocol from the American Society of Brewing Chemists (ASBC) using an isocratic elution of the mobile phase (1.5% v/v phosphoric acid solution and methanol) at a flow rate of 1.0 mL/min. Prior to injection, all samples were filtered (0.22 µm); quantification was based on external calibration curves generated from certified reference standards. All statistical analyses were conducted using RStudio (Version 2025.05.0; Posit, PBC, Boston, MA).

3. Results and Discussion

3.1 Selection of the plant matrix for PDEVs extraction

Figure 1 shows the physical characterization of the isolated nanovesicle fractions (NVs), as determined by Nanoparticle Tracking Analysis (NTA) of hops, green tea and pomegranate (A1).

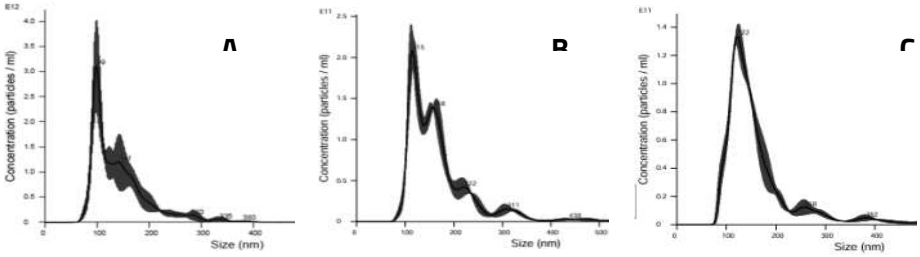


Figure 1. *Particle size distribution of nanovesicles isolated from hop (Humulus lupulus L) (A); green tea (Camellia sinensis) (B) and pomegranate (Citrus paradisi) (C).*

Evaluation of particle concentration revealed significant differences among the sources, being hops the matrix with higher yield, with the greatest concentration of NVs compared to both green tea and grapefruit. Results of the mean particle diameter of the NV fractions also showed source-dependent variations with hop- and green tea-derived vesicles representing the smallest (156 nm) and largest (205 nm) populations, respectively and grapefruit an intermediate size (177 nm). These results led to select hops for the following investigations on PDEVs health and techno-functionality. Hops were also selected also based on the growing interest of this plant source beyond the brewery sector and its significant scientific novelty.

3.2 Hops quality properties and physical, health, and techno-functional properties of the derived PDEVs

Being hops selected, two factors potentially affecting the quality and techno-functionality of PDEVs were investigated: pelletization (vs. dried cones) and cultivar. Pelletization is carried out at industrial level to enhance convenience in brew process, while cultivars are known present different composition of hops bioactives and flavours. The results of the bioactivity assays are summarized in Figure 2. In general microvesicle (MV) fractions consistently showed significantly higher Total Phenolic Content (TPC) and antioxidant capacity (FRAP, TEAC) than their corresponding exosome-like (EXO) fractions. Moreover, Hercules cones-MVs showed the highest in vitro bioactivity, confirming the relevance of the variety also for this study. The effect of pelletization on the Cascade variety was dependent on the specific assay. While no significant difference was observed in the TPC of MVs from cones versus pellets, FRAP and TEAC results were significantly lower in vesicles from pellets. These functional trends were confirmed by the composition of selected hops bioactive compounds by HPLC analyses (data not shown). Particle concentration, Zeta potential, showed significant differences attributable to both the hop cultivar and the processing type. A two-way Analysis of Variance (ANOVA) revealed that the hop source, the vesicle type (MV vs. EXO), and their interaction all had a highly significant effect ($p < 0.001$) on the results.

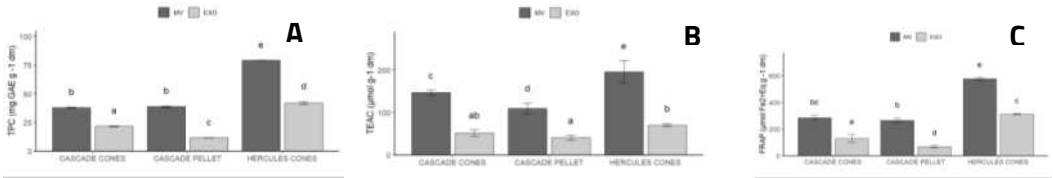


Figure 2. Resuts of the Total Phenolic Content (TPC) (A), Trolox Equivalent Antioxidant Capacity (TEAC) (B) and Ferric Reducing Antioxidant Power (FRAP) (C) of MV and EXO fractions of cones and pellets of two hops varieties. Data with different letters are statistically different at p< 0.001.

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Characterization of Pea Proteins, Soft Wheat Cultivars, Pomegranate and Derivatives to Design Products for Preconceptional and Pregnant Women (P-II-59)

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A comprehensive screening of selected raw materials was carried out in the second phase of the PhD project, to detect those richest in key nutrients (e.g. folate, iron) and develop healthy foods for preconceptional and pregnant women. Two pea protein concentrates, several pomegranate matrices – juice, squeezed juice and arils - some cultivars of soft wheat, and their by-products were analyzed. The aim was to evaluate their nutritional profiles, particularly in terms of micronutrients, to select the most promising ingredients. These results will guide the later stages of product formulation, improving the nutritional composition of the prototype

Caratterizzazione di proteine del pisello, *cultivar* di grano tenero e derivati di melagrana per la formulazione di alimenti per donne in fase preconcezionale e in gravidanza

Nella seconda fase del progetto di dottorato è stato effettuato uno screening completo di materie prime selezionate per identificare quelle più ricche di nutrienti chiave (ad esempio folati, ferro), con l'obiettivo di sviluppare alimenti sani per le donne in fase preconcezionale e in gravidanza. Sono stati analizzati due concentrati di proteine di pisello, diverse matrici di melograno - succo, spremuta e arilli - e *cultivar* di grano tenero e loro sottoprodotti. L'obiettivo è stato valutare i loro profili nutrizionali e selezionare gli ingredienti più promettenti. I risultati guideranno le fasi della formulazione del prodotto, permettendo una composizione ottimale del prototipo.

Key words: Pregnancy nutrition, plant-based ingredients, folate, whole grain, food design.

1. Introduction

Women in the periconceptional period have specific nutritional requirements essential to ensure the health of both mother and child. Ensuring an adequate supply of micronutrients - particularly folate, iron - remains crucial for proper foetal development (Barchitta, 2020). The objective of this work is to present the initial screening of some raw materials, to select those naturally rich in key micronutrients (such as folate and minerals) and bioactive compounds, for the formulation of foods tailored to the needs of women in the periconceptional period.

2. Materials and Methods

Three soft wheat cultivars: Coyote, Garbino and Providence, supplied by Florimond Desprez, from consecutive harvest seasons (2022/2024) were analysed. Two pea protein concentrates were from “Martino Rossi SpA”. Pomegranate juice and arils were kindly provided by “Parco Agricolo” (Rome), and organic squeezed juice was supplied by “Maira Bio” (Sicily). Kernels were ground with a Bühler laboratory mill MLI 204. To evaluate the effect of debranning process on Folate content, two Soft Wheat cultivars — Providence and Garbino, harvest year 2022, underwent three debranning levels: 9%, 12%, and 15%. These varieties were chosen for their promising preliminary results. Total Folate Content was determined with *VitaFast® Folic Acid kit from R-Biopharm*. The Total Polyphenol Content was measured using the Folin-Ciocalteu method. Mineral content was analysed by ICP-OES, fibre by enzymatic-gravimetric method. A one-way analysis of variance (ANOVA) employing the Kruskal–Wallis non-parametric test was carried out at a significance level of 5%.

3. Results and Discussion

3.1 Folate Content in Soft Wheat Cultivars: A Comparison Across Harvest Years and Milling Fractions

Table 1 *Folate content in soft wheat cultivars across three different harvest seasons. N.B. Different letters on the same row indicate values that are significantly different (P < 0.05).*

	Folate (µg/100 g)		
Harvest year	**2022**	**2023**	**2024**
Soft wheat grains, cv Coyote	21.5 ± 1.8a	38.9 ± 3.3b	35.6 ± 2.3c
Soft wheat grains, cv Garbino	22.7 ± 1.6a	36.0 ± 6.0b	34.3 ± 3.0b
Soft wheat grains, cv Providence	28.1 ± 4.4a	40.3 ± 5.9b	32.0 ± 3.6a

The folate content is given in Table 1. The value differs between wheat cultivar and between years. In 2022, levels were generally lower than in 2023, with Providence showing the highest value (28.1 ± 4.4). In 2023, all varieties

reached peak values with consistently higher folate levels. The highest value was observed in Providence (40.3 ± 5.9). In 2024, folate content decreased compared to 2023 but remained higher than the value recorded in 2022. These fluctuations reflect environmental factors.

Table 2 *Comparison of two soft wheat cultivars (Garbino and Providence), highlighting the folate content in debranned grains and corresponding debranning by-products at different levels.*
N.B. Different letters on the same row indicate values that are significantly different (P < 0.05)

Cv Garbino 2022	Folate (µg/100 g)	Cv Providence 2022	Folate (µg/100 g)
9% Debranned Grain	20.3 ± 5.0 a	**9% Debranned Grain**	21.0 ± 4.1a
9% Debranning by-Products	103.6 ± 4.6 a	**9% Debranning by-Products**	84.0 ± 0.3b
12% Debranned Grain	26.6 ± 5.1a	**12% Debranned Grain**	22.2 ± 3.3a
12% Debranning by-Products	52.6 ± 7.1a	**12% Debranning by-Products**	69.7 ± 10.1b
15% Debranned Grain	26.7 ± 5.4a	**15% Debranned Grain**	17.3 ± 2.6b
15% Debranning by-Products	73.2 ± 7.0a	**15% Debranning by-Products**	78.8 ± 13.4a

The results (Table 2) show that debranning by-products from Garbino and Providence have much higher folate levels than debranned grains, especially at 9%. This suggests potential for using milling by-products to formulate flour blends naturally enriched in micronutrients. Debranning processes are currently underway on the 2023 and 2024 harvests to complete the screening.

3.2 Protein, Folate, Polyphenols and Minerals content of Pea Concentrates and Pomegranate derivatives

The results (Table 3) show that Pea protein concentrates have high levels of folate, polyphenols, protein and key minerals such as magnesium and zinc. Pomegranate products vary in nutrient content, with arils having the highest levels of folate. Squeezed juice contains more nutrients than classical juice. Each product offers specific nutritional characteristics that can support different functional food applications.

Table 3 *Protein, Folate Content, Total Polyphenols Content (TPC), Mineral Profile in Pea Protein Concentrates and Pomegranate derivatives. (nd= not detected; ND = not determined).*

	Protein %	Folate (µg/100g)	TPC (g GAE/100 g)	Magnesium (µg/g)	Iron (µg/g)	Calcium (µg/g)	Zinc (µg/g)
Pea Protein Concentrate I	60.3	28.4 ± 1.6	213.5 ± 3.0	1644.9 ± 49.6	67.8 ± 3.9	860.8 ± 23.3	54.6 ± 1.7
Pea Protein Concentrate II	55.1	18.1 ± 0.6	166.6 ± 8.7	1424.0 ± 55.6	71.7 ± 4.7	900.6 ± 33.6	48.6 ± 1.3
Pomegranate juice	nd	2.2 ± 0.1	0.2 ± 0	52.1 ± 1.2	nd	47.2 ± 1.4	1.4 ± 0
Pomegranate squeezed juice	0.6	23.7 ± 0.5	0.5 ± 0	55.8 ± 1.1	nd	22.35 ± 7.51	1.1 ± 0
Pomegranate arils	ND	28.9 ± 1.6	2.6 ± 0.1	ND	ND	ND	ND

The selection of naturally nutrient-rich ingredients is a key step in the development of functional, tailor-made food products for pregnant women, in accordance with the design thinking methodology. The ingredient screening was conducted in parallel with a target group analysis, combining laboratory data with an understanding of the users' specific needs and perceptions. This transdisciplinary approach enables the creation of meaningful solutions that go beyond conventional product development.

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Valorisation of Brewer's Spent Grain as a Functional Ingredient to Improve Nutritional and Health Quality in Dry Pasta Formulation (P-II-60)

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Fresh BSG were dried to obtained flour (BSGF) and a physical, chemical, nutritional, and technological characterization was carried out. Three formulations of semolina pasta extruded in bronze in the "tagliatelle" format enriched with three different percentages of BSGF (PBSG25, PBSG40, PBSG50) were created. The formulations were compared with a positive control consisting of semolina (PS) and a negative control consisting of wholemeal semolina (PW). Pasta samples were dried at low temperatures, specifically 40°C for 2 hours, in other to preserve the functional characteristics of the pasta. The different formulations of dry pasta were characterized from a chemical-physical, nutritional and technological point of view. To evaluate the beneficial potential for the body, preliminary in vitro digestion tests of the pasta and inhibition tests of the alpha glucosidase enzyme, essential for digestive purposes, and alpha amylase were conducted.

Valorizzazione di trebbie di birra come ingrediente funzionale per migliorare la qualità nutrizionale e salutistica di diverse formulazioni di pasta secca

Le trebbie (BSG) conferite presso il laboratorio di Tecnologie Alimentari del Di3A sono state essiccate per ottenere la farina (BSGF) ed è stata effettuata una caratterizzazione chimico-fisica, nutrizionale e tecnologica. La farina ottenuta è stata impiegata per la formulazione di pasta. In particolare, sono state realizzate tre formulazioni di pasta di semola trafilata al bronzo nel formato "tagliatelle" arricchita con tre diverse percentuali di BSGF (PBSG25, PBSG40, PBSG50). Le formulazioni sono state confrontate con un controllo positivo costituito da semola (PS) e un controllo negativo costituito da semola integrale (PW). I campioni di pasta sono stati essiccati a basse temperature, nello specifico a 40°C per 2 ore, al fine di preservare le caratteristiche funzionali della pasta. È stata condotta una caratterizzazione chimico-fisica, nutrizionale e tecnologica di tutti i campioni di pasta realizzati al fine di valutarne le caratteristiche qualitative e funzionali. Per valutare il potenziale benefico per l'organismo, sono stati condotti prove preliminari di digestione in vitro della pasta e test di inibizione dell'enzima α- glucosidasi, cruciale ai fini digestivi, e α- amilasi.

Keywords: by-product**,** brewers' spent grain, functional pasta, formulation, enzymatic inhibition, upcycling

1. Introduction

In accordance with the PhD thesis project previously described, this poster reports the main results of the first activities concerning:

- Storage and drying of BSG to obtain flour (BSGF) and determination of chemical-physical and nutritional characteristics such as moisture, water activity, proteins, ashes, lipids, reducing sugars, total dietary fibers, ß-glucan, and total carbohydrates and technological characteristics such as water binding capacity (WBC) and oil binding capacity (OBC) for the formulation of dry pasta.
- Evaluation of the chemical-physical and nutritional characteristics of the 5 formulated dry pasta and of the technological properties such as: Optimal Cooking Time (OCT), Water Absorption (WA) and Cooking Loss (CL) and yellow index.
- Evaluation of the health properties of pasta through in vitro digestion tests, inhibition of α-glucosidase and α-amylase.

2. Materials and methods

Brewers' spent grain (BSG) was provided by the "24 Baroni" brewery, a craft brewery located in Nicosia (EN), Italy. The sample provided by the company consisted of 60% barley malt (Pilsner) and 40% wheat and was used to produce top-fermented Blond Ale. The content of proteins, ashes, lipids, and reducing sugars were also analysed according to the standard methods described by the Association of Official Analytical Chemist (AOAC, 2007). Total Dietary Fibre (TDF) content was measured using an enzymatic kit for fibre determination (Bioquant, Merck, Darmstadt, Germany) according to the Official Method 991.42 (AOAC, 1995). β-glucan content was evaluated by the Megazyme Mixed-Linkage Beta-Glucan kit (EBC method 4.16.1, 1998), according to the instructions. The color parameter of PS, PW, and different dried pastas were analysed on all the formulations made. The determinations were carried out using a portable colorimeter Konica Minolta CM-2500d (Bremen, Germany) using the CIELab color space coordinates L (brightness), a* (red-green chromaticity) and b* (yellow-blue chromaticity) and the illuminant D65. For cereal products, browning is generally expressed as 100-L. Pasta cooking quality was assessed by cooking test, according to the AACC method 66-50.01 (AACC, 2009). In vitro digestion was performed according to Goñi et al. (1997), while α-amylase inhibition activity was estimated according to the method of Anisha et al. (2023) and the α-glucosidase inhibition assay was performed as reported by Sferrazzo et al. (2022) with which modification

3. Results and Discussion

3.1 Physicochemical and nutritional characterization of pasta formulations

The high protein (20.13 ± 0.37%), and fiber content (65.00 ± 0.06%) of BSGF has been used as a reference for the different formulations. Table 3 shows the physical-chemical and nutritional characteristics of the pasta formulations. It is interesting to note the moisture (%), similar to that established by law for semolina pasta, proteins and fibres content and in particular the β-glucan of PBSG50 compared to PS. These values are higher than those reported by Nocente et al., 2019, and allow us to define this pasta as functional and high in fiber and protein.

Table 3. Results of physico-chemical characterization of different dry pasta formulations

Parameters	PS	PBSG25	PBSG40	PBSG50	PW
Moisture (%)	12.50±0.00^{a}	12.50±0.00^{a}	12.50±0.00^{a}	12.50±0.00^{a}	12.50±0.00^{a}
Activity water (Aw)	0.65±0.04^{b}	0.55±0.04^{a}	0.6280.07±^{b}	0.67±0.08^{b}	0.64±0.06^{b}
Lipids (%)	1.6±0.22^{b}	1.8±0.12^{b}	1.9±0.23^{b}	2.0±0.12^{b}	3.5±0.09^{a}
Proteins (%)	12.00±0.00^{d}	16.43±0.11^{c}	18.04±0.17^{b}	20.18±0.32^{a}	14.06±0.11^{d}
Ashes (%)	0.91±0.07^{c}	0.81±0.03^{d}	1.29±0.05^{b}	1.62±0.05^{a}	0.95±0.06^{c}
Reducing sugar (%)	0.53±0.10^{c}	0.62±0.04^{b}	1.00±0.06^{a}	1.25±0.08^{a}	0.72±0.05^{b}
β-glucans (%)	0.25±0.03^{c}	0.43±0.07^{d}	0.63±0.05^{c}	0.83±0.10^{b}	1.53±0.04^{a}
Total dietary fibers (%)	3.56±0.32^{c}	20.03±0.11^{b}	29.96±0.25^{a}	36.50±0.22^{a}	16.43±0.11^{b}
Total carbohydrates (%)	84.2±0.23^{a}	81.4±0.13^{a}	79.6±0.14^{a}	78.5±0.24^{a}	64.3±0.35^{b}

Data expressed as the mean ± standard deviation. In each column, values followed by different letters are significantly different (p < 0.05).

3.3 Technological parameters of pasta formulations

Table 4 shows how PS, i.e. 100% semolina, requires the longest cooking time (8 min), due to the more robust gluten structure and the greater quantity of starch (Chillo et al., 2011). PS shows a lower loss (CL) than pasta added with BSG flour, except for the 25% one. PBSG40 and PBSG50 show higher values of cooking loss (Table 4), presumably due to the high fibre content which weakens the gluten matrix (Nocente et al., 2021). WA increases with increasing OCT, therefore this variable also influences the reported WA values.

Table 4. Results of technological properties of different dry pasta formulations

Samples	OCT	CL	WA
PS	8 min	6.13%^{b}	130.67%^{a}
PBSG25	5 min	5.25%^{c}	109.26%^{c}
PBSG40	5 min	8.31%^{a}	103.14%^{c}
PBSG50	5 min	8.56%^{a}	95.68%^{d}
PW	6 min	5.33%^{c}	119.50%^{b}

Data expressed as the mean ± standard deviation. In each column, values followed by different letters are significantly different (p < 0.05).

Data expressed as the mean ± standard deviation. Data expressed as the mean ± standard deviation. In each column, values followed by different letters are significantly different (p < 0.05).

Table 5. Colorimetric parameters of pasta formulation

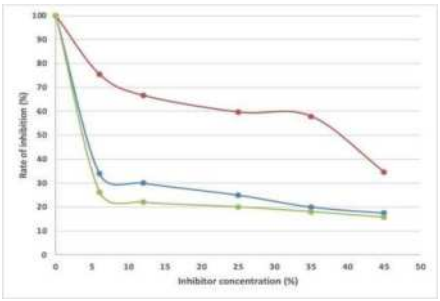
		b*	SD	100-L*	SD	a*	SD
PS		13.68^{b}	0.77	29.84^{d}	0.65	1.25^{c}	0.08
PBSG25		14.23^{b}	0.19	41.17^{b}	0.45	2.00^{b}	0.06
PBSG40		15.20^{a}	0.29	44.11^{a}	0.46	2.65^{a}	0.14
PBSG50		15.37^{a}	0.23	44.87^{a}	0.35	2.76^{a}	0.08
PW		14.83^{a}	0.50	39.66^{c}	0.61	2.61^{a}	0.61

PBSG50 is much darker than PS, as shown in Table 5: in fact, the 100-L* value goes from 28.44 ± 0.65 to 44.87 ± 0.35. It is also important to note that the pasta with the highest percentage of substitution (PBSG50) does not differ much from the negative control (PW) produced with wholemeal semolina, especially in the values b* (15.37 ± 0.23 for the 50% pasta and 14.83 ± 0.50 for the negative control) and a* (2.76 ± 0.08 for the 50% pasta and 2.61 ± 0.61 for the negative control). Nocente et al., 2019 used a semolina whose yellow (b* = 21.7) and brown (100-L*=10.7) indices are in the good quality range for semolina.

3.5 Preliminary evaluation of α-glucosidase and α-amylase enzymatic inhibition

Figures 2a and 2b show preliminary results of the inhibitory activity of α-glucosidase as a function of the substrate concentration (%) of PBSG50 and a linearization of the inhibition, respectively. PBSG50 had an inhibitory activity of about 15% on the enzyme α-amylase, however, tests are underway to optimize the method to demonstrate in vitro the functionality of this paste in postprandial digestion and reduction of the glycemic index. In conclusion, the possibility of using PBSG50 to inhibit α-amylase and alpha-glucosidase represents a very interesting and promising strategy for the management of postprandial glycemia and a complementary or alternative approach in the prevention and treatment of type 2 diabetes.

a)



b)

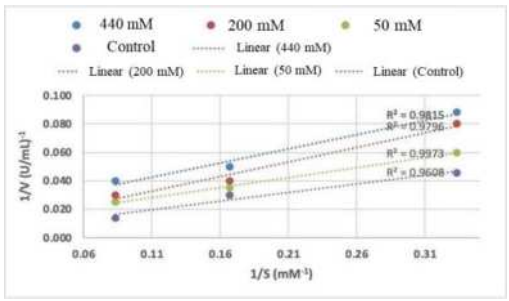


Figure 2. a) Inhibition of PBSG α-glucosidase in relation to substrate concentration (%); b) Linearization of inhibition of PBGS50 α-glucosidase

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Modulatory Effects of Nutritional Supplements on Gut Microbiota: An *In vitro* Study on Crohn's Disease and Healthy Donors (P-II-61)

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PhD Program: Agricultural, Environmental and Food Sciences and Technologies
Topic: Food Science and Biotechnology; Doctoral Cycle: XXXIX
Tutor: Andrea Gianotti; Co-tutor: Lorenzo Nissen

This PhD study employs the in vitro intestinal model MICODE to investigate the effects toward the gut microbiota of dietary supplements formulated to support gut health.

Effetti modulatori di integratori alimentari sul microbiota intestinale: Uno studio *in vitro* su individui con morbo di Crohn e individui sani.

Questo studio di dottorato utilizza il modello intestinale *in vitro* MICODE per esaminare gli effetti verso il microbiota intestinale umano di integratori alimentari formulati per il mantenimento del benessere intestinale.

Key words: Gut Microbiota, Crohn disease, *in vitro* gut model.

1. Introduction

In accordance with the PhD thesis project previously described, this poster reports the main results:

(A1) Modulation of microbial population and production of microbial metabolites after digestion and colonic fermentation with GM of healthy subjects.

(A2) Modulation of microbial population and production of microbial metabolites after digestion and colonic fermentation with GM of subjects affected by non-communicable Chron's disease.

2. Materials and Method

Human gut microbiota (GM) was sourced from fecal samples, the collection followed established protocols from previous studies (Nissen *et al*., 2023). For sample collection, donors were supplied with a collection kit, which included a stool collector (Sarstedt AG & Co. KG, Nümbrecht, Germany) and an anaerobic jar containing an O2-consuming catalyst (Oxoid, Thermo Fisher Scientific, Waltham, MA, USA). A 10% (w/v) gut microbiota (GM) suspension was freshly prepared from human fecal donations by homogenizing 6 g of stool in 54 mL of phosphate-buffered saline (PBS) (Cattivelli *et al*., 2023; Wang *et al*., 2020). Since the nutritional supplements were gastro-resistant, oro-gastro-duodenal digestion was bypassed, and fermentation was performed directly. Samples were collected at three time points: baseline (BL), intermediate (T1 = 4 h), and endpoint (EP = 24 h), and were used to assess microbial populations and metabolite production. Fermentations were performed in MICODE, simulating proximal colon conditions (Gianotti *et al*., 2025), following standardized procedures (Connolly *et al*., 2012). Supernatants were stored for SPME-GC-MS analysis, while pellets were used for DNA extraction. qPCR was performed using SYBR Green I chemistry (Tamargo *et al*., 2022; Tanner *et al*., 2014; Westfall *et al*., 2018), targeting key bacterial taxa. Volatile analysis was carried out using an Intuvo 9000 GC coupled with a 5977B mass spectrometer (Agilent Technologies, USA) (Di Cagno *et al*., 2011). Samples were added with internal standards and analyzed after SPME fiber exposure. VOCs were identified using spectral libraries (NIST 11, NIST MS Search 2.0) and quantified based on relative abundance (LOD = 0.001 mg/kg) (Bonfrate *et al*., 2020). Statistical analysis and graphics were generated using Rstudio software (Posit, Boston, MA, USA).

3. Results and Discussion

3.1 Quantification of microbial populations and production of metabolites

Preliminary data on microbial population modulation suggest that both essential oil-based supplements lead to a slight increase in beneficial taxa (Fig. 1A) and a modest reduction in potentially harmful bacteria (Fig. 1B). This trend is consistent with previous studies on the activity of essential oils such as D-limonene and lavender (Nissen et al., 2021; Hawrelak et al., 2009). For beneficial metabolites (short-chain fatty acids) no significant increase was observed in (Fig. 2). Sample distribution analysis indicates that time is the main differentiating factor (Fig. 3C). Samples collected at different timepoints show clear separation, suggesting a marked temporal evolution. Regarding molecular distribution (Fig. 3D), compounds such as D-limonene, β-pinene, γ-elemene, and camphene appear to contribute more than caryophyllene and umulene (Nissen et al., 2021).

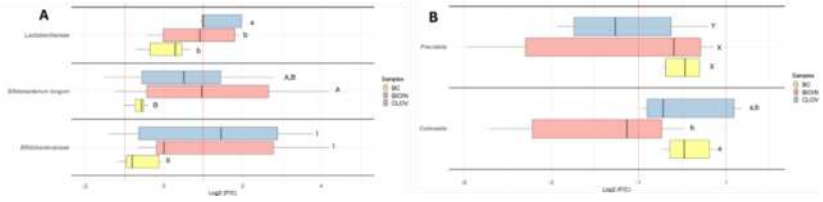


Figure 11 *Box plots of Log_2(F/C) shifts of A) beneficial groups and B) opportunistic groups of the gut microbiota.*

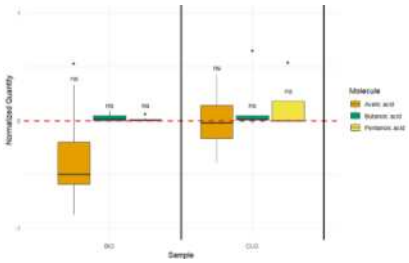


Figure 12 *Change during colonic fermentation of positive microbial metabolites.*

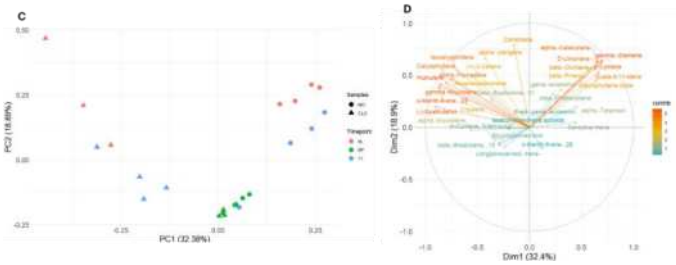


Figure 13 *Plots of Principal component analysis (PCA) C) of cases and D) dependent variables.*

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Harnessing microbial resources for innovations in cocoa fermentation (P-II-62)

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The activities of this PhD project aim to utilize the cocoa microbiota as an element of innovation in chocolate production. The work presented focuses on the first two activities of the project. First, phenotypic characterization of strains to understand their fermentative potential using novel and conventional methods. Next, the shortlisted strains were used for fermentation. The goal was to ferment novel, non- cocoa metrices, understanding growth dynamics of native cocoa strains.

Sfruttamento delle risorse microbiche per fermentazioni innovative nel cacao

Le attività di questo progetto di dottorato mirano a utilizzare il microbiota del cacao come elemento di innovazione nella produzione di cioccolato. Il lavoro presentato si concentra sulle prime due attività del progetto. In primo luogo, la caratterizzazione fenotipica dei ceppi per comprenderne il potenziale fermentativo utilizzando metodi innovativi e convenzionali. In secondo luogo, i ceppi selezionati sono stati utilizzati per fermentazione. L'obiettivo era quello di sviluppare nuove metriche non legate al cacao, comprendendo le dinamiche di crescita delle varietà di cacao autoctone.

Key words: cocoa, fermentation, metabolic activity, carob, BIOLOG.

1. Introduction

In accordance with the PhD project previously described, this poster reports the following:

(A2) Phenotypic characterization using novel technologies to map the mechanism of action and biochemical characteristics of the identified yeast and bacteria isolated from cocoa farms: (A2.1) metabolic activity of microorganisms (A2.2) carbon source utilization assays for understanding strain-substrate corelation.

(A3) Development of inoculations using the most promising strains: (A3.1) growth dynamics of strains on novel raw material (A3.2) impact of microorganisms on the organoleptic properties of raw material

2. Materials and Methods

2.1 Strain Screening

One-hundred and two yeast strains and forty-three lactic acid bacteria (LAB) from cocoa plantations in West Africa were explored in this study. The yeasts were investigated by high throughput sequencing 26S rRNA genes. The identified isolates were screened qualitatively for 5 enzymatic activities (Vasileios et al., 2015). The yeasts with enzymatic activities were then explored qualitatively and quantitatively for their ability to metabolize over 90 substrates using the OMNILOG technology. The LAB strains were biotyped using rep-PCR to group the most abundant strains in the collection. The shortlisted strains were tested for their ability to use different carbon sources, their ability to acidify a medium and produce citric lyase by degrading citric acid.

2.2. Starter Culture Selection and Fermentation

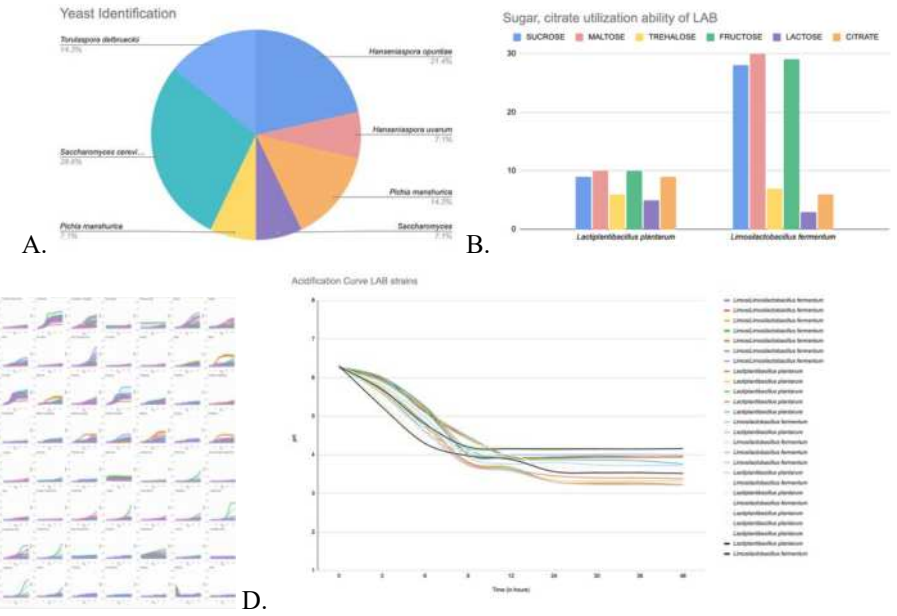
Fermentation with select strains was carried out on raw carob (2mm sized pieces). Preliminary study of microbial growth across time and pH was carried out. Water content was optimized for minimal rehydration needed to support microbial growth for each substrate to carry out Solid State Fermentation (SSF). A sensory analysis of the fermented materials was carried out to detect desirable outcomes.

3. Results and Discussion

3.1 Strain Screening

13.7% of the yeasts were positive for protease, esterase, pectinase and/or β-Glucosidase activity. The DNA extracts from these yeasts were used to amplify the variable D1/D2 region at the 5' end of the large-subunit (26S) ribosomal DNA (rDNA) using primers NL1 (5′ - GCATATCAATAAGCGGAGGAAAAG - 3′) and NL4 (5′ - GGTCCGTGTTTCAAGACGG - 3′) for species confirmation (Kurtzman & Robnett, 1997). These were then compared with sequences present in the NCBI database using nucleotide BLAST (Fig. 1A). These strains were then analysed for their ability to utilize 96 carbon sources (Fig.1C). All LAB strains were able to utilise fructose, sucrose and maltose while 34.88% were able to produce citric lyase and 90%, 18.6%, 32.5% were able to metabolise fructose, lactose and trehalose respectively (Fig. 1B). The LAB strains also reduced the pH of the medium from 6.4 to a range of 4.17 to 3.21 in 48hrs (Fig. 1D).

Figure 1.
A. Yeast Species identification
B. LAB carbon utilization profile
C. Yeast substrate utilization profile
D. LAB acidification curve

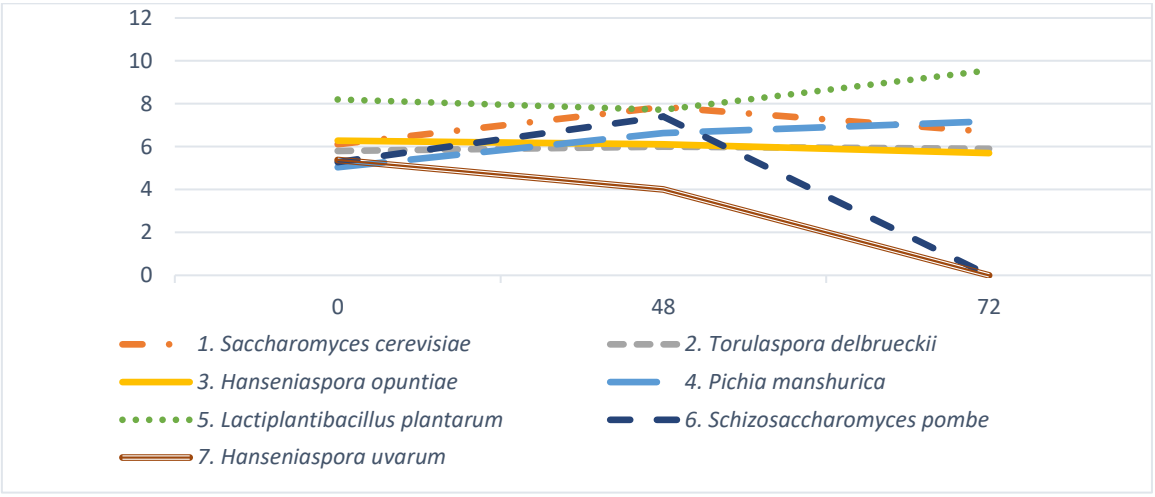


A. B. C. D.

3.2 Starter Culture Selection and Fermentation

The strains tested showed varying fermentation efficiency in a novel matrix of raw carob (rehydrated to over A_w 0.9). The rehydrated raw material was inoculated with 10^6 log for yeast and 10^8 for LAB at 25°C in sterile plastic containers. The growth was observed at the time of inoculation as well as after 48 hours and 72 hours. The growth was observed on Wallerstein Laboratory Nutrient Agar and De Man-Rogosa-Sharpe agar (MRS agar) for yeasts and LAB respectively for the plate count (CFU/mL). In Fig. 2 below, the growth curves in log CFU/mL (Y axis) are illustrated for the different strains and mixes inoculated across 72 hours (X axis).

Figure 2. Log Growth of Strains in Raw Carob across 72 hours (sampling at Time 0, after 48 hours and after 72 hours)



It is therefore possible to conclude that the raw material can support the growth of certain strains. None of the fermentations showed a significant change in pH compared to control. The next step is to analyse the chemical and functional changes in the fermented material. The preliminary sensory analysis also indicated that each strain had some impact on the smell of the fermentative material, compared to control across time. To properly assess the impact of fermentation on the sensory quality, it is important to further induce Millard reaction. This releases the volatile compounds from the flavour pre-cursors developed during fermentation. The next step of the project would further explore this by conducting drying and roasting to measure the impact of the fermentation compared to control using GC/MS and a more detailed sensory analysis of products made using the fermented matrix. This knowledge can aid in the development of novel food products using carob that enhance taste and texture while promoting responsible consumption, as carob is a sustainable crop that requires fewer land and water resources.

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Development of probiotics and post-biotics for the modulation of skin microbiome (P-II-63)

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The first two activities of the PhD thesis project are described. Bacteria normally residing on human skin were isolated and characterized, selecting both beneficial bacteria (*Lactobacillus* strains) and potentially pathogenic ones (*Cutibacterium acne*). The beneficial and protective effects of *Lactobacillus* strains, in the form of postbiotics (exosomes isolation), were analyzed in relation to the skin and their antagonistic activity against *C. acne*.

Sviluppo di probiotici e post-biotici per la modulazione del microbioma della pelle

Le prime due attività del progetto di tesi di dottorato sono descritte. Sono stati isolati e caratterizzati batteri che normalmente vivono sulla pelle dell'uomo, selezionando batteri benefici (*Lactobacillus* strains) e batteri potenzialmente patogeni (*Cutibacterium acnes*). Sono stati analizzati gli effetti positivi dei lattobacilli sulla pelle e protettivi nei confronti di *C. acnes* sotto forma di post-biotico mediamente l'isolamento degli esosomi.

Key words: Skin bacteria, *lactobacillus*, post-biotics, exosomes, skin health.

1. Introduction

This poster reports the main results of the first two activities concerning:

(A1) the studying of the skin microbiome and the isolation of different skin bacterial species to select specific microorganisms that can interact with the skin cells and with the other skin bacteria

(A2) the selection of *Lactiplantibaccilus plantarum* e *Cutibacterium acnes* to study their interaction and effect. The analysis of the *Lactobacillus* beneficial effect on the human skin through their exosomes and their potential protection against *C.acnes*

2. Materials and Methods

2.1 Isolation of new skin bacteria and their characterization.

Human skin was used to isolate new bacteria strains. 55 human research participants were sampled on the face and on the back. Participants were healthy volunteers, aged 18–50, with no history of chronic skin conditions or autoimmune diseases. The participants were sampled from the face and the back by swabbing with cotton swabs saturated in 50 mM Tris (Amresco), 1 mM EDTA (Amresco), 0.5% Tween 20 (Sigma) in nuclease-free water for 30 s. For all participants, swabs were plated immediately on the surface of selective Lactobacillus media (de Man Rogosa Sharpe agar, MRS, Oxoid) and on Reinforced clostridial medium (Oxoid), incubated at 37°C in anaerobic and aerobic condition for 48h. After incubation, colonies with distinct morphologic characteristics were streaked onto MRS and RCM agar and incubated in the same conditions for other identifications. Once obtained pure cultured, the preliminary identification was done using classic microbiology tests including morphology by microscopy, Gram-staining, and catalase. The remaining strains of interest were used to amplify the 16S rRNA. A single colony for every different bacterium was inoculated in 10ml of MRS and incubated for 48h at 37°C in anaerobic condition using AnaeroGen 2.5L (Thermo Scientific). 1,8ml of these cultures were used to do DNA extraction using the DNeasy UltraClean Microbial Kit (Qiagen). We amplified the 16S rRNA with the universal primer. 2ul DNA, 5ul buffer, 2,5ul MgCl2, 0,5ul dNTPs 25uM, 0,1ul for each primer 100uM, 0,5ul Taq, up to 50ul Nuclease-free water were used for the reaction mix. The Sanger sequencing was used to analyze the rRNA and only the DNA of the bacteria resulted as the *Lactobacillus*, *Cutibacterium*, strains were used to sequence the entire genome.

2.2 Exosomes isolation

L. plantarum was cultured in 1 L of MRS broth and incubated overnight at 37°C under aerobic conditions at 80rpm. After incubation, the culture was centrifuged at 4,000 × g for 15 minutes at 4°C to pellet the bacterial cells. The resulting supernatant was carefully collected.

2.3 Antioxidant tests.

For this process, 1.8 × 104 HaCaT were seeded in 96-well plates and grown for 20 h. The cells were then treated for 2 h with 10^6/mL exosomes of *L. plantarum* or with 500 µM ascorbic acid, used as the positive control. After that, they were washed in PBS and incubated at 37 °C with 100 µL/well of a solution containing: 10 mM of Hepes, 1.3 mM CaCl2, 1 mM MgSO4, 5 mM of glucose and 5 µM CM-H2DCFDA. After 45 min, a PBS wash was performed and the baseline fluorescence intensity of the cells was measured at 535 nm (excitation 485 nm), using the instrument EnVision (PerkinElmer, Waltham, MA, USA). Then, the oxidative stress was induced by adding *C.acnes* OD: 0,5 and the fluorescence of the samples measured after 30 min. Data were obtained with five biological replications.

2.4 Wound healing. For scratch test, HaCaT keratinocytes were seeded on 6-well tissue culture plates. When cells

reached the confluence, a scratch was introduced into the monolayer by using a sterile p10 pipette tip. Medium were then replaced with fresh DMEM medium plus TGFb (positive control samples) or DMEM medium containing 10^6/mL exosomes of *L. plantarum*. After 20h, scratches were monitored, and pictures were acquired. Quantification of scratch closure was performed by comparing the area of the scratch at the beginning of the experiment and the time point at the end of evaluation. Data were obtained from three independent experiments.

3. Results and Discussion

3.1 *L.p.* exosomes isolatation

Extracellular vesicles (EVs) were successfully isolated from *L. plantarum* using tangential flow filtration (TFF). Nanoparticle tracking analysis (NTA) was performed using the ZetaView® PMX-120 system (Particle Metrix, Germany), equipped with a 488 nm laser (40 mW) and a CMOS camera sensor, to determine the size distribution and concentration of the vesicles. The EVs exhibited a median particle size of 89.8 ± 6.2 nm, consistent with the expected dimensions for bacterial extracellular vesicles. The particle concentration was estimated at 1.5×10^{11} particles/mL, corresponding to a total yield of approximately 7.7×10^{12} particles for the entire preparation.
Protein concentration in the final EV preparation was measured at 0.02 mg/mL. The overall purity of the EV sample, defined as the ratio of particles to protein content, was calculated to be 6.9×10^{12} particles per milligram of protein, indicating a highly purified vesicle preparation.

3.2 *L. plantarum* exosomes showed antioxidant effects against *C.acnes*

To assess the antioxidant potential of *L. plantarum*-derived exosomes, intracellular reactive oxygen species (ROS) levels were measured in HaCaT keratinocytes subjected to oxidative stress induced by *C.acnes*. Cells were pre-treated for 2 hours with either L. plantarum exosomes or a positive control (ascorbic acid). Oxidative stress was then triggered by *C. acnes* exposure. Upon oxidation, the fluorescent probe formed a stable adduct, allowing quantification of intracellular ROS levels. *C. acnes* exposure led to a significant increase in cytosolic ROS, with fluorescence intensity rising by approximately 40% compared to unstimulated controls. Pre-treatment with *L. plantarum* exosomes resulted in a 20% reduction in ROS levels, while cells treated with ascorbic acid showed a 15% decrease relative to the *C. acnes*-only group. These findings indicate that *L. plantarum*-derived exosomes exert a measurable antioxidant effect in keratinocytes under bacterial-induced oxidative stress, outperforming the conventional antioxidant control in this model.

3.2 *L. plantarum*-derived exosomes promote keratinocyte re-epithelialization in a Scratch Assay

The wound healing potential of *L. plantarum*-derived exosomes was assessed using an in vitro scratch assay on human keratinocyte monolayers. Following mechanical disruption of the confluent cell layer, exosomes were added to the culture medium, and the extent of wound closure was evaluated over time by quantifying the re-epithelialized area. Treatment with *L. plantarum* exosomes accelerated scratch closure compared to the untreated control group. Quantitative image analysis revealed that 79% of the initial wound area was re-epithelialized in the exosomes-treated condition, whereas the control group exhibited markedly lower closure rates. The difference was statistically significant ($p < 0.0001$, $n = 3$), indicating a robust enhancement of keratinocyte migration and/or proliferation in response to bacterial vesicles.

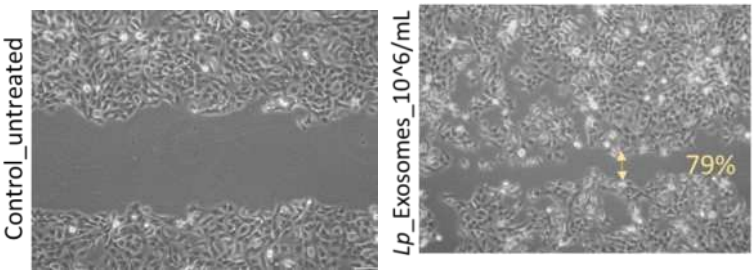


Figure 1 *Lp exosomes promote keratinocyte re-epithelization compared to the untreated control.*

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Impact of chemical pollutants on the gut microbiome of pollinators (P-II-64)

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The first activity of the PhD thesis project assessed how chemical pollutants affect the gut microbiome of pollinators. The impact of sublethal exposure to the fungicides boscalid and copper (II) chloride dihydrate, the herbicide glyphosate and the veterinary product ivermectin was studied considering *Apis mellifera* and *Osmia bicornis*. qPCR analyses showed a significant reduction of bacterial abundance in *O. bicornis* following exposure to the fungicides, while glyphosate and ivermectin had no effect. In *A. mellifera*, bacterial abundance was not affected by the treatments, apart from one colony susceptible to copper (II) chloride dihydrate; the fungal load decreased significantly after boscalid treatment. These results suggest that fungicides can disrupt the gut microbiome of pollinators.

Impatto degli inquinanti sul microbioma intestinale degli impollinatori

La prima attività del progetto di tesi di Dottorato ha riguardato lo studio dell'effetto degli inquinanti chimici sul microbioma intestinale degli impollinatori. È stato studiato l'impatto di dosi subletali dei fungicidi boscalid e cloruro di rame, dell'erbicida glifosato e del farmaco veterinario ivermectina, considerando *Apis mellifera* e *Osmia bicornis*. I risultati delle qPCR hanno rivelato una riduzione significativa dell'abbondanza batterica in *O. bicornis* in seguito all'esposizione ai fungicidi, mentre glifosato e ivermectina non hanno avuto un effetto. In *A. mellifera* non sono stati riscontrati cambiamenti nell'abbondanza batterica a seguito dei trattamenti, ad eccezione di una famiglia, suscettibile al cloruro di rame; la carica fungina è significativamente diminuita a seguito del trattamento con boscalid. I risultati suggeriscono un potenziale impatto negativo dei fungicidi sul microbioma intestinale degli impollinatori.

Key words: Insect gut microbiome, chemical pollutants, pollinators, *Apis mellifera*, *Osmia bicornis*.

1. Introduction

Exposure to chemical pollutants, particularly agrochemicals, has been recognized as one of the leading factors contributing to the decline of pollinator populations recorded in recent years (Goulson *et al*., 2015). Exposure to agrochemicals may indirectly compromise insect health by disrupting the gut microbiome, which plays crucial roles for insect physiology (Motta and Moran, 2020; Engel and Moran, 2013). Most studies assessing the effects of chemical pollutants on pollinators have focused on honeybees (*Apis mellifera*) and bumblebees and have evaluated a limited number of substances, mainly insecticides, at typical field application levels (Motta *et al*., 2018). Thus, there is a significant knowledge gap regarding the effects of sub-lethal concentrations of various chemical pollutants on different pollinator species. In this context, in accordance with the PhD thesis project previously presented, this poster concerns the main results of activity A1: study of the influence of chemical pollutants on the structure of the gut microbial community of the red mason bee *Osmia bicornis* and of the honeybee *Apis mellifera.* Experiments are ongoing also on the hoverfly *Eristalis tenax*. These species were selected because they are among the most important crop pollinators, with social (*A. mellifera*) or solitary (*O. bicornis, E. tenax*) behaviour. The chemicals studied are boscalid, copper (II) chloride dihydrate, glyphosate and ivermectin. Boscalid and copper (II) chloride dihydrate act as fungicides, while glyphosate is used as herbicide and ivermectin is a veterinary drug. These compounds have been previously detected as residues in nectar and pollen, making them relevant for studying the potential impacts on the gut microbiome of pollinators (Wallner, 2009; Lewis *et al*., 2016; Thompson *et al*., 2014; Sands and Noll, 2022).

2. Materials and Methods

A. mellifera and *O. bicornis* adults were subjected to chronic oral toxicity tests using sublethal doses of boscalid, copper (II) chloride dihydrate, glyphosate and ivermectin, administered singularly and in combination. The tested concentrations were 2 mg/kg for boscalid (Wallner, 2009), 7,2 mg/kg for copper (II) chloride dihydrate (Nikolić *et al*., 2016), 30 mg/kg for glyphosate (Thompson *et al.,* 2014) and 0,16 µg/ml for ivermectin (Guseman *et al*., 2016). For *O. bicornis*, individuals were subjected to a period of three days of pollen-feeding for microbiome acquisition, while for *A. mellifera* microbiome acquisition was achieved by feeding the specimens with a gut homogenate preparation for five days. In both species, exposure to chemicals lasted four days. To account for potential genetic variability in susceptibility to chemicals, three distinct *A. mellifera* colonies (F3, F5, and L1) were selected for exposure to boscalid and copper (II) chloride dihydrate. After surface sterilization, insects were dissected and their guts were extracted under sterile conditions (Callegari *et al*., 2021). Total genomic DNA was extracted from guts with QIAamp PowerFecal Pro DNA Kit (Qiagen). The gut bacterial community of *O. bicornis* and *A. mellifera* was investigated by molecular analyses in terms of bacterial abundance using quantitative PCR

targeting the phylogenetic marker 16S rRNA gene (Callegari *et al*., 2021). Additionally, the gut microbiome of *A. mellifera* was characterized by studying the abundance of fungal species, through quantitative PCR targeting ITS1 region, a reference taxonomic marker for fungi (Callegari *et al*., 2021).

3. Results and Discussion

3.1 Quantification of gut bacterial abundance in *O. bicornis* and *A. mellifera*

In *O. bicornis*, qPCR results targeting the 16S rRNA gene showed that gut bacterial abundance was significantly reduced when specimens were exposed to boscalid, copper (II) chloride dihydrate, and their combination (50:50 mixture), suggesting that fungicides are detrimental for the gut bacterial community. Gut bacterial abundance is not affected by treatments with glyphosate, ivermectin and the mixture of the two compounds. A metataxonomic analysis targeting the 16S rRNA gene is ongoing to evaluate the effect of the chemicals on the structure of the bacterial community of *O. bicornis* (Callegari *et al*., 2021). For *A. mellifera*, no differences among the different treatments in terms of gut bacterial abundance were recorded, apart from colony L1, which was susceptible to treatment with copper (II) chloride dihydrate. This result indicates that the response to chemicals may vary between colonies. qPCR analyses targeting the main bacterial taxa of *A. mellifera* are ongoing to assess whether exposure to the agrochemicals has affected the core microbiome (Callegari *et al*., 2021).

3.2 Quantification of fungal load in the gut of *A. mellifera*

Considering individuals from F3 colony, qPCR results targeting ITS1 region showed that exposure to boscalid and to 50:50 boscalid:copper mixture led to a reduction in fungal abundance present in the gut. In F5 and L1 colonies, a significant reduction in fungal load was observed when specimens were fed with boscalid. These findings suggest a potential negative impact of boscalid on the gut-associated fungal community of *A. mellifera*, confirming that the response to chemicals can differ between colonies. Exposure to glyphosate, ivermectin and to the mixture of the two compounds had no effect on the abundance of fungal species. A metataxonomic analysis targeting the ITS2 region will be carried out to assess the effect of the chemicals on the composition of the fungal community of *A. mellifera* (Callegari *et al*., 2021).

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Human and food microbiome: interactions explored *in vitro* (P-II-65)

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The goal of this PhD thesis research project is to investigate how the human microbiota interacts with the enteric nervous system health, in order to provide useful information to improve human health, increasing the education quality and offering new economic perspectives. The present project will provide a basis to investigate the interaction between diet, bacteria and neurons in a comprehensive way.

Microbioma dell'uomo e degli alimenti: interazioni esplorate *in vitro*

L'obiettivo di questo progetto di tesi di dottorato è indagare come il microbiota umano interagisca con la salute del sistema nervoso enterico, al fine di fornire informazioni utili per migliorare la salute umana, aumentando la qualità dell'istruzione e offrendo nuove prospettive economiche. Il presente progetto fornirà le basi per studiare l'interazione tra dieta, batteri e neuroni in modo completo.

Key words: *transcriptome*, RNA-seq, *PRCN, Caco-2,* Lactiplantibacillus plantarum*, gene expression*

1. Introduction

In accordance with the previously described PhD thesis project (Binello *et al*., 2024), this poster reports the main results of the first activities concerning the investigation on adhesion tests and transcriptome analysis of *L. plantarum* when exposed to the different eukaryotic cell lines; namely primary rat cortex neurons (PRCN) and human enterocytes (Caco-2).

2. Materials and Methods

2.1 Preparation of the *L. plantarum* working culture

A strain of *L. plantarum* previously isolated from olives by Botta *et al.* (2014) was used in this study. A colony of *L. plantarum* was suspended in 10 mL of MRS broth and incubated for 24 h at 30°C. Subsequently, 10 µL of the suspension were diluted in 10 mL MRS and incubated for 18h at 30°C to obtain the working culture.

2.2 Adhesion tests involving PRCN

For the adhesion test with PRCN, neurons were incubated for 14 days at 37°C with 5% CO_2 in 0.5 mL Neurobasal Plus Medium with 2% B27, 0.25% Glutamax and 1% Penicillin Streptomycin. Every 2-3 days 0.3 mL of medium from each well were removed and replaced with the same volume of fresh medium. On the day of the experiment, the medium was replaced by fresh medium without antibiotic, and 0.5 mL of bacterial working culture were added. After 30' incubation at 37°C with 5% CO_2, and two rinses with PBS at room temperature, 0.2 mL PBS with 0.25% Triton X at 4°C were added, and after 30' incubation at 37°C with 5% CO_2, and 10" scratching, the content of the well was collected in an Eppendorf. The *L. plantarum* viable count was determined by the spot technique on MRS medium. This procedure was repeated in total 7 times (biological replicates for both neurons and bacteria).

2.3 Adhesion tests involving Caco-2

For the adhesion test involving Caco-2, enterocytes were prepared as described by Buzzanca *et al.* (2023).
On the day of the experiment, 1 mL of bacterial working culture was centrifugated for 5' at 5'000 rpm, resuspended in DMEM with FBS 10%, diluted 1:100 and incubated at 37°C for 30'. 0.5 mL of this suspension were put in a well with Caco-2 after old medium removal and two rinses with PBS at room temperature. The co-culture was manipulated as described in paragraph 2.2. The procedure was repeated 9 times in total (biological replicates for both enterocytes and bacteria).

2.4 Preparation of *L. plantarum* transcriptome samples

Concomitantly to the adhesion tests, samples were collected to analyze the transcriptome of *L. plantarum* and compare the impact of two different conditions: exposure to PRCN and to Caco-2. For the preparation of these samples, the procedure described in paragraph 2.2 was followed with PRCN and Caco-2 until the end of the two rinses with PBS but involving 16 wells instead of one. After that, 0.2 mL PBS at 4°C were added to each well, and after 10" scratching, the content of the wells was collected in 2 mL Eppendorf. After 10' centrifugation at 15'000 rpm and removal of supernatant, 0.5 mL of RNA later were added to each Eppendorf. Samples were sent to a commercial facility (Genewiz, Germany) for RNA sequencing.

The procedure was repeated in total 7 times for neuronal samples and 9 times for enterocyte samples (biological replicates for bacteria).

2.5 Statistical analysis

At the present moment, statistical analyses are being performed with R studio 2024.12.1+563. We consider in our calculations two groups: data obtained from replicates involving PRCN and data obtained from replicates based on Caco-2. Raw data were normalized according to the FPKM method.

3. Results and Discussion

3.1 Adhesion tests

Adhesion data appear to be consistent to a previous study with the *same L.* plantarum strain on Caco2 cells (Botta *et al.* 2014). In fact, the collected samples of *L. plantarum* exposed for 30' to either PRCN or Caco-2 showed values of CFU/mL between one and two magnitude order smaller than the ones measured in the working culture before the contact with eukaryotic cells (Fig. 1). More in detail, bacterial suspension exposed to PRCN showed an initial concentration of 2.81*10^7 CFU/mL, which decreased to 1.90*10^6 CFU/mL at the end of the experiment. Samples of *L. plantarum* exposed to Caco-2 provided values of 1.50*10^7 CFU/mL and 6.10*10^5 CFU/mL respectively.

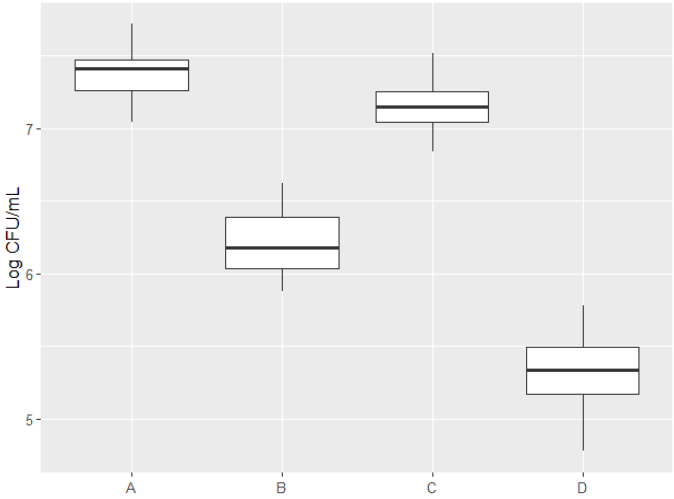


Figure 14*: Results of* in vitro *adhesion tests. A and B:* L. plantarum *before and after exposition to PRCN, respectively. C and D:* L. plantarum *before and after exposition to Caco-2, respectively. Values on y-axis are expressed as Log CFU/mL*

3.2 Transcriptome analysis

In the genome of *L. plantarum*, 3145 genes are described. In this study, 88 of these genes were not expressed in any sample. Of the remaining 3057 genes, 606 showed significant differences in the expression between the group of PRCN-samples and Caco-2-samples.
Normalized data indicate that significantly differentially expressed genes are mostly associated to higher values in the PRCN-group than in the Caco-2 group.
Further statistical analyses are underway to identify eventual outliers, and more investigations are necessary to attribute a biological explanation to the expression differences between PRCN and Caco-2 groups.

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(P-II-66)

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The astAGEani project aims to investigate the association between the consumption of ultra-processed foods (UPFs) and the prevalence of chronic non-communicable diseases in individuals over the age of 65. The study also considers demographic, lifestyle, anthropometric, and clinical factors that may influence food choices and health outcomes.

Consumo di alimenti ultraprocessati e impatto sulla salute pubblica negli adulti over 65

Il progetto astAGEani mira a indagare l'associazione tra il consumo di alimenti ultraprocessati (UPF) e la presenza di malattie croniche non trasmissibili nella popolazione over 65. Lo studio prende in considerazione anche fattori demografici, comportamentali, antropometrici e clinici che possono influenzare le scelte alimentari e la salute.

Key words: Nutrition, public health, ultra-processed foods, NCDs, elderly

1. Introduction

Chronic non-communicable diseases (NCDs), including cardiovascular diseases, type 2 diabetes, and certain types of cancer, represent the leading cause of mortality and disability among older adults. Lifestyle and environmental factors, particularly nutrition, are key contributors to the onset and progression of these conditions. In recent years, growing attention has been directed toward ultra-processed foods (UPFs), a category of products characterized by high industrial processing and poor nutritional quality. UPFs are often energy-dense and rich in added sugars, salt, and saturated fats, while being low in fiber, vitamins, and essential nutrients. Their excessive consumption has been linked to adverse health outcomes such as obesity, hypertension, dyslipidemia, and insulin resistance. Older adults may be especially vulnerable to the effects of UPFs due to age-related physiological changes and the high prevalence of multimorbidity in this age group. Despite this, data on UPF intake among the elderly population remain scarce, particularly in Italy. The astAGEani study aims to address this gap by investigating the dietary habits of individuals over 65 years old and exploring potential associations between UPF consumption and clinical parameters indicative of chronic disease.

2. Materials and Methods

The astAGEani project is a cross-sectional observational study conducted in the province of Asti, Italy. Recruitment focused on individuals aged 65 and above, who provided informed consent and completed a structured set of questionnaires. Data collection included: a sociodemographic questionnaire; the Short Form-12 (SF-12) to assess physical and mental health-related quality of life; the Physical Activity Scale for the Elderly (PASE) to evaluate physical activity levels; and the TASTE questionnaire to explore food preferences and sensory perception.

Additional data included anthropometric measures (weight, height, waist circumference), handgrip strength, physical activity, and clinical history (diagnosed NCDs, medication use). Dietary assessment was performed using a validated quantitative Food Frequency Questionnaire (NFFQ), which records both the weekly frequency and portion size for a wide range of food items. For the purpose of this analysis, 16 food groups were selected and classified as ultra-processed according to the NOVA classification system. These included packaged snacks, sugary beverages, industrial baked goods, ready meals, processed meats, and sweetened dairy products. Weekly UPF consumption was estimated by multiplying the reported frequency by the standard portion size for each item and summing across all UPF categories.
Descriptive statistics were computed for the entire sample. Consumption levels were analyzed using measures of central tendency and dispersion (mean, median, standard deviation, interquartile range), and preliminary comparisons were performed to explore variability by age group and sex.

3. Results and Discussion

The present analysis includes data from 685 older adults, aged between 64 and 89 years. The mean age of the participants was 73.3 ± 5.7 years, with a mean Body Mass Index (BMI) of 26.3 ± 5.9 kg/m², suggesting a prevalence of overweight status within the population. The gender distribution was approximately balanced, with a slight predominance of women (53.9%).
Weekly UPF consumption varied widely across participants. The mean total intake was 2.9 ± 4.2 portions per week, with a median of 2 portions (interquartile range: 1–3.5). Notably, while the majority of individuals reported low to moderate consumption, a small subset of participants exceeded 20 weekly portions, reaching 82 portions per week. These high intakes may reflect habits involving regular use of packaged snacks, sweetened yogurts, or

sugary drinks, which could contribute significantly to poor metabolic outcomes.

Variable	Mean ± SD	Median (IQR)	Range
Age (years)	73.3 ± 5.7	73 (69–78)	64–89
BMI (kg/m²)	26.3 ± 5.9	25.0 (22.2–28.5)	15.7–55.2
Weekly UPF intake (portions)	2.9 ± 4.2	2 (1–3.5)	0–82

Table 1 *Descriptive statistics of age, BMI, and weekly ultra-processed food intake in the study sample (n = 685)*

These preliminary findings demonstrate the feasibility of the study protocol and provide valuable insights into dietary patterns among older adults in northern Italy. The heterogeneity observed in UPF consumption highlights the importance of stratifying the population by risk profiles in future analyses. Subsequent phases of the project will include inferential analyses to examine the associations between UPF intake and the prevalence of chronic conditions, physical function decline, and multimorbidity indicators. Results will support the development of targeted dietary recommendations and public health strategies to promote healthy aging.

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Application of a new semi-quantitative food frequency questionnaire for evaluating seasonal (poly)phenol intake (P-II-67)

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The first two activities of the PhD thesis project are described. Firstly, a new semi-quantitative food frequency questionnaire, namely FitoQ, was validated, to evaluate the (poly)phenol intake in the Italian population. Secondly, this new tool was applied in a wider research project, which is being conducted on a cohort of Italian adults, for the assessment of (poly)phenol intake across seasons, with the final aim to elucidate the role of in-season (poly)phenol consumption on possibly associated metabolic phenotypes.

Applicazione di un nuovo questionario di frequenza di consumo semi-quantitativo per la valutazione del consumo stagionale di (poli)fenoli

Le prime due attività del progetto di tesi di dottorato sono descritte. La prima attività ha riguardato la validazione di un nuovo questionario di frequenza di consumo semi-quantitativo, chiamato FitoQ, sviluppato per la valutazione del consumo di (poli)fenoli nella popolazione italiana. Nel contesto della seconda attività, questo nuovo strumento è stato utilizzato in uno studio osservazionale, che si sta svolgendo su una coorte di adulti italiani, per la valutazione del consumo stagionale di (poli)fenoli con il fine ultimo di valutare il ruolo del consumo stagionale di (poli)fenoli sui possibili fenotipi metabolici a loro associati.

Key words: (poly)phenols, dietary assessment, seasonality.

1. Introduction

In accordance with the PhD thesis project previously described (Gallina, PhD Dissertation Project, 2024), this poster reports the main results on the validation of a new semi-quantitative food frequency questionnaires (sFFQ), namely FitoQ, developed to evaluate the (poly)phenol intake in the Italian population (A1). Preliminary results focusing on the characteristics of the study population from the ongoing 9-month observational study (A2) are also presented.

2. Materials and Methods

A total of 128 healthy subjects were recruited from different Italian regions, including Emilia-Romagna, Lombardy, Piedmont, Abruzzo and Campania. The FitoQ was administered twice: once initially and again after 2/4 weeks, in order to evaluate the temporal stability. Moreover, FitoQ validity was assessed through a 7-day dietary record completed in the same week as the initial FitoQ administration. An in-house database based on Phenol-Explorer implemented with the most updated scientific literature (Ziauddeen et al., 2019) was used and linked to the data recorded in both FitoQs and in dietary records, to establish (poly)phenol intake (expressed as milligrams per day) of each participant.
The validated FitoQ was then applied in a wider research project, which aims to evaluate the association between seasonal dietary choices and adherence to the Mediterranean Diet (primary outcome), and to shed lights on how these choices could influence the human microbiota composition and, consequently, the metabolic phenotype possibly associated to (poly)phenol metabolism (secondary outcomes). To do this, a 9-month multicentre observational study is being conducted on a cohort of 500 Italian adults. Of these, 84 subjects were recruited in Parma. After eligibility evaluation (V1), volunteer dietary habits were monitored every three months at the midpoint of each season, through 4 periodic visits (V2, V3, V4, V5) by using a 3-day dietary record. FitoQ was administered at the beginning (V2) and at the end (V5) of the observational phase. Moreover, 24-h urine samples were collected at each visit.

3. Results and Discussion

3.1 Validation of a new sFFQ for (poly)phenol intake evaluation in the Italian population (FitoQ)

A total of 112 participants, with a mean age of 37±16 years, completed the two FitoQ administrations and for these subjects the dietary record was adequately completed. A total of 16 subjects were excluded due to missing data. FitoQ temporal stability was assessed comparing the (poly)phenol intake data obtained from FitoQ administered at baseline with the data obtained from the second administration. Spearman correlation coefficient and *p-value* were calculated for the total intake of (poly)phenols and for the main (poly)phenolic classes, including flavonoids, phenolic acids, lignans and others. The results are shown in Table 1, demonstrating that good temporal stability has been obtained for the consumption of all analysed (poly)phenol classes through the developed FFQ.

Table 1. Evaluation of FitoQ temporal stability, comparing the first (T0) and the second (t1) administration. Values are reported as median (IQR) (n=112).

Compounds	Daily intake from FitoQ (t0) (mg/die)	Daily intake from FitoQ (t1) (mg/die)	Spearman Rho	*p-value*
TOTAL (POLY)PHENOLS	1221(860-1703)	1231(872-1626)	0.930	***<0.001***

Flavonoids	653(458-817)	647(430-773)	0.911	***<0.001***
Phenolic acids	434(295-684)	451(305-651)	0.937	***<0.001***
Lignans	1(1-2)	1(1-2)	0.940	***<0.001***

Following the same approach, the estimated (poly)phenol intake using the FitoQ at t0 were compared with the data collected through the 7-day dietary records, in order to validate the FitoQ. The correlation resulted statistically significant for all (poly)phenol classes evaluated (Table 2).

Table 2. Validity evaluation comparing data obtained from FitoQ at t0 and data extrapolated from the 7-day food diary. Values are reported as median (IQR) (n=112).

Groups	Daily intake from FitoQ (t0) (mg/die)	Daily intake from 7-day dietary records (mg/die)	Spearman Rho	*p-value*
TOTAL (POLY)PHENOL	1221(860-1703)	621(446-866)	0.386	***<0.001***
Flavonoids	653(458-817)	369(204-551)	0.406	***<0.001***
Phenolic acids	434(295-684)	220(137-313)	0.505	***<0.001***
Lignans	1(1-2)	1(1-2)	0.525	***<0.001***

Daily total (poly)phenol intake for all food groups considered (1221 mg/day) is similar to that reported in a previous study (Zamora-Ros et al., 2016) and flavonoids were found to be the main contributors to total (poly)phenol intake among all evaluated classes, while lignans were the minor contributors.

3.2 Running a 9-month observational study

The current progress of the study, related to the volunteers recruited in Parma, is summarized in Figure 1, while the anthropometric characteristics of the study population are reported in Table 3. A total of 84 subjects with a mean age of 40±13 was enrolled and completed V2. At the moment, 13 participants dropped out due to different reasons, mainly related to antibiotic use, unavailability, and ineligibility. To date, 21 subjects have completed all the visits and concluded the study, while 50 subjects are currently involved.

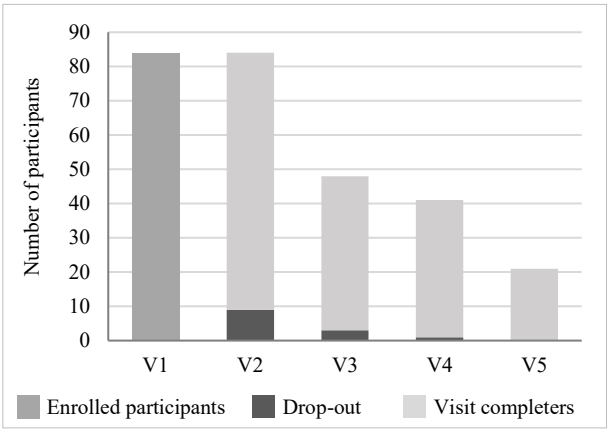


Figure 1. Participants status across visits (V1-V5): participants who completed the visit (light) and dropouts (dark) shown.

Parameter	Weight (kg)	Height (m)	BMI (kg/m²)
Visit 1 (n=84)	65±9	1.68±0.09	22.9±2.4
Visit 2 (n=27)	63±10	1.67±0.08	22.6±2.8
Visit 3 (n=4)	67±8	1.72±0.08	22.6±0.8
Visit 4 (n=19)	69±9	1.72±0.08	23.2±2.2
Visit 5 (n=21)	64±10	1.68±0.07	22.4±2.2

Table 3. Anthropometric data (mean ± standard deviation) for subjects enrolled at Visit 1 and for subjects currently in each subsequent visit phase (n = number of subjects per visit)

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Woman's nutrition: eating habits, nutritional status and main sex-related risk factors in the physiological phases of female life (P-II-68)

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This PhD thesis research project aims to evaluate the impact of dietary habits, nutritional status, and nutritional knowledge on key sex-related nutritional risk factors across major physiological stages of women's lives, with a focus on pregnancy. The collected data will be the basis for developing evidence-based nutrition education tools for women and for drafting nutrition guidelines specific to these physiological phases.

Nutrizione della donna: abitudini alimentari, stato nutrizionale e principali fattori di rischio legati al sesso nelle fasi fisiologiche della vita della donna

Questo progetto di tesi di dottorato mira a valutare come le abitudini alimentari, lo stato nutrizionale, e la conoscenza nutrizionale influiscono sui principali fattori di rischio nutrizionale sesso-specifici nelle principali fasi fisiologiche della vita della donna, in particolare durante la gravidanza. I dati raccolti saranno la base per lo sviluppo di strumenti per l'educazione alimentare delle donne e per la stesura di linee guida nutrizionali specifiche per questi stadi fisiologici.

Key words: Nutrition education, women's nutrition, pregnancy, educational tool, eating behaviour.

1. Introduction

Among the five activities outlined in the PhD thesis project, the results of the first three are presented below:

(A1) **Systematic review of global Food-Based Dietary Guidelines (FBDGs)** to assess the presence of recommendations for pregnant and lactating women and categorize the different topics covered by them.

(A2) **Cross-sectional study using an online survey** to assess food habits, attitudes, nutrition knowledge, and the main determinants of a healthy eating behaviour in a representative sample of Italian pregnant women.

(A3) **Creation of an evidence-based educational tool for Italian pregnant women**.
Focus group will be performed to assess the consumers' perception and thoughts about the new tool.

2. Materials and Methods

2.1 Systematic review of global Food-Based Dietary Guidelines (FBDGs) for pregnant and lactating women

All available FBDGs were retrieved from the FAO repository (FAO, 2025) and through Internet searches. Inclusion criteria were being a country-specific FBDG, containing recommendations for pregnancy or breastfeeding, and being available in English or translatable. For each guideline, country, region, year of publication, language, source, document, and target population were collected. All documents were reviewed, and nutrition recommendations for pregnancy and breastfeeding were categorized by topic.

2.2 Cross-sectional study using an online survey

The cross-sectional study was conducted between July and September 2024 using an online survey developed with Qualtrics software (Qualtrics, 2024). The minimum sample size was set at 543, considering a confidence level of 98% and a confidence interval of 5%, based on Italian birth data (n=386,253) (Ministry of Health, 2023). To account for potential dropouts, more than 600 participants were invited. Inclusion criteria were being pregnant, aged 18-49, being Italian or resident in Italy for at least 5 years, a minimum B2 level of Italian language, and absence of chronic or pregnancy-related complications. The survey consisted of 9 sections regarding sociodemographic and pregnancy characteristics, use of supplements, nutrition knowledge, dietary habits, food-related habits, attitudes, and physical activity. Adherence to the Italian dietary guidelines was assessed using the Adherence to the Italian Dietary Guidelines Indicator (AIDGI) (Grant and Rossi, 2022), which categorizes adherence as low (0–18), medium-low (19–20), medium-high (21–23), or high (>23). Non-parametric analysis and logistic regression were performed to explore associations with adherence levels.

2.3 Creation of an evidence-based educational tool for Italian pregnant women

Two prototypes of the tool were developed based on survey results and the Italian dietary guidelines (CREA, 2018). Focus group were conducted to collect data on perceptions and opinions of pregnant women on the prototypes. Pregnant women were recruited via flyers in gynaecological clinics and counselling centres, which included a QR code for eligibility screening. Inclusion criteria were being pregnant, aged 18-49 years, Italian or resident in Italy for at least 5 years, and nulliparous. Each focus group (2-10 participants) last about 90 minutes and followed a structured outline including also projective techniques (word association, complete the sentence, editing). A qualitative analysis will be performed, and results will be used to create the final version of the tool.

3. Results and Discussion

3.1 Systematic review of global Food-Based Dietary Guidelines (FBDGs) for pregnant and lactating women

A total of 82 FBDGs were included in the review: 58 from FAO repository and 24 from additional sources. The

majority were from Europe (n=28), followed by Asia and the Pacific (n=17), Latin America and the Caribbean (n=17), Africa (n=12), Near East (n=6), and North America (n=2). 17 FBDGs were specifically targeted at pregnant and breastfeeding women, while the remaining were addressed to the general population but including recommendations for specific groups. 73 guidelines included recommendations for both pregnancy and breastfeeding, 6 focused solely on pregnancy, and 3 only on breastfeeding. Due to the volume of data, the current analysis focuses on pregnancy-related recommendations. Preliminary findings show that most macronutrient recommendations focus on increased energy needs during pregnancy (n=57), as well as folic acid (n=56) and iron intake (n=54). Similarly, supplementation recommendations primarily concern folic acid (n=40) and iron (n=38). Other macro- and micronutrient recommendations were less frequently addressed. Among other topics, most guidelines provided recommendations on alcohol consumption (n=50), general advice on maintaining a healthy and balanced diet during pregnancy (n=49), appropriate weight gain (n=43), and water intake (n=39).

3.2 Cross-sectional study using an online survey

A total of 589 Italian pregnant women were included in the final data analysis. The mean age was 31 years. Most participants had a high sociodemographic profile: 63% were graduates, 89% were employed or on maternity leave, and 82% reported no financial difficulties. Overall, 62% had at least one previous pregnancy, and 48% breastfed for at least 6 months, aligned with WHO recommendations. Notably, 26% reported never having taken folic acid supplements during pregnancy. Adherence to the Italian FBDGs was low in 50% of the sample, particularly among multiparous women. The most critical food groups were milk and yogurt, as almost 90% not meeting the recommended three daily servings. Conversely, processed and cured meats, red meat, salty snacks, and sugary drinks were consumed in excess. The median (IQR) NK score was 9 (7-13) out of 15, indicating sufficient knowledge. Multiparous women scored higher (12 (8–10)) than nulliparous ones (8 (7–10)). Logistic regression identified key predictors of high adherence to the Italian Dietary Guidelines during pregnancy. Previous pregnancy was linked to lower adherence, while actively avoiding harmful foods and a positive attitude toward the Mediterranean Diet were associated with higher adherence. Familiarity with food was negatively associated, suggesting that openness to dietary change may support better compliance.

3.3 Creation of an evidence-based educational tool for Italian pregnant women

The tool is a graphical representation of a circular sectioned plate designed to visually communicate recommended food group proportions, consumption frequencies, and key pregnancy-related dietary and culinary messages. Half of the plate is dedicated to fruit and vegetables, while cereals and protein-rich foods each occupy a quarter. The centre emphasises folic acid supplementation, while a surrounding ring includes information on fats. Two outer concentric circles provide recommendations on hydration and additional nutritional advice. The prototypes differ on the number of information available on the front side. In addition, the back side of one prototype is blank, while the other offers further details on food groups and portion sizes. Preliminary results from two focus groups (n=8 first-time pregnant women) were positive. Prototype A (front only) was preferred by participants, who found it clearer and more focused than Prototype B (front and back). Overall, both prototypes were positively evaluated for their clarity, comprehensiveness, and ease of use. Participants suggested enhancing the content by providing more detailed information on cooking methods and specific foods to include or avoid.

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Mapping the complexity of the diet: circulating metabolites of plant bioactives and food-gut microbiome-host interactions (P-II-69)

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The first activity of the PhD thesis project, focused on the development and validation of a method for (poly)phenol metabolites in urine, was performed last year. This second year is focused on performing the development of terpenoid methods in different human biological samples. Moreover, the focus is also on another milestone related to the data integration pipeline for the assessment of the drivers of inter-individual variability for different plant bioactives, and their association with cardiometabolic health markers. In particular, it has already been developed and tested for plasma carotenoids.

Mappatura della complessità della dieta: metaboliti circolanti di composti bioattivi vegetali e interazione cibo-gut microbiota-ospite

La prima attività del progetto di tesi di dottorato focalizzata sullo sviluppo e la validazione di un metodo analitico per metaboliti fenolici in urina è stata portata a termine. Focus di questo secondo anno è lo sviluppo di metodi analitici per diversi composti terpenoidi in diversi campioni biologici umani. Inoltre, il focus sarà anche sulla pipeline di integrazione dei dati, finalizzata all'identificazione dei fattori che guidano la variabilità interindividuale nella risposta a diversi composti bioattivi di origine vegetale e alla loro associazione con i marker di salute cardiometabolica. Questo workflow è stato già sviluppato e applicato per i livelli di carotenoidi del plasma.

Key words: Plant bioactives, inter-individual variability, gut microbiota, personalised nutrition, metabolomics.

1. Introduction

Following the PhD thesis project previously described, the first activity, focused on the development and validation of an analytical method for (poly)phenol metabolites, has been performed. This poster presents two key milestones of the ongoing PhD project:

- the development of analytical terpenoid methods (only for carotenoids so far);
- the assessment of the drivers of inter-individual variability of multiple phytochemicals in biological samples, and their association with cardiometabolic health markers.

2. Materials and Methods

2.1. (Poly)phenol metabolite method development and validation

The (poly)phenol metabolite method development has been performed based on a hybrid UPLC-MS-based targeted metabolomic approach. Then, method validation has been performed following FDA guidelines.

2.2. Carotenoid method development, sample processing and data acquisition

Multi-level, multi-modal data coming from a cohort of 298 deeply-phenotyped subjects has been collected. Carotenoid individual levels were assessed using a UPLC-MS-based targeted metabolomic approach. Different dietary patterns were assessed using the EPIC food frequency questionnaire. A genome-wide association (GWAS) targeted and untargeted study for genotyped and imputed data was employed to assess genetic variability in the metabolism and absorption of carotenoids. STRING, PLINK 1.9/2.0, and FUMAGWAS tools have been used for genomic analysis. Gut microbiota composition was profiled by using internally transcribed spacer (ITS) rRNA profiling. Metagenomic data has been analysed using Rstudio tool. Cardiometabolic health status was evaluated through clinical biomarkers and cardiometabolic risk scores. Data normality and data differences have been assessed using Shapiro-Wilk, Wilcoxon. and Kruskal-Wallis tests through the Rstudio tool. Plasma carotenoids extraction has been perfomed following Hrvolová et al. (2016) method, but with some modification. In particular, 200 µL of plasma samples were mixed with 200 µL of methanol with fucoxanthin as internal standard. Liquid-liquid extraction was performed by adding 400 µL of hexane containing BHT (100 mg/L), followed by vortexing and centrifugation. The upper hexane phase was collected in amber vials, and the extraction was repeated. The two organic phases were combined. Samples were evaporated and stored at –80 °C. On the day of analysis, the residues were reconstituted in 100 µL of acetonitrile/isopropanol solvent (1:1, v/v). Plasma carotenoid analysis has been performed, and data is still being processed and integrated.

3. Results and Discussion

3.1 Development of a UPLC-MS-based targeted metabolomic approach for carotenoids

A fast UPLC-QqQ-MS/MS method (20 min) has been developed for the analysis of 20 representative dietary carotenoids in human plasma, including: *E*-canthaxanthin, *E/Z*-phytoene, *E*-zeaxanthin, *E/Z*-phytofluene, *E*-lutein, *E*-α-carotene, *E/13Z/9Z*-β-carotene, *E*-γ-carotene, *E*-ζ-carotene, *E/13Z/5Z*-lycopene, *E*-astaxanthin, *E*-fucoxanthin, *E*-α-cryptoxanthin, *E*-β-cryptoxanthin, *E*-β-apo-8'-carotenal, and *E*-β-carotene 5,6-epoxide. The method was developed and optimized using commercially available standards, *E*-fucoxanthin as internal standard,

and human plasma samples for different tests. Quantification was performed using external calibration curves created for each standard covering a concentration range of 0.001-15.00 μM, considering also the different limits of quantification and identification for each compound. Calibration curves were constructed using at least five concentration levels, and the linearity was assessed with R^2 values greater than 0.99. For each analyte, peak integration was accepted only if the chromatographic peak was defined by a minimum of ≥12 points per peaks.

3.2 Data integration approach for the assessment of inter-individual variability drivers of plant bioactive individual levels and their association with cardiometabolic health markers

The development of the pipeline for data integration of the potential drivers of inter-individual variability for plant bioactives has been carried out using carotenoid data. Plasma carotenoid concentrations have been associated with the relative dietary intake using the Spearman correlation. Then, plasma concentrations have been divided into tertiles to test the statistical differences between the tertile with the highest plasma concentration (HIGH) and the tertile with the lowest one (LOW). Statistical differences between the two tertiles have been tested for different demographic and lifestyle information, anthropometric data, dietary data, cardiometabolic health biomarkers and risk scores, α- and β- diversity data for metagenomics, and GWAS targeted and untargeted study for genomic data. This pipeline will be further applied for all the plant bioactives considered in this PhD project.

3.3 Data integration for *E*-lycopene: inter-individual variability assessment

Plasma *E*-lycopene was selected as the initial compound for the development of the integration pipeline, with the aim of extending the approach to the full panel of carotenoid analysed. All the results reported here are preliminary results, and other tests will be performed in the coming months. Plasma *E*-lycopene concentration ranged from 0.0018 to 0.5391 μmol/L of plasma. No strong correlation between *E*-lycopene dietary intake and its plasma concentration has been observed. Plasma concentration data have been divided into tertiles, testing statistical differences between the two extreme tertiles, HIGH versus LOW. Demographic and lifestyle information has highlighted a statistically significant higher percentage of young people in the HIGH tertile than the LOW one. Physical activity showed a significant difference, with a higher percentage of individuals in the moderate physical activity level of the LOW tertile, and a higher percentage in the low level of the HIGH tertile. No significant differences in the anthropometric measurements have been observed between the two tertiles. Similarly, no statistical differences have been observed in the dietary intake data for macro- and micronutrients. Also, dietary data for the different food group intake did not show statistical differences. For the different dietary pattern scores tested, statistically significant differences have been shown between the two tertiles for the plant-based diet index (PDI) and the pro-vegetarian food pattern score (PVG). In particular, the healthy PDI and PVG level resulted higher in the tertile LOW, and the unhealthy PVG level was higher in the tertile HIGH. Different biomarkers and risk scores for cardiometabolic health status have been tested, resulting in a better profile of the tertile HIGH than the tertile LOW, in particular, for LDL-cholesterol, total cholesterol/HDL ratio, disposition index, and systolic blood pressure, as well as for some Framingham cardiovascular disease and coronary heart disease risk scores, QRISK3 and SCORE2. Metagenomic data highlighted a higher α- and β-diversity in the tertile HIGH than in the tertile LOW. To understand the potential sources of genomic differences for carotenoid levels, literature searches were performed on >50 papers, and >200 genes related to carotenoid genomics. A targeted GWAS study highlighted statistically significant differences between the two tertiles for different single nucleotide polymorphisms (SNPs) related to genes such as SCARB1, SLIT3, and SLC27A6. The untargeted GWAS has shown significant differences in SNPs interacting with intergenic and intronic regions of the genome. Using the FUMAGWAS *in silico* tool, we tested how these SNPs interact with the different chromosomes. It has been highlighted how these SNPs not-coding for specific proteins but interacting with these poorly characterized regions in the chromosomes 1, 2 and 11 could have potential regulatory effect on proteins. Further statistical approaches covariating for different potential confounding factors such as age, sex, etc. are guaranteed.

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Nutritional Quality and Environmental Impact of Menus in the Italian Institutional Catering Sector: Potential for Optimization and Sustainability

(P-II-70)

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The activities performed during the second year of the PhD project are described. First, a scoping review was conducted regarding the nutritional quality (NQ) and environmental impact (EI) of institutional canteen menus and meals. This was done to identify the status of knowledge and gaps in data availability on such topic. Second, a nutritional and environmental analysis of canteen menus collected from catering companies in different cities in Italy is currently being carried out. The goal of this activity is to optimize these menus and meals to make them more sustainable. Finally, the nutritional content of plant-based fish and seafood analogs (PBFSA) was analysed and compared to their animal-based counterparts. Given their increasing popularity in the Italian market and their sensory similarities to animal-based foods, the aim was to start analysing such products to explore their feasibility as part of nutritious, climate-friendly, and acceptable menus, which may further encourage plant-based eating in institutional settings.

Recupero di alcuni metabolici microbici da soluzioni modello per osmosi inversa

Le attività svolte durante il secondo anno del progetto di dottorato sono descritte di seguito. In primo luogo, è stata condotta una scoping review sulla qualità nutrizionale (QN) e l'impatto ambientale (IA) dei menu e dei pasti serviti nella ristorazione collettiva. L'obiettivo era identificare lo stato delle conoscenze e le lacune nella disponibilità di dati su questo tema. In secondo luogo, è attualmente in corso un'analisi nutrizionale e ambientale dei menu delle mense, raccolti da aziende di ristorazione operanti in diverse città italiane. Lo scopo di questa attività è ottimizzare tali menu e pasti per renderli più sostenibili. Infine, è stato analizzato il contenuto nutrizionale degli analoghi vegetali del pesce e dei prodotti ittici (PBFSA) e confrontato con quello dei corrispettivi di origine animale. Considerata la loro crescente popolarità nel mercato italiano e le somiglianze con i prodotti animali da un punto di vista sensoriale, l'obiettivo era iniziare ad analizzare questi prodotti per valutarne la fattibilità come parte di menu nutrizionalmente validi, a basso impatto ambientale e accettabili, contribuendo così a promuovere un'alimentazione più vegetale anche nei contesti istituzionali.

Key words: Canteen menus, Plant-based alternatives, Sustainable healthy diet, Nutritional Evaluation, Linear optimization

1. Introduction

This poster reports the main results of the first three activities concerning:

(A1) the analysis of existing evidence on the NQ and EI of menus and meals offered in various institutional canteens

(A2) the nutritional and environmental analysis of canteen menus collected from catering companies in different cities across Italy, for the future goal of optimizing such menus and meals to improve their sustainability.

(A3) the nutritional evaluation of plant-based fish and seafood alternatives (PBFSAs) sold in Italy, for the future goal of exploring the feasibility of plant-based analogs within a sustainable healthy menu

2. Materials and Methods

For the first activity, a literature review was performed using carefully crafted syntaxes in the PubMed and Scopus databases, specifying the search from 2013 to 2023. The inclusion criteria focused on quantitative articles written in English and evaluating NQ and/or EI of menus served in institutional canteens. The extracted data included publication year, country and setting of the study, and the nutritional and/or environmental components it considered. Conclusions regarding nutritional and environmental adequacy were also extracted from the articles. For the second activity, school menus were collected from catering companies that provide lunch meals to schools in different Italian cities. The nutritional data of the menus were analyzed using the Metadieta Software (MètaDieta professional 4.1.1 METEDA Srl–Roma, Italy), while the environmental data were collected using the Su-EATABLE LIFE (SEL) database (Petersson et al., 2021), and when specific data were missing, the AGRIBALYSE 3.2 database. Data is in the process of being compared to the Dietary Reference Values for the Italian Population (LARN) for adequacy analysis, while the environmental data is being compared to the EAT-Lancet cut-offs and results from another published research (Ferrari et al., 2020; Tucci et al., 2022). Regarding the third activity, main PBFSA products were selected from retail grocery stores in Italy and were compared to their animal-based counterparts. Nutritional declarations, ingredient lists, and claims were collected from the on-pack labels of PBFSA. To determine nutritional quality, Nutri-Score of PBFSA products and animal-based counterparts were also assigned.

3. Results and Discussion

3.1 Results of literature review

Seventy-six studies were included in the scoping review. Most studies evaluated NQ (n = 58) in comparison to EI (n = 7), with only a few studies evaluating both (n = 11). Among the menus and meals, there was a trend of nutritional inadequacy in terms of fiber and other micronutrients, including vitamins D and E, calcium, and iron, as well as high sodium levels. Regarding EI, articles often only considered greenhouse gas (GHG) emissions with less focus on other indicators. The emphasis on GHG emissions may be attributed to their higher availability of data, their key role in increasing global temperature, and their distribution in the atmosphere making it easier measure compared to other environmental indicators that vary more by region and supply. Most of the studies were conducted in schools or nurseries, which may be due to the importance of ensuring that children have access to adequate and sustainable diets to promote optimal growth, development, and better health later in life. However, there was still great variability in study settings. Most of the studies were conducted in high-income developed countries, with the US (n = 17), Spain (n = 8), and Brazil (n = 7) being the most common. The studies being conducted in developed countries, along with the variability in settings and target populations, limited generalizability of the results. Based on these findings, future research should aim to improve the nutritional adequacy of canteen menus and meals, include a broader range of environmental components in their assessments, and take developing countries into consideration to improve generalizability and comparability of data. The review is currently submitted for publication.

3.2 Nutritional and Environmental Evaluation of Italian Canteen Menus

As a main part of the PhD project, menus were collected from catering companies in different cities across Italy. This part of the project is still ongoing. The nutritional and environmental data collection part of the menu from the first city is completed and the data is in the analysis phase. Once data is analysed, the next step, which is planned to be completed between October 2025 and March 2026, is to identify an optimized solution for the menus using linear optimization to improve sustainability while considering cultural, nutritional, and environmental constraints.

3.3 Nutritional Evaluation of PBFSA

Since they have been gaining popularity in the Italian market, plant-based analogs were nutritionally evaluated with an end goal of exploring their potential to be included in nutritious, environment-friendly, and acceptable meals. This further supports the more general goal of encouraging plant-based eating in institutional settings. This part of the project, with PBFSA, acts as a continuation of previously published research where the plant-based meat and dairy analogs were nutritionally evaluated (Cutroneo et al., 2022; Angelino et al., 2020). Fifty-one PBFSA products were collected. The results showed that their nutritional content differed from that of their animal-based counterparts. PBFSA were generally higher in carbohydrates, fiber, and salt levels, but lower in protein. Regarding claims, products with fiber or fat nutrition claims did not necessarily indicate better levels of fiber or fat, while products with a protein claim presented higher protein levels. The animal-based products, which were used for comparison, often had a better Nutri-Score than the PBFSA, and this was mainly attributed to the lower salt levels found in the counterparts. Despite this research showing that PBFSAs currently do not nutritionally equate animal-based fish and seafood, they can still contribute to diversifying plant-based diets. The findings of this research, along with the nutritional data of other analogs collected from previous research (Cutroneo et al., 2022; Angelino et al., 2020) and the upcoming data collection of EI and social factors, will help in assessing the viability of plant-based analogs as part of the optimized menus mentioned in section 3.2.

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Valorization of Apulian black chickpea and Senatore Cappelli durum wheat for the production of foods with high nutritional and functional value (P-II-71)

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The study involved the spontaneous fermentation of model doughs made from Apulian black chickpea flour (BC) and "Senatore Cappelli" durum wheat flour (SC), as well as the monitoring and characterization of the microbiota during fermentation through culture-based techniques. The physicochemical and technological properties of the doughs were also monitored. Autocthonous lactic acid bacteria (LAB) and yeasts were isolated from model doughs subjected to spontaneous fermentation. The profile of microbial metabolites produced during fermentation was characterised.

Valorizzazione del cece nero pugliese e del grano duro Senatore Cappelli per la produzione di alimenti ad elevato valore nutrizionale e funzionale

Le attività svolte hanno riguardato la fermentazione spontanea di impasti modello composti da farina di cece nero della Puglia (BC) e farina di grano duro "Senatore Cappelli" (SC), il monitoraggio e la caratterizzazione del microbiota durante la fermentazione mediante tecniche coltura-dipendenti, e il monitoraggio delle caratteristiche chimico-fisiche e tecnologiche dell'impasto. Inoltre, è stato effettuato l'isolamento di batteri lattici (LAB) e lieviti autoctoni da impasti modello sottoposti a fermentazione spontanea. È stata svolta anche una caratterizzazione del profilo dei metaboliti microbici prodotti durante la fermentazione.

Key words: Apulian black chickpea, durum wheat "Senatore Cappelli", spontaneous fermentation, culture-dependent techniques, craterisation dough, microbial isolation, and characterization of microbial metabolite profiles.

1. Introduction

In line with the objectives of the project, this study presents the main activities undertaken, including:
(A1) spontaneous fermentation of model doughs using BC and SC;
(A2) culture-dependent monitoring and characterization of the microbiota;
(A3) evaluation of dough physicochemical and technological properties during fermentation;
(A4) isolation of autochthonous LAB and yeasts;
(A5) characterization of microbial metabolite profiles.

2. Materials and Methods

Durum wheat semolina "Senatore Cappelli" was purchased from CA.ME.MA mill (Altamura, Italy). Apulian black chickpea (*Cicer arietinum* L.) grains were obtained from "Sementificio Pellegrino" (Altamura, Italy) and milled to wholemeal flour using a 0.21 mm sieve. The black chickpea flour composition was determined by official AACC methods. Doughs were prepared using 187.5 g of flour (SC, BC, or SCBC [70:30, w/w]) and 112.5 mL water (DY = 160), following a traditional sourdough protocol without added starters (Di Cagno et al., 2014; Minervini et al., 2012). Fermentations lasted 5 h daily at 25 °C (8 h initially), with 16 h storage at 4 °C between cycles. Propagation was by backslopping with 25% (w/w) of the previous sourdough mixed with fresh flour and water, repeated for 10 days.
Microbial counts were performed on 10 g dough homogenized with 90 mL sterile NaCl (0.9%) using a Stomacher (Perri et al., 2020). Presumptive LAB were enumerated on selective media (mMRS, modified M17, SDB) after anaerobic incubation at 30 °C for 48 h; media were supplemented with cycloheximide to inhibit fungi (Calasso et al., 2023; Minervini et al., 2015). Other microbial groups were enumerated on selective media as described in the literature. For each sampling point, at least seven presumptive lactic acid bacteria and seven yeast colonies were isolated from the highest dilution plates. Bacterial isolates displaying rod or coccal morphology, Gram-positive staining, and catalase-negative reaction were cultured in broth medium at 30 °C for 24 hours and streaked onto the same agar medium. Only isolates capable of acidifying the medium were selected for further characterization. Yeast colonies were cultured in YPD broth and streaked onto YPD agar for subsequent analyses. pH was measured by pH meter; total titratable acidity (TTA) was determined by titration to pH 8.3. Leavening capacity, the dough volume increase from CO_2 retention, was measured after fermentation in 500 mL beakers. Volatile organic compounds were analyzed using a Dynamic Headspace System (DHS) combined with Thermal Desorption Unit (TDU) and GC-MS. Samples (4 g) were sealed and stored at –80 °C, then thawed overnight at 4 °C. Headspace extraction was performed at 30 °C after incubation, with helium purging (30 mL/min, 300 mL total) at 120 °C. Volatiles were trapped on Tenax and moisture removed by dry purging. Thermal desorption occurred in the TDU

(30–290 °C) with transfer to a cold injection system (CIS) cryo-focused at –100 °C, then rapidly heated to 270 °C. GC separation used a polar capillary column (HP-Innowax) with a temperature program from 40 °C to 250 °C. MS detection was done by electron impact (70 eV), and compounds identified via NIST library (≥75% match) with manual curation.

3. Results and Discussion

The culture-dependent approach showed that before fermentation (T0) the lactic acid bacteria cell density was low. For instance, lactobacilli on mMRS were 3.99 ± 0.12 log CFU/g for BC, 4.27 ± 0.21 for SC, and 4.55 ± 0.08 for SCBC. After two refreshments, the density significantly increased to approximately 9.5 log CFU/g, remaining stable during subsequent refreshments, thus confirming a stable bacterial community (De Vuyst and Neysens, 2005). The pH progressively decreased, from initial values of 6.19 ± 0.01 (BC) to 4.22 ± 0.01 (SC) at T10, while total titratable acidity increased from 6.69 ± 0.13 to 19.58 ± 0.12 (BC) (Calasso et al., 2023; Rizzello et al., 2014). *Enterobacteriaceae* increased after the first refreshment from 3.17 ± 0.25 to approximately 5.75 ± 0.16 log CFU/g for SCBC, then decreased to around 2 log CFU/g at T10 (Arora et al., 2022; Ercolini et al., 2013). Enterococci grew from 3.99 ± 0.19 to 9.14 ± 0.13 log CFU/g, while yeasts increased up to about 3.6 log CFU/g, remaining constant in subsequent refreshments (De Angelis et al., 2019). The number of isolates obtained during the 10 daily refreshments of BC, SC, and SCBC doughs was 789 for presumptive lactic acid bacteria and 221 presumptive for yeasts.

Analysis of volatile organic compounds (VOCs) revealed an increase in aromatic complexity, with the number of compounds rising from 73.5 ± 4.8 to 84.3 ± 4.6 in BC, from 27.4 ± 3.3 to 50.3 ± 2.8 in SC, and from 44.5 ± 4.9 to 65.6 ± 5.1 in SCBC. This increase is indicative of microbial metabolic activity (Birch et al., 2014). Higher alcohols derive from the Ehrlich pathway, while organic acids produced by lactic acid bacteria promote the formation of esters responsible for fruity and floral notes (Pico et al., 2015). Ketones and aldehydes, resulting from catabolic processes such as deamination and β-oxidation, influence the aroma. Under specific conditions, aldehydes are converted into less pungent and more pleasant compounds. The VOC dynamics reflect the evolution of the microbial community and the fermentative potential of the sourdough.

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Selection of *Lactiplantibacillus plantarum* strains for γ-amino butyric acid (GABA) production in a legume-based fermented beverage (P-II-72)

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A selected activity of the second year of the PhD project is described. A collection of 20 *Lactiplantibacillus plantarum* strains, isolated from fermented foods, was studied for the ability to produce γ-amino butyric acid (GABA) *in vitro* and *in situ* during a lab-scale fermentation aimed at producing a legume-based beverage. Firstly, culture conditions were optimized, considering parameters such as time and temperature for growth and concentration of monosodium glutamate (MSG), used by strains as GABA precursor, by combining colorimetric, GABAse enzymatic assay and UPLC/MS-MS analysis. Based on the results obtained, 4 strains were selected and combined as starters to naturally enrich a legume-based beverage with GABA. UPLC/MS-MS analyses showed a clear strain-specific GABA production both *in vitro* and in the real food system during the lab-scale fermentation.

Selezione di ceppi di *Lactiplantibacillus plantarum* per la produzione di acido γ-amminobutirrico (GABA) in una bevanda fermentata a base di legumi

Un' attività selezionata del secondo anno del progetto di dottorato è descritta. È stata studiata una collezione di 20 ceppi di *Lactiplantibacillus plantarum*, isolati da alimenti fermentati, per la loro capacità di produrre acido γ-amminobutirrico (GABA) *in vitro* e *in situ* durante un processo di fermentazione finalizzato alla produzione di una bevanda a base di legumi. In primo luogo, sono state ottimizzate le condizioni di coltura, considerando parametri come il tempo e la temperatura di crescita e la concentrazione di glutammato monosodico (MSG), utilizzato dai ceppi come precursore del GABA, mediante l'impiego di un test colorimetrico, di un saggio enzimatico (GABAse) e analisi tramite UPLC/MS-MS. In base ai risultati ottenuti, sono stati selezionati 4 ceppi e utilizzati come colture starter per arricchire naturalmente in GABA una bevanda a base di legumi.
Le analisi UPLC/MS-MS hanno evidenziato una produzione di GABA chiaramente ceppo-specifica sia *in vitro* che nel sistema alimentare durante la fermentazione.

Key words: *Lactiplantibacillus plantarum*, starter cultures, strain selection, fermented legume—based beverage, GABA

1. Introduction

In accordance with the PhD thesis project, this reports the main results of the activities concerning:
(A1) selection and characterization of *Lpb. plantarum* starter cultures
(A2) screening of *Lpb. plantarum strains* for GABA production (colorimetric assay)
(A3) optimization of culture conditions for GABA production
(A4) *in vitro* quantification of GABA production by *Lpb. plantarum* strains (GABase enzymatic assay)
(A5) *in vitro* quantification of GABA production by UPLC-MS/MS
(A6) selection of an innovative vegetable substrate for the fermentation
(A7) optimization and monitoring of the lab-scale fermentation
(A8) quantification of GABA production with UPLC-MS/MS

2. Materials and Methods

The *Lpb. plantarum* strains investigated in this study belong to the Culture Collection of the University of Teramo. Previously, these strains were genetically and phenotypically characterized for various probiotic traits, (Prete et al., 2017, Garcia-Gonzalez et al., 2018, Prete et al., 2020a, Prete et al., 2020b). In order to assess the potential of *Lpb. plantarum* strains to produce γ-amino butyric acid (GABA), an initial qualitative screening was performed using the colorimetric glutamate decarboxylase assay (GAD assay), according to Edalatian Dovom et al. (2023). Subsequently, the enzymatic activity was assessed by observing the development of a bluish colour (Santos-Espinosa et al., 2020). To optimize GABA production through culture conditions, all the tested strains were cultured in MRS broth with different concentration of monosodium glutamate (MSG) (0 mM, 25 mM, 50 mM, 160 mM, 270 mM, and 400 mM) for 96 hours at 30°C, since MSG, temperature and incubation time influence the microbial production of GABA (Villegas et al., 2016). The GABA production by *Lpb. plantarum* strains after the incubation at different MSG concentrations was evaluated using the GABase enzymatic assay, measuring absorbance at 340 nm every 5 minutes for 1 hour with a Multimode Plate Reader Enspire (PerkinElmer, Waltham, MA, United States) (Tsukatani et al., 2005). Based on the enzymatic assay results, MRS broth supplemented with 400 mM MSG has been selected as the optimal concentration, and the microbial GABA production has been confirmed by UPLC-MS/MS analysis using a UPLC Acquity H-class chromatographic system (Waters, Milford, Massachusetts, USA) coupled with a Sciex 4500 Qtrap mass spectrometer (Toronto, ON, Canada) equipped with a Turbo V electrospray ionization (ESI) source. In addition, the precursor amino acid (glutamic acid) and its related neurotransmitter (GABA) were also identified and quantified by UPLC-MS/MS analysis. Then, to assess GABA production by *Lpb. plantarum* strains in a real food model system, a legume-based matrix was selected as an innovative substrate to obtain a fermented beverage. Fermentation was carried out by incubation at 30 °C for 24 hours, while monitoring pH (using a pH meter, pH 80+ DHS® Instruments), titratable acidity (with phenolphthalein as indicator) and performing viable count to confirm the viability of the inoculated *Lpb. plantarum* strains at 30°C for 48 hours under microaerophilic conditions. As previously described, an UPLC-MS/MS analysis was performed for the identification and quantification of glutamic acid and its derived neurotransmitter (GABA) in the unfermented and fermented legume-based beverage.

3. Results and Discussion

3.1 Colorimetric screening of *Lpb. plantarum* strains for GABA production (GAD assay)

Due to the strain-specific nature of microbial functional properties, including the ability to produce bioactive molecules and/or neurotransmitters, a screening step is crucial to detect these properties (Binda et al., 2020). In line with that, *Lpb. plantarum* strains were initially subjected to a qualitative colorimetric screening for GABA production, using the GAD assay (Santos-Espinosa et al., 2020). The results showed that all strains had the potential to produce GABA, with differential capabilities to transform the substrate from green to blue. Based on those differences, the strains were initially categorized into three different groups: low, medium, and high GABA production, confirming the strain-specific nature of this property.

3.2 *In vitro* quantification of GABA production using GABAse enzymatic assay

Subsequently, GABA production was confirmed and quantified for all strains using the GABAse enzymatic assay (Tsukatani et al., 2005; Villegas et al., 2016). Since the concentration of the precursor MSG is one of the major factors influencing GABA production, the enzymatic assay was performed in MRS, testing GABA production at 6 different MSG concentrations (0, 25, 50, 160, 270, and 400 mM) (Villegas et al., 2016). The result of the enzymatic assay confirms differential GABA production among the 20 tested strains across all MSG concentrations, identifying 400 mM as the optimal condition for GABA production. This assay led to the careful selection of four strains (*Lpb. plantarum* C9S2, C9O4, LAB62, and LT52) as the most efficient in converting MSG to GABA (Figure 1).

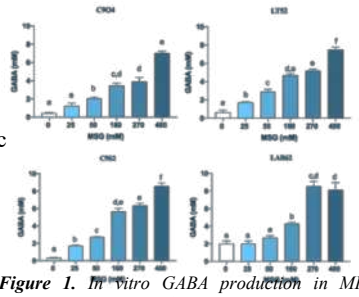


Figure 1. *In vitro GABA production in MRS medium by selected Lpb. plantarum strains.*

3.3 Quantification of GABA production with UPLC-MS/MS

To evaluate the potential of combining the selected strains and use them as co-inoculum, GABA production in MRS broth with 0 mM and 400 mM MSG was quantified using UPLC-MS/MS. Two combinations were tested based on individual strain GABA production: *Lpb. plantarum* C9O4 and LT52 (C1) and *Lpb. plantarum* C9S2 and LAB62 (C2). As shown in Figure 2, a significant (*p<0.05) different GABA production was observed between the two combinations C1 and C2, with C2 showing higher production (468.27 ± 34.49 mg/L, approximately 4.54 mM) and C1 showing a lower production (261.34 ± 19.83 mg/L, approximately 2.53 mM). However, both concentrations exceed 2.1 mM. which is the amount reported for the strain *Lb. brevis* NBRC 12005, known for being an excellent GABA producer (Santos-Espinosa et al., 2020).

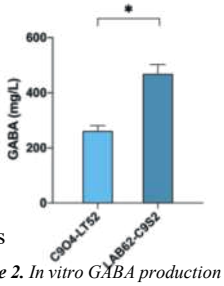


Figure 2. *In vitro GABA production in MRS medium by the two selected combination for co-inoculation (C1 and C2). C1= Lbp. plantarum C9O4+LT52; C2 = Lpb. plantarum C9S2+LAB62.*

3.2 Quantification of GABA by UPLC-MS/MS in the real food system

With the aim of formulating a functional fermented beverage, the legume flour used as food matrix for fermentation was preliminary characterized with UPLC-MS/MS to assess the presence of glutamate. The analysis revealed a high concentration of glutamic acid (355.81±34.1 mg/L), known as precursors of GABA, confirming this substrate as a valid substrate to produce GABA during fermentation with selected starters. Moreover, by conducting fermentation without MSG addition, the GABA detected in the fermented samples can be largely attributed to the enzymatic activities of the selected *Lpb. plantarum* mixtures (C1 and C2) inoculated as starters. At the end of the fermentation process, UPLC-MS/MS analysis was performed to verify the actual production of GABA in the real food model by the two selected mixtures of *Lpb. plantarum* strains (Figure 3). The results indicate a significantly different production (****p < 0.0001) between both the fermented C1 and C2 beverages compared to the unfermented control, confirming the strains' ability to produce GABA *in situ* in a real food system. Overall, our study shows that GABA production by 20 *Lpb. plantarum* strains is clearly a strain-specific feature. Moreover, since the direct addition of GABA to foods is not permitted, microbial biosynthesis of GABA during fermentation could represent a successful approach to naturally enrich foods with GABA and, in turn, produce final clean-label, healthy fermented foods.

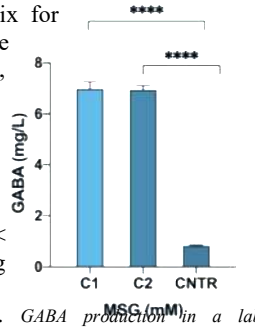


Figure 3. *GABA production in a lab-scale fermentation of a legume-based matrix by C1 and C2. CNTR= unfermented matrix.*

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Sustainable chemical processes for the valorization of agri-food residues (P-II-73)

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The PhD thesis project aims to identify molecules with high antimicrobial activity starting from agri-food chain residues (e.g., grapevine, citrus fruits) for potential use in crop protection. During the second year, in order to have access to a sufficient quantity of the selected bioactives and to conduct structure-activity relationship studies (antifungal and antibacterial), chemo-enzymatic synthetic procedures were developed to obtain polyphenols, their metabolites, and synthetic analogues. The results obtained during this phase of the research will be highly relevant for the targeted selection of the most promising agri-food residues and the isolation of the desired bioactives.

Processi chimici sostenibili per la valorizzazione di residui agro-alimentari

Il progetto di dottorato ha l'obiettivo di identificare nei residui della filiera agroalimentare (es. vite, agrumi) molecole dotate di elevata attività antimicrobica per un potenziale impiego nella protezione delle colture. Durante il secondo anno, per avere accesso ad una quantità sufficiente dei bioattivi di interesse e condurre studi di relazione struttura-attività biologica (antifungina ed antibatterica), sono state sviluppate procedure chemo-enzimatiche per la sintesi di polifenoli, loro metaboliti e analoghi sintetici. I risultati ottenuti in questa fase della ricerca saranno di grande importanza per la selezione mirata dei residui di filiera più promettenti e l'isolamento dei bioattivi di interesse.

Keywords: natural compounds, polyphenols, chemo-enzymatic processes, antimicrobials

1. Introduction

According to FAO website, agri-food industries generate significant amounts of food loss and waste, especially from fruit and vegetable processing. These include peels, seeds, leaves, and stems rich in high-value natural compounds that are interesting for different industrial sectors (i.e. agri-food, nutraceutical, cosmetic and pharmaceutical). Resveratrol is a natural phytoalexin, a small defensive molecule with biocidal activity, whose *de novo* synthesis is induced by stress, which is found in high concentration in grapes, as reported by L. Mattio et al. (2019). It has been intensively studied for its variety of biological activities, as well as its glycosylation, methoxylation, hydroxylation, prenylation and oligomerization derivatives. However, the scarce quantities of resveratrol analogues and metabolites, combined with their difficult, time-consuming and expensive isolation from natural matrices, have hampered more extensive biological evaluation. A versatile synthetic approach has been developed to attain a collection of 14 resveratrol metabolites and derivatives in sufficient amounts for biological evaluation and systematic structure-activity relationship studies. The collection was designed combining modifications associated with phase I metabolism (i.e. reduction of the double bond, dehydroxylation and methylation of phenolic hydroxyl groups). Thanks to this approach, the impact of different substitution patterns, shape and geometry on the biological activities will be highlighted. Given the rising interest in the bidirectional interaction between gut microbiota and polyphenols, which affects human health, the antibacterial properties were initially evaluated considering a representative panel of Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Salmonella enterica* Enteritidis and *Pseudomonas aeruginosa*). To expand the potential application of resveratrol metabolites also to the agri-food sector, the biological activity was evaluated against relevant phytopathogenic fungi (*Pyricularia oryzae*, *Botrytis cinerea*, and *Fusarium culmorum*) considering both mycelium growth inhibition and the inhibition of spore germination and appressoria formation (only for *Pyricularia oryzae*).

2. Materials and Methods

General procedure for the synthesis of styrenes
To a solution of methyltriphenylphosphonium bromide (2.6 eq) in dry THF (24 mL), *t*BuOK (2.8 eq) was added in several portions, and stirred for 15 min. The aldehyde (1 eq) was added, and stirring was kept for another 2 h. The reaction mixture was quenched with acetone (1.6 eq), diluted with diethyl ether (50 mL), washed with water and brine (2 x 2 mL each), dried (Na_2SO_4), and the solvents were evaporated under reduced pressure. The crude product was purified by chromatography (silica gel, cyclohexane/ethyl acetate mixtures) and the product was isolated in good yields (85-90%).

General procedure for the Heck reactions
A mixture of aryl iodide (1 eq), tri(*o*-tolyl) phosphine (0.225 eq), Pd(II) acetate (0.05 eq) and dry TEA (2.1 eq) in dry ACN was stirred under nitrogen at r.t for 30 min, and then the styrene (1.2 eq) was added. The reaction mixture

was stirred under reflux for 18 h. The reaction was cooled to room temperature, quenched by the addition of hydrochloric acid (1 N) and extracted with EtOAc three times. The organic phases were dried (Na_2SO_4), filtered, the solvent evaporated, and the crude product was subjected to chromatography (silica gel, hexane/ethyl acetate mixtures) and isolated in moderate yields (40-60%).

General procedure for the reductive hydrogenation
A solution of each precursor stilbenoid (50 mg) in EtOH (5 mL) was stirred at room temperature under H_2 pressure in the presence of 10% Pd/C (10 mg); the reactions were monitored by TLC and stopped after 4 – 16 h., by filtering the catalyst off through a celite pad. The filtrate was evaporated in vacuo, and the product was obtained in quantitative yield.
All synthesized derivatives were stored in dry form at −20 °C and dissolved in DMSO at a final concentration of 20 mM prior to use in biological assays.

3. Results and Discussion

3.1 Chemistry

Deoxyrhapontigenin (**1**) and compound **2** were synthesized following the procedure reported by L. Mattio et al (2019), previously developed in our research group. Due to the low selectivity of this protocol, an alternative approach has been developed, exploiting the peracetylation of resveratrol, followed by the selective deprotection of the acetyl group in the *para* position catalysed by lipase from *C. antarctica*. Subsequently, the methylation with iodomethane followed by the basic hydrolysis of the acetyl group allowed to obtain the desired compound **1**. However, during the methylation attempts, the migration of the acetyl from C3 to C4'was observed, resulting in a mixture of compounds among which compound **2** was isolated after the hydrolytic step.
The other synthetic strategies employed Wittig reactions, Heck reactions, methylation reactions, and Pd/C catalyzed hydrogenation. The resulting small library consists of 14 monomeric stilbenoids with different methylation patterns.

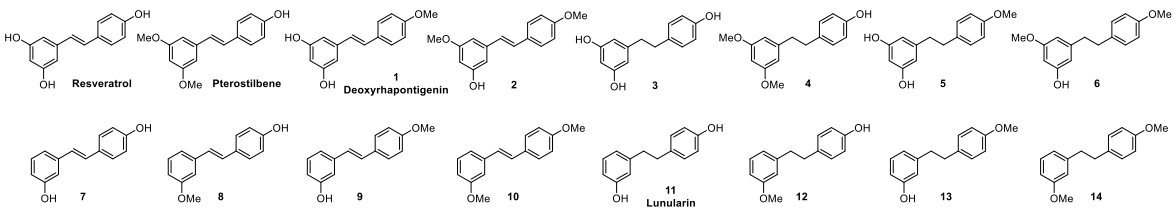


Figure 1: *Structures of the synthesized stilbenoids.*

3.2 Biological evaluation

The obtained compounds were then evaluated as potential antibacterial agents on a panel of Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli*, *S. enterica* Enteritidis and *P. aeruginosa*). Compound **8** showed a minimum inhibitory concentration (MIC) of 8 µg/mL against *S. aureus,* 16 folds lower than resveratrol and 4 folds lower than pterostilbene (natural 3,5-di-*O*-methylated derivative of resveratrol). Other interesting compounds against *S. aureus* and/or *P. aeruginosa* were compounds **5**, **6**, and **7** (MIC 64 µg/mL). Further investigations are currently ongoing.
In parallel, the compounds were also evaluated for their antifungal properties with promising preliminary results. The inhibition of mycelium growth of four fungal strains (*P. oryzae*, strobilurin sensitive and resistant strains, *B. cinerea*, and *F. culmorum*) was evaluated at 200 µM. The results showed that eight compounds out of fourteen were able to inhibit the mycelia growth more than 50%, with compound **2** inhibiting almost 90% of *P. oryzae* strobilurin sensitive strain. Additionally, the compounds were tested for their ability to inhibit spore germination in *P. oryzae*. This process is indeed crucial for the formation of the appressorium, a specialized dome-shaped structure, essential for the rice cuticle penetration and the fungal infection stage, as described by A. Kunova et al (2023). In this context, most of the synthesized metabolites effectively inhibited both spore germination and appressorium formation, even at concentrations as low as 50 µM. Further tests at lower concentrations (10 µM and 1 µM) are currently ongoing.

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Toxicological profile and preliminary therapeutic effects of a green extra-virgin olive oil extract in the zebrafish model (P-II-74)

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The first activities of the PhD thesis project are described. Firstly, the toxicological profile of a green bioactive extra-virgin olive oil (EVOO) extract containing the secoiridoids oleocanthal (OLC), oleacein (OLE), hydroxytyrosol (HYT) and tyrosol (TYR) in the zebrafish model (*Danio rerio*) was determined. Secondly, sublethal concentrations of the extract have been chosen to test for their therapeutic potential on wild type larvae treated with lipopolysaccharide (LPS) and pentetrazole (PTZ).

Profilo tossicologico ed effetti terapeutici preliminari di un estratto ecosostenibile di olio extravergine di oliva nel modello zebrafish

Le prime attività del progetto di tesi di dottorato sono descritte. In primo luogo, è stato determinato il profilo tossicologico di un estratto di olio extra vergine d'oliva (EVOO), ecosostenibile e bioattivo, contenente i secoiridoidi oleocantale (OLC), oleaceina (OLE), idrossitirosolo (H-Tyr) e tirosolo (TYR) nell'organismo modello *Danio rerio*. Successivamente sono state scelte delle concentrazioni subletali dell'estratto da testare per il loro potenziale terapeutico su larve wild type trattate con lipopolisaccaride (LPS) e pentetrazolo (PTZ).

Key words: EVOO, zebrafish model, secoiridoids, toxicological profile, therapeutic effects

1. Introduction

In accordance with the PhD thesis project previously decribed (Damiano, 2024), this poster reports the main results of the activities concerning:

A1) Toxicological profile of extract
  1) FET TESTs
  2) Toxicological endpoints
A2) Evaluation of anti-inflammatory properties of the extract
  1) Creation of inflammatory zebrafish model
  2) FET TESTs
A3) Evaluation of anti-inflammatory properties of single bioactive compound
  1) FET TESTs
A4) Assessment of therapeutic/modulatory effects of the polyphenols in exam
  2) Molecular assay (PCR)

2. Materials and Methods

The lethal and sub-lethal effects after exposure of zebrafish embryos to EVO oil extract for 96 hours post-fertilization (hpf) were evaluated through the Fish Embryo Acute Toxicity (FET) test. The tests were conducted according to the guidelines for chemical testing (OECD TG no.236) (OECD, 2013). In order to make assessments regarding the toxicological profile and therapeutic potential of the EVOO extract, the following reagents and solvents were used:

• Green EVO oil extract [18mg/mL];
•Solvent betaine: propylene glycol (molar ratio 1:3.3);
• LPS from *Escherichia Coli* 0111:B4;
• PTZ Number CAS 54-95-5 purchased from Sigma-Aldrich (Co. St. Louis, MO);
• Dilution Water (DW) was used as indicated by OECD TG 203 guidelines and as a diluent for the stock solution of the EVOO extract. DW was prepared and adjusted to pH (7.75);
• 4% of 3,4-dichloroaniline (purity >98%; Sigma-Aldrich; Co. St. Louis, MO).

For the tests, embryonated eggs from adult zebrafish (*Danio rerio*) of the wild-type AB strain, raised at the Facility of the University of Teramo, were used. The test, conducted within one-hour post-fertilization (hpf) and not later than 3 hpf, lasted a total of 96 hours. Eight concentrations of EVOO extract were tested: 0.05 mg/L, 0.1 mg/L, 0.5 mg/L, 1 mg/L, 2 mg/L, 5 mg/L, 10 mg/L, and 50 mg/L. Additionally, a negative control (embryos exposed to DW) and a positive control (4 mg/L of 3,4-dichloroaniline) were tested. For each concentration and control, 20 embryos were exposed, and the substance was tested in parallel in 3 independent replicates. The lethal effects were determined by comparison with the controls through daily observations, and the ones taken into consideration for the evaluation of the tests were: embryo coagulation, lack to form somites, lack to detach the tail, and absence of heartbeat.

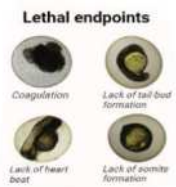


FIG1. *Lethal alterations assessed for FET test*.

To evaluate the anti-inflammatory and neuromodulatory properties of the oil extract and the individual molecules of particular interest represented in the extract, oleocanthal and oleacein, two pathology models of Danio rerio were used:
- a model of inflammation caused by exposure to E. Coli LPS;
- a model of epilepsy induced by exposure to a concentration of PTZ.

Regarding the inflammatory model, zebrafish larvae were treated with oil extract or natural deep eutectic solvent (NaDES) [0.5 mg/L] for 72h and then exposed to DW or LPS [10 ug/mL] for 24h. Two concentrations of Oleocanthal [0.18 mg/L], [0.36 mg/L] and Oleacein [0.17 mg/L], [0.35 mg/L] were used to pretreat zebrafish larvae for 72h and then exposed to DW or LPS [10 ug/mL] for 24h. The control groups (CTRL) were exposed to DW only and DMSO. Finally, the sublethal concentration of 0.5 mg/L of EVO oil extract was tested as a pretreatment for 96h before exposing the larvae to a concentration of 2 g/L of PTZ for 30 minutes. At the end of the treatment of all zebrafish larvae, they were frozen in eppendorf tubes containing TrizolTM, working on ice (cold sedation of the larvae) to proceed with subsequent freezing at -80°C; at this point, molecular analysis was conducted using the Real-Time PCR technique. The gene expression levels of key genes involved in inflammatory and neromodulatory pathways were examined (e.g. interleukins, cytokines, *bdnf, c-fos*, etc.) using *β-actin* as the housekeeping gene. The PrimerBlast tool was used for primer design with reference to the GRCz11 genome, given that the animal model is zebrafish.

3. Results and Discussion

3.1 Determination of toxicological profile of EVOO extract

The toxicological profile of the EVO oil extract under consideration was evaluated through the determination of its LC50 value, using specific software (ToxRat Solutions GmbH, Germany; EFSA web tool), which was found to be 3.7 mg/L (95% CI 2.845-4.851 mg/L) at 96 hours post-fertilization.

Concentrations (mg/L)	LC50 (mg/L)
0.05, 0.1, 0.5, 1, 2, 5, 10, 50	3.7

Table 1. *Concentrations of the tested EVO oil extract and the determined LC50 value.*

3.2 Assessment of neuromodulatory and anti-inflammatory properties of EVO oil extract

The sublethal concentration of 0.5 mg/L was chosen to investigate the neuromodulatory power of this extract by assessing the changes in the expression of important neuromodulatory genes in the zebrafish epilepsy model. Statistical differences are considered such * $p < 0.05$, ** $p < 0.005$, and *** $p < 0.0005$ (for reasons of confidentiality, specific data are not fully reported here). A significant reduction was observed in the increased expression of the *c-fos, bdnf, serta, oxtrb* genes induced by PTZ exposure, following pretreatment with 0.5 mg/L of EVOO extract. In zebrafish, *c-fos* upregulation is a known marker of neuronal activation in response to stress or seizures. PTZ exposure markedly increases *c-fos* expression, as shown by RT-PCR and in situ hybridization, highlighting its role in epilepsy pathogenesis (Baraban *et al*., 2005). Treatment with PTZ significantly elevates the levels of expression of this gene in this case, while pre-treatment with EVO oil extract restores gene expression almost to the level of the negative control. The decrease in gene expression levels of *bdnf*, on the other hand, is associated with neurodegenerative diseases; its expression is also found to be strongly altered in stressful conditions, as observed in larvae treated with PTZ (Baraban *et al.,* 2005). The neuroprotective effect of the extract is also visible on plates pre-treated with oil, which show a lower expression of the *bdnf* gene following treatment with PTZ. An upregulation of *serta* gene expression also has been observed, indicating a potential compensatory activation of the serotonergic system in response to seizures. This finding aligns with the modulation seen in other genes associated with epileptiform activity (Torres-Hernández *et al*., 2016); furthermore, pretreatment with extract oil was found to exert the expected protective effects. Previous studies have shown that oxytocin has an anticonvulsant effect on PTZ-induced seizures and that is why its expression levels are elevated in the presence of the drug (Erbas *et al.,* 2013). Also in this case, pretreatment with the EVO oil extract reduces the expression levels of this gene (*oxtrb*), demonstrating promising neuromodulatory properties of the extract. The same sublethal concentration of 0.5 mg/L was used to investigate the anti-inflammatory/antioxidant properties of EVO oil extract on the LPS inflammatory zebrafish model. In zebrafish, *gpx1a* is expressed in multiple tissues and is predicted to be involved in the hydrogen peroxide catabolic process and the cellular response to oxidative stress (https://zfin.org/ZDB-GENE-030410-1#summary). Like gpx1a, *sod1* helps limit inflammation by reducing ROS levels. Elevated ROS can trigger inflammatory cascades, so sod1 activity is protective against inflammation-driven tissue damage (Da Costa *et al*., 2014). Mutations or reduced activity of *sod1* in zebrafish lead to increased oxidative stress, neuromuscular defects, and heightened sensitivity to environmental insults, as seen in models of neurodegenerative diseases like Amyotrophic lateral sclerosis (Ramesh *et al.,* 2010). Preliminary results of this research showed that OLC and OLE significantly decreased LPS-induced expression of anti-inflammatory and antioxidant genes, particularly *gpx1a* and *sod1*, which aligns with previous *in vitro* studies (Carpi *et al.,*2019). On the other hand, the EVO oil extract resulted in a modulation of the target genes, although the effect was not significant.

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Antifungal Effects of Essential Oils (EOs) and Pulsed Light (PL)on Cheese Spoilage Fungi: Inhibition, Gene Expression, and Cellular Disruption (P-II-75)

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The PhD thesis includes two research activities focused on controlling fungal spoilage in Caciocavallo cheese: the use of essential oils (*Salvia rosmarinus*, *Origanum vulgare*, *Laurus nobilis*) and pulsed light (PL) technology. The first one investigates the antifungal efficacy of these plant-based EOs against *Penicillium*, *Fusarium*, *Aspergillus*, and *Alternaria* strains isolated from the cheese. The second examines PL as a non-thermal treatment to control *Alternaria alternata*, focusing on growth inhibition, cellular disruption and related gene expression changes.

Antifungal effects of essential oils and pulsed light on cheese spoilage fungi: inhibition, gene expression, and cellular disruption

Cheese spoilage by fungi, especially in products like Caciocavallo, is a major issue impacting quality, safety, and economic value. Common spoilage genera include *Penicillium*, *Aspergillus*, *Fusarium*, and *Alternaria*, which pose health risks and economic losses. Conventional control using chemical preservatives raises concerns about safety and sustainability. Consequently, interest is increasing in natural alternatives like EOs and non-chemical approaches such as PL technology.
Key words: Essential oils, Pulsed light, Cheese spoilage, Gene expression, Fungal inhibition

1.Introduction

The research is divided into two main experimental approaches to control mold spoilage in Caciocavallo cheese.
(A) This study evaluated the antifungal activity of EOs rich in α-pinene, camphor, and 1,8-cineole against molds isolated from Caciocavallo. Their effects on mycelial growth and spore germination were tested using CAM media
(B) The second part investigated PL treatment to control Alternaria alternata. Using short bursts of intense light, PL's impact was assessed through growth inhibition, spore germination, and RT-qPCR analysis of key genes (PKS, CHIT, ALM1, PP2A) involved in fungal growth and stress response.

2. Materials and Methods

Molds isolated from Caciocavallo cheese were identified through PCR and sequencing of 18S rRNA and β-tubulin genes. EOs from *Salvia rosmarinus*, *Origanum vulgare*, and *Laurus nobilis* were extracted by steam distillation, characterized by GC-MS, and tested for antifungal activity on a newly optimized Cheese Agar Medium (CAM) at concentrations ranging from 0.14% to 7%. Their efficacy was evaluated based on inhibition of mycelial growth and spore germination. For PL treatment, *Alternaria alternata* strains were cultured on CAM and PDA, and exposed to 30 and 120 pulses using a Xenon X-100 system. The effects on mycelial growth, spore germination, and gene expression were evaluated, with RT-qPCR targeting 22 genes related to metabolism, stress response, and virulence. Cellular damage post-PL exposure was assessed using confocal microscopy.

3. Results and Discussion

3.1 EOs as Antifungal Agents: The antifungal activities of the EOs were evaluated by their inhibition of mold mycelial growth and spore germination isolated from sampling during 3 seasons. The EOs showed varying degrees of inhibition, with *Salvia Rosmarinus* showing the highest inhibition against *Aspergillus flavus* (44% inhibition at 7% concentration. The optimized CAM medium supported robust mold growth, mimicking natural cheese conditions, and was effective in testing the EOs' antifungal potential. These results suggest that essential oils could be a viable alternative to chemical preservatives in cheese production in Table1.

Table1. Essential Oil (EO)	**Mold Species**	**Concentration (%)**	**Mycelial PI (%)**	**Spore Germination PI (%)**
SR-CV3-23 (*Salvia rosmarinus*)	*P. camemberti, P. Commune, A. alternata, F. avenaceum, A. flavus*	7	45	66
SR-CV6-23 (*Salvia rosmarinus*)	*P. camemberti, P. Commune, A. alternata, F. avenaceum, A. flavus*	7	**46**	**82**
SR-CV2-23 (*Salvia rosmarinus*)	*P. camemberti, P. Commune, A. alternata, F. avenaceum, A. flavus*	7	41	**83**
Ln-23 (*Laurus nobilis*)	*P. camemberti, P. Commune, A. alternata, F. avenaceum, A. flavus*	7	**58**	**77**
OV-C-23 (*Origanum Vulgare*)	*P. camemberti, P. Commune, A. alternata, F. avenaceum, A. flavus*	7	43	**83**

3.2 Pulsed Light-Induced Fungal Disruption: PL treatment significantly inhibited *Alternaria alternata* growth, with 22-37% inhibition in mycelial growth and 78-83% inhibition in spore germination at higher pulse doses. Confocal microscopy revealed substantial damage to the fungal mycelia, spores, membrane, mitochondria, and nucleus, as well as oxidative stress markers like ROS/RNS accumulation (Fig1). Gene expression analysis showed that PL treatment downregulated key genes associated with fungal growth and virulence (e.g., *PKS*, *CHIT*, *ALM1*) immediately post-treatment (Fig 2). Over time, adaptive upregulation was observed in stress response genes such as *PP2A*, *CAL*, and *TES*. These findings suggest that PL causes both structural and genetic disruption of the fungus, making it a promising non-thermal method for fungal control in dairy products.

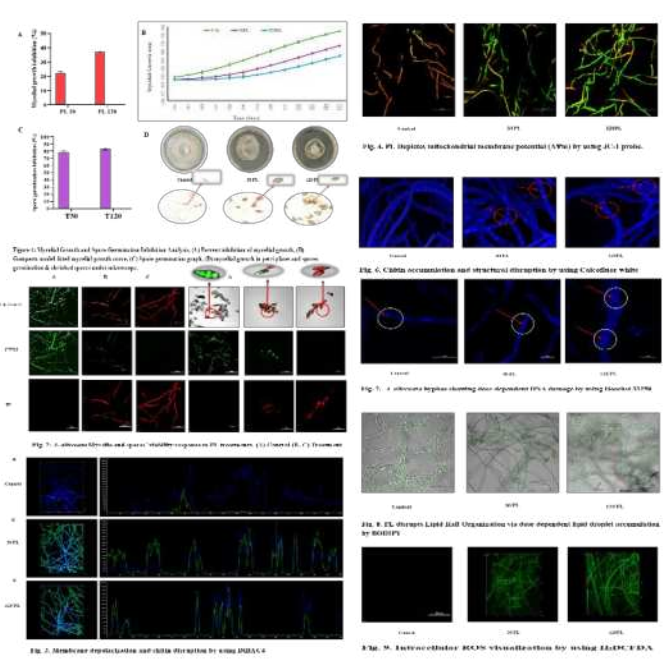
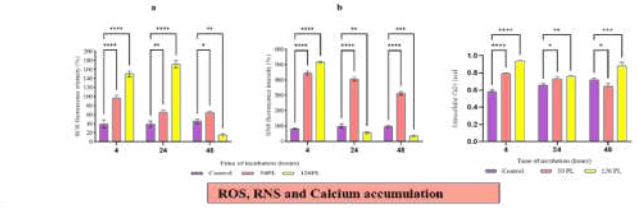
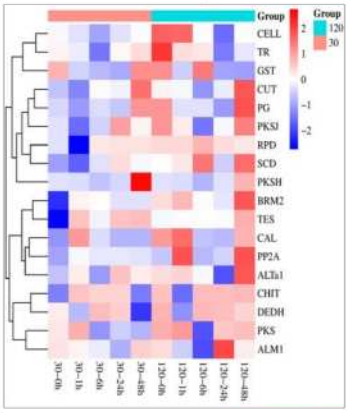


Fig1 : Unraveling the Mechanism of PL in Controlling *Alternaria alternata* by using different molecular probe under Nikon confocal microscopy



ROS, RNS and Calcium accumulation



	30-0h	30-1h	30-6h	30-24h	30-48h	120-0h	120-1h	120-6h	120-24h	120-48h
BRM2	-2.35	1.85	1.63	1.06	1.11	2.29	2.90	1.54	1.18	4.86
CAL	-2.64	1.89	-1.01	-1.99	-1.96	1.81	2.85	-1.55	-1.85	1.30
CELL	-1.80	-2.30	-3.57	-2.30	-1.73	1.47	1.40	-1.74	-4.72	-1.64
CHIT	-51.15	-5.02	-8.30	-8.30	-52.77	-5.11	-55.62	-4.09	-3.08	-1.66
CUT	-2.67	-3.86	-1.45	-1.65	1.47	-1.19	-1.57	-3.58	-1.82	1.98
DEDH	-10.95	-23.88	-4.57	-6.90	-42.21	-2.85	-29.90	-3.56	-4.38	-7.40
GST	1.02	-1.10	-1.54	-1.80	1.66	1.68	-1.16	2.03	-1.89	-1.99
PG	-1.41	-2.48	-1.34	-1.63	1.10	1.11	-1.17	-1.18	-2.49	2.31
PKS	-2.39	-1.71	-3.91	-3.27	-3.56	-1.70	-1.45	-4.77	-2.05	-1.91
PKSH	-1.10	-1.16	-1.85	-1.31	5.80	-1.09	-1.28	-2.22	-1.11	1.59
PKSJ	-2.50	-6.03	-2.87	1.27	-1.26	1.60	-1.03	-5.66	-1.87	2.12
PP2A	-2.17	-1.10	-1.25	-1.30	-1.32	-1.68	1.29	-1.88	-1.53	1.31
RPD	-5.28	-19.23	-2.05	-1.88	-1.82	-1.26	-1.28	1.02	-1.53	-2.20
SCD	-3.93	-5.24	-2.43	-1.08	-1.97	-1.82	-1.30	1.08	-2.93	1.30
TES	-11.18	1.16	-1.62	1.27	1.49	-1.39	-1.23	-1.03	-1.27	2.09
TR	-1.67	-2.18	-3.95	-1.81	-1.43	1.05	-1.40	-1.56	-3.03	-2.21
ALM1	-1.88	-2.21	-2.82	-3.99	-1.53	-1.85	-3.37	-5.03	1.09	-2.09
ALTa1	-4.10	-2.27	-4.96	-1.36	-2.23	-1.90	-1.63	-2.83	-6.71	1.18

Fig 2. Change in Gene Expression

Genes related to secondary metabolism, cell wall integrity, allergen production, DNA repair, calcium signaling, cell cycle, carbohydrate metabolism, and stress response show significant expression changes. Clustering was performed using Pearson correlation and average linkage. Heatmap illustrates gene expression in treated samples (30, 120 PL) compared to control (C) across timepoints (0, 1, 6, 12, 48 h). Red indicates upregulation, blue indicates downregulation.

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Characterization and Authentication of Alcoholic Products with Geographical Indications (P-II-76)

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This work reports two research activities carried out as part of the PhD project. The first activity analyzed the impact of different oak wood treatments on the volatile composition of the Geographical Indication (GI) *Brandy Italiano,* determined by SPE-GC-MS and HPLC-DAD analysis. The second activity focused on the application of volatile fingerprinting, obtained through Flash GC (FGC) and analysed using multivariate statistical methods, with the aim of differentiating GI *Grappa* samples produced from four distinct grape varieties: Muscat, Brunello, Prosecco, and Barolo.

Caratterizzazione ed autenticazione di prodotti alcolici a indicazione geografica

Questo lavoro presenta due attività di ricerca svolte nell'ambito del progetto di dottorato. La prima ha analizzato l'impatto di diversi trattamenti del legno applicati alle botti di rovere utilizzate per l'invecchiamento sul contenuto in composti volatili del *Brandy Italiano* a Indicazione Geografica (IG), determinato mediante analisi SPE-GC-MS e HPLC-DAD. La seconda attività si è focalizzata sull'applicazione del profilo dei composti volatili, ottenuto mediante Flash GC (FGC) e analizzato attraverso metodi statistici multivariati, con l'obiettivo di differenziare campioni di *Grappa* a Indicazione Geografica (IG) prodotti da quattro diverse varietà di uva: Moscato, Brunello, Prosecco e Barolo.

Keywords: Geographical Indication, Authentication, *Grappa*, Flash-GC, *Brandy Italiano*, Volatile Compounds.

1. Introduction

In accordance with the PhD thesis project previously described (Arduini, 2024), this poster reports some results of the following two activities concerning:
(A1) Application of "traditional" analytical techniques (SPE/GC/MS and HPLC) on alcoholic products with GI for their characterization and for the identification of potential category-marker compounds.
(A2) Application of "fingerprint" analytical techniques, including Flash-GC coupled with chemometrics, on alcoholic products with GI to develop classification models for authentication purposes.

2. Materials and Methods

2.1 Influence of wood treatments on the volatile composition of GI *Brandy Italiano* aged in oak casks.

Four brandy samples, produced from the same Trebbiano Romagnolo wine distillate, were analysed. The brandies were aged for eight months in 350-L new Limousin French oak barrels, differing for wood seasoning duration and toasting level: L24 (24 months natural seasoning, light toasting), L36 (36 months seasoning, light toasting), H24 (24 months seasoning, heavy toasting), and H36 (36 months seasoning, heavy toasting). After ageing, 50 mL of each sample was collected from each barrel, diluted to 40% vol., and analysed in triplicate using HPLC-DAD and SPE-GC-MS. HPLC-DAD analysis was carried out without sample pretreatment following the EU method for wood compound analysis in spirits (The Commission of the European Communities, 2000). For SPE-GC-MS analysis, 10 mL samples were diluted to 10% vol. and spiked with 2-octanol (internal standard) before extraction. The method described by Lopez et al. 2002 was then applied. Compounds were identified by reference standards or libraries (reverse match >800) and quantified with respect to 2-octanol (µg/L). Two-way analysis of variance (ANOVA) with significance at $p = 0.05$ was carried out with the software XLSTAT (Addinsoft, New York, USA).

2.2 Volatile fingerprint for the differentiation of monovarietal *Grappa* using Flash-GC and chemometrics.

A total of 45 grappa samples from Muscat (13), Barolo (11), Brunello (10), and Prosecco (11) grape varieties were analyzed; 33 out of 45 provided by AssoDistil and the remaining purchased from the market. One mL of each sample was diluted with distilled water to 3 mL and two replicate vials were prepared. Vials were incubated at 40 °C for 20 minutes while agitating (500 rpm) to concentrate volatiles in the headspace. A 5 mL headspace aliquot was then adsorbed onto a Carbowax trap at 40 °C for 60 s before GC analysis. Hydrogen was the carrier gas. The trap was heated to 240 °C (93 s) for desorption and injection. Volatile profiles were recorded with a Heracles II GC (Alpha M.O.S., France) equipped with parallel non-polar MXT-05 and slightly polar MXT-1701 columns (both 10 m × 180 µm), each coupled to a FID. Oven program was the following: 40 °C (2 s), ramp to 80 °C at 1 °C/s, then to 250 °C at 3 °C/s (hold 101 s) with a total runtime of 200 s. Multivariate statistical analysis was performed with the software Matlab (The Mathworks, Natick, MA).

3. Results and Discussion

3.1 Influence of wood treatments on the volatile composition of GI *Brandy Italiano* aged in oak casks.

A total of 107 compounds, including both endogenous components and wood-derived substances, were identified through SPE-GC-MS and HPLC-DAD analyses. With regard to fermentative compounds, ageing in heavily toasted wooden barrels led to a significant reduction (up to 20%) in total alcohols and esters compared to brandy aged in lightly toasted barrels, independently of the wood seasoning duration. Interestingly, specific combinations of toasting intensity and seasoning length showed differentiated effects on volatile compound composition. As for wood-extracted compounds, heavy toasting yielded the highest concentrations of lignin, hemicellulose, and tannin-derived compounds. Sinapaldehyde showed up to a 20-fold increase, while coniferaldehyde and syringaldehyde exhibited increases of up to 10-fold compared to light toasting (Fig. 1a). Heavy toasting also promoted the formation of 5-acetoxymethyl furfural, a furfural derivative identified for the first time in *Brandy Italiano*, potentially serving as a marker of the use of highly toasted wood in short-term ageing processes. The duration of natural wood seasoning significantly influenced both gallic and ellagic acid concentrations, as well as the extraction of cis-β-methyl-γ-octalactone. For barrels with the same toasting level, those made from wood seasoned for 3 years contained 50% more cis-β-methyl-γ-octalactone than those seasoned for only 2 years (Fig. 1b). The same samples are currently being analyzed using other techniques, namely NIR, electronic nose, and electronic tongue, to correlate these findings with results obtained from rapid methods.

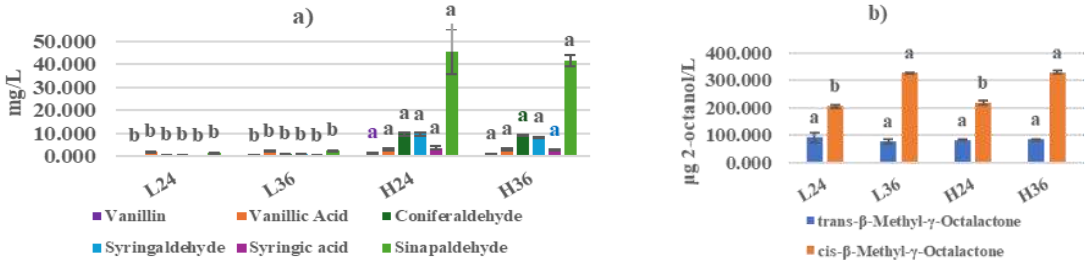


Figure 1 *a) Lignin Derivatives*; *b) Oak lactones. Error bars represent standard deviation of three replicates. For a same compound, values displaying different letters are significantly different (Tukey's test, p = 0.05).*

3.2 Volatile fingerprint for the differentiation of monovarietal *Grappa* using Flash-GC and chemometrics.

The volatile profiles of the grappa samples obtained by FGC were analyzed through unsupervised Principal Component Analysis (PCA) and supervised Partial Least Squares-Discriminant Analysis (PLS-DA). The PCA score plot of PC1 versus PC3 (Fig. 2) revealed a clear discrimination between Barolo and Brunello varieties along PC3, with a less pronounced separation between Prosecco and Muscat. PC1 effectively differentiated grappa samples produced from white grape varieties from those derived from red grape varieties. PLS-DA model showed classification results with high predictive accuracy both in regression (96.6%, Table 1a) and in cross-validation (91.0 %, Table 1b). In the next year of the PhD, these results will be correlated with SPE-GC-MS data already acquired on the same samples.

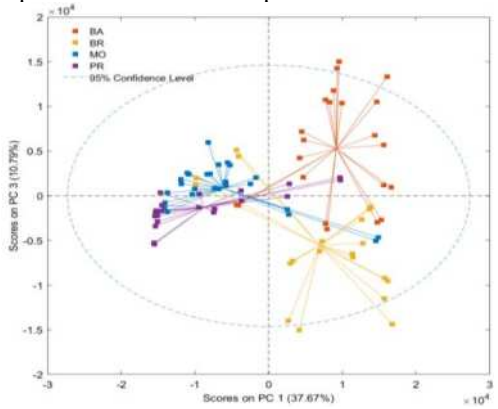


Figure 2 *PCA Score Plot. BA: Barolo; BR: Brunello; MO: Muscat; PR: Prosecco.*

a)

	Actual class			
	Barolo	**Brunello**	**Muscat**	**Prosecco**
Predict as Barolo	22	0	0	1
Predict as Brunello	0	20	0	0
Predict as Muscat	0	0	25	1
Predict as Prosecco	0	0	1	19

b)

	Actual class			
	Barolo	**Brunello**	**Muscat**	**Prosecco**
Predict as Barolo	21	0	0	1
Predict as Brunello	1	20	0	0
Predict as Muscat	0	0	23	3
Predict as Prosecco	0	0	3	17

Table 1 *PLS-DA Confusion Matrix in a) Regression and in b) Cross-Validation.*

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Release and perception of γ-nonalactone and massoia lactone in the red wine matrix: impact of ethanol and acidity (P-II-77)

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The first activity of the PhD thesis project is described. The main objective of this study was to investigate, for the first time, the sensory impact of matrix-aroma interactions in red wine matrices simulating chemical compositional changes expected under future climate change (CC) scenarios. The perceptual interaction with the matrix, the sensory perceptibility and the release of γ-nonalactone (γ-C9) and massoia lactone (ML), was evaluate in wine matrices with different chemical conditions, using sensory and GC-MS analysis. This work provides new insights into how CC-induced wine matrix compositional changes modulate the aroma expression.

Rilascio e percezione del γ-nonalattone e massoia lattone in matrici di vino rosso: impatto dell'etanolo e dell'acidità

La prima attività del progetto di tesi di dottorato viene descritta. L'obiettivo principale dello studio è stato quello di indagare, per la prima volta, l'impatto sensoriale delle interazioni tra matrice e aroma in matrici di vini rossi che simulassero i cambiamenti compositivi chimici potenzialmente previsti in futuri scenari di cambiamento climatico (CC). In particolare, sono stati valutati le interazioni percettive con la matrice, la percettibilità sensoriale e il comportamento di rilascio del γ-nonalattone (γ-C9) e del massoia lattone (ML), in matrici modello di vino caratterizzate da differenti condizioni chimiche. Le analisi sono state condotte attraverso tecniche sensoriali e GC-MS. Questo studio mostra come le variazioni compositive della matrice del vino indotte dal cambiamento climatico, possano influenzare l'espressione aromatica.

Key words: climate change, perceptual interactions, alcohol, pH/total acidity, aroma, wine sensory quality

1. Introduction

In accordance with the PhD thesis project previously described (Di Fede, 2024), this poster reports the main results of the first activity concerning:
(A1) **Study of perceptual interactions and matrix effect on perceptibility and release of γ-C9 and ML:** The results show the effects of the wine matrix (pH, TA, EtOH) on the release and perception of odorous markers previously correlated to CC (γ-C9 and ML) (Allamy et al., 2023) and the effects on the taste and mouthfeel balance of wine. Analytical sensory methods (e.g. triangle test, RATA) and chemical analysis (SPME/GC/MS) have been performed. This integrative approach combining chemical quantification and sensory analysis provided insights into how CC may alter wine sensory profiles by modifying matrix composition.

2. Materials and Methods

A total of nine wine matrices (WM) were reconstituted to simulate different climate change (CC) scenarios. Red table wine was first deodorized through rotary evaporation at 32 °C under reduced pressure (Pittari et al., 2020). The matrices were then reconstituted based on a full factorial design that combined three ethanol concentrations (12, 14, and16% v/v) with three pH/total acidity (TA) ratios (3.2/8, 3.6/6.5, 4/5), representing plausible future oenological conditions associated with CC.

All matrices were spiked with two CC-related volatile organic compounds (VOCs), γ-C9 ML, previously identified as markers of CC in wine aroma with the characteristic notes of dried fruits.

The nine wine matrices underwent both discriminative (triangle test) and descriptive (Rate-All-That-Apply, RATA) sensory evaluations. Thirty-five panellists participated in the discrimination tests, and 16 of them were further trained (Pittari et al., 2020) for the descriptive evaluation. The two lactones were quantified using HS-SPME-GC/MS in SIM mode, following matrix-matched calibration. The release and perception of γ-C9 and ML were measured across the different matrices to evaluate the impact of varying ethanol and acidic profile on aroma expression.

3. Results and Discussion

3.1 Sensory discrimination in wine matrix

Triangle tests were conducted to assess whether differences in ethanol content (12, 14, 16% v/v) and acid profile (pH/TA = 3.2/8, 3.6/6.5, 4/5) modulate the olfactory perception of γ-C9 (logP = 1.942) and ML (logP = 2.540). Results indicate that both matrix parameters significantly influence aroma perception. In particular, γ-C9 was

consistently perceived in all matrices with a pH/TA ratio of 3.2/8, regardless of ethanol content, suggesting that higher ionic strength may enhance its volatility (Arvisenet et al., 2019). For ML, significant discrimination occurred only in matrices with 14% ethanol and pH/TA = 4/5 and 16% ethanol and pH/TA = 3.2/8 suggesting that ML release may be more affected by ethanol concentration rather than ionic strength.

3.2 Volatile release analysed by HS-SPME-GC/MS

HS-SPME-GC/MS analyses aimed to determine whether the outcomes of the triangle tests were linked to physicochemical phenomena—specifically, differences in aroma release due to varying chemical equilibria driven by ethanol content and acid-related ionic strength across wine matrices. Analysis of variance (ANOVA, Tukey's HSD, $\alpha < 0.05$) revealed a significant increase in the release of both γ-nonalactone and massoia lactone in the matrix with 16% v/v ethanol and a pH/TA ratio of 3.2/8. This finding aligns with sensory discrimination results and suggests that, in red wines affected by climate change with elevated alcohol levels, acidification—an increasingly necessary practice to ensure chemical and microbial stability as well as taste balance (Payan et al., 2023)—may enhance the perceptibility of these climate-related aroma markers.

3.3 Perceptual interaction between aroma, taste, and matrix composition

The descriptive analysis was carried out to evaluate both taste and aroma profiles. Regarding taste profile, the PCA in figure 1a explained 94% of the variance. The first component differentiated matrices with bitterness and sweetness features from those associated with acidity and saltiness. According to (Frost et al., 2017) as the pH/TA ratio increases, acidity and saltiness decrease. This means that wines with 16% ethanol and low acidity (CC's effect) showed increased bitterness indicating gustatory imbalance. About aroma evaluation, the PCA in figure 1b explained 82.49% of the variance, and matrices shifted from citric and tropical notes to sweet, full-bodied with rising pH/TA and ethanol levels. These findings suggest that acidity and alcohol jointly modulate retro-nasal aroma and mouthfeel, reducing freshness and altering wine identity.

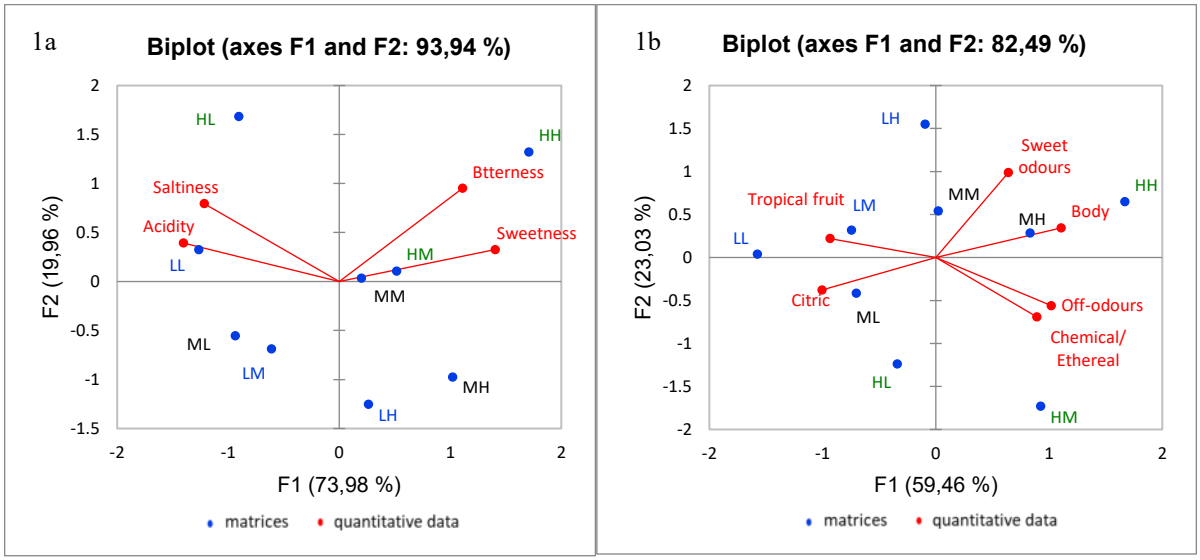


Figure 1. Principal Component Analysis of sensory descriptive data for reconstituted wine matrices spiked with γ-nonalactone and massoia lactone. a) taste profile b) aroma profile.

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Valorization of Polysaccharides and Oligosaccharides from Food Waste (P-II-78)

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This PhD research project aims to develop strategies for the valorization of polysaccharides and oligosaccharides derived from agro-industrial waste and by-products. Among possible sources, olive stones (a lignocellulosic biomass composed mainly of lignin, cellulose, and hemicellulose) were selected as matrix of interest, considering the large amounts generated during olive oil production. The project aims to establish a solid basis for recovering valuable compounds from lignocellulosic materials and using them in food products as innovative, high-value ingredients with specific technological and health-promoting properties. Designing strategies to valorize waste and by-products is a crucial step towards more sustainable diets that not only could reduce environmental impact but also promote a healthy lifestyle for current and future generations.

Valorizzazione di polisaccaridi e oligosaccaridi da scarti alimentari

Questo progetto di dottorato ha l'obiettivo di sviluppare strategie per la valorizzazione di polisaccaridi e oligosaccaridi derivati da scarti e sottoprodotti agroindustriali. Tra le possibili fonti, i noccioli di oliva (una biomassa lignocellulosica costituita principalmente da lignina, cellulosa ed emicellulosa) sono stati selezionati come matrice di interesse, considerate le ingenti quantità generate durante la produzione dell'olio di oliva. Il progetto mira a costruire solide basi per il recupero di composti ad alto valore aggiunto da materiali lignocellulosici, da impiegare in prodotti alimentari come ingredienti innovativi con specifiche proprietà tecnologiche e funzionali. La progettazione di strategie per la valorizzazione degli scarti e sottoprodotti rappresenta un passo fondamentale verso diete più sostenibili, capaci non solo di ridurre l'impatto ambientale, ma anche di promuovere uno stile di vita sano per le generazioni presenti e future.

Key words: Olive stones, Up-cycling, Lignocellulose, Bioactive compounds, Agro-industrial waste valorization.

1. Introduction

In accordance with the PhD thesis project previously described (Trevisiol, 2024), this poster reports the main results of the activities concerning:

(A1) Ultrasound-assisted water extraction of the water-soluble fraction components from olive stones;
(A2) Characterization of the chemical, physicochemical and functionalities, such as emulsifying and antioxidant properties, of the aqueous extracts.

2. Materials and Methods

Olive stones were collected from oil mills, dried (40 °C, 2 h), milled (25 Hz, 2 min), and sieved (ϕ < 0.5 mm) to obtain a powder (OSP). Ultrasound-assisted extraction was applied to aqueous dispersions of OSP (5% w/w) at 24 kHz for 0, 30, 60, or 120 min. Aqueous extracts (AE) were recovered by centrifugation and filtration, then stored frozen or freeze-dried. Microstructure of OSP and AE was analyzed by scanning electron microscopy (SEM). Chemical characterization was performed by ^{1}H NMR in D_2O. Dry matter was measured gravimetrically. The pH of AE was measured at room temperature using a calibrated pH meter. Free sugars were quantified by HPLC-RI. Total phenolic content (TPC) was determined by the Folin-Ciocalteu method and expressed as mg of gallic acid equivalents (GAE) per g of dry matter of AE. Antioxidant capacity was assessed using DPPH and FRAP assays, expressed as mg Trolox equivalents (TE) per g of dry matter of AE. Emulsifying properties were evaluated by preparing oil-in-water emulsions (0.5% and 1.0% w/w oil) homogenized at 150 MPa. Emulsion stability was visually monitored over 30 days. All data were expressed as mean ± SD (n=2), analyzed by ANOVA with Tukey's HSD test (p < 0.05). All methods were performed according to established literature protocols.

3. Results and Discussion

3.1 Chemical characterization of the aqueous extracts (AE)

Table 1 presents the dry matter, pH, free sugar, and total phenolic content of aqueous extracts obtained by applying ultrasound (US) for increasing time to a 5% (w/w) olive stone dispersion. Ultrasound treatment progressively disrupted the lignocellulosic matrix, facilitating the release of smaller, water-soluble components. This is confirmed by the increase in dry matter content and the concurrent pH decrease, which suggests the release of acidic substances. SEM analysis clearly confirmed the structural degradation of the biomass, revealing particles with diverse morphologies, including lamellar, tubular, and globular forms (data not shown). Regarding the chemical composition of AE, the ^{1}H NMR spectra revealed the presence of aromatic protons, attributable to phenolic compounds and of anomeric signals associated with sugars (data not shown). The presence of sugars was further assessed by HPLC, which identified glucose and mannitol as the only free sugars present in all samples.

Both sugars showed a decrease after 30 minutes of ultrasound treatment, followed by a partial recovery after 60 minutes, likely reflecting a balance between enhanced extraction efficiency and degradation due to prolonged sonication (AlYammahi et al., 2022). Moreover, in line with NMR and dry matter results, the progressive increase in TPC with treatment time further supports the release of phenolic compounds induced by ultrasound treatment.

Table 1 *Dry matter content, pH, free sugars, and total phenolic content (TPC) of aqueous extracts from olive stone powder dispersion sonicated for 0, 30, 60, and 120 minutes.*

US treatment time (min)	**0**	**30**	**60**	**120**
Dry matter (mg/g)	0.51 ± 0.01^{b}	0.64 ± 0.01^{a}	0.65 ± 0.03^{a}	0.69 ± 0.02^{a}
pH	7.14 ± 0.01^{a}	6.58 ± 0.01^{d}	6.67 ± 0.01^{b}	6.62 ± 0.01^{c}
Glucose (mg/mL)	1.28 ± 0.01^{a}	1.04 ± 0.01^{c}	1.14 ± 0.01^{b}	1.12 ± 0.02^{b}
Mannitol (mg/mL)	1.30 ± 0.01^{a}	0.99 ± 0.01^{b}	1.12 ± 0.01^{ab}	0.98 ± 0.10^{b}
TPC (mg GAE/ g_{dw})	247.5 ± 1.0^{d}	276.2 ± 1.7^{c}	310.9 ± 0.8^{b}	367.3 ± 0.7^{a}

Different letters mean statistically different values within the same row ($p < 0.05$).

3.2 Functionalities of the aqueous extracts (AE)

Figure 1 shows the antioxidant activities of the extract obtained after the application of increasing sonication times.

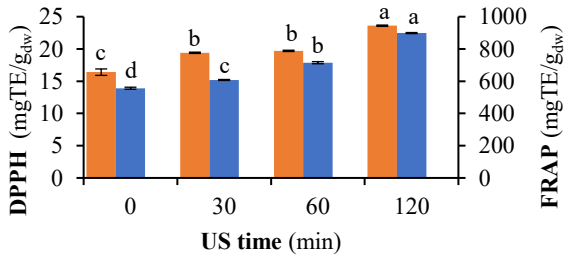


Figure 1 *Antioxidant activity of aqueous extracts at different sonication treatment times (0, 30, 60, 120 minutes). DPPH (blue) and FRAP (orange). Different letters mean statistically different values within the same assay ($p < 0.05$).*

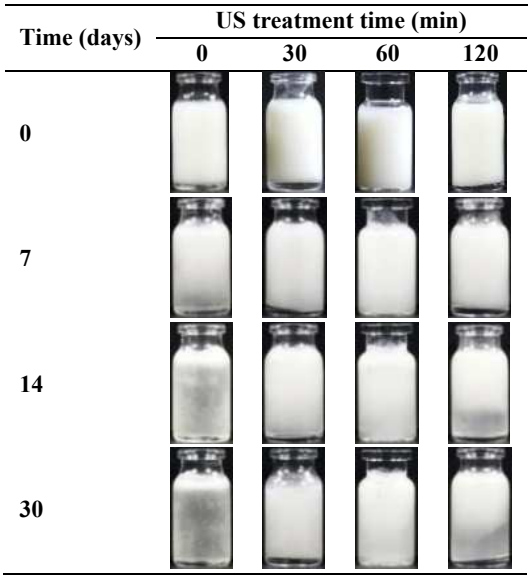


Figure 2 *Stability over time of emulsions (1% w/w oil) with aqueous extracts at different sonication treatment times (0, 30, 60, and 120 minutes.*

DPPH and FRAP assays progressively increased with increasing sonication time, confirming the effectiveness of ultrasound-assisted extraction in enriching the extracts with antioxidant compounds, likely associated with polyphenols.

Considering the possible surface activity of components derived from lignocellulosic matrices (Mikkonen, 2020), the emulsifying capacity of the aqueous extract was determined. Table 2 presents the stability of emulsions containing AE over time, assessed by the extent of phase separation. Emulsions prepared with untreated aqueous extract (0 minutes) showed the lowest stability, with phase separation occurring after 7 days. In contrast, emulsions prepared with samples sonicated for 30 and 60 minutes remained stable up to 30 days. Finally, those obtained with AE sonicated for 120 minutes showed phase separation after 14 days. These differences are likely attributable to the content of polyphenols and other water-soluble compounds, which may adsorb at the oil-water interface and modulate emulsion stability in a dose-dependent manner (Tian et al., 2021).

4. Conclusions

Ultrasound treatment effectively enhanced the extraction of water-soluble compounds from olive stones. The complex mixture of extracted compounds exhibited increased antioxidant activity and contributed to improved emulsion stability, particularly for sonication times between 30 and 60 minutes.

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Development of new functional foods derived from the honeybees' world (P-II-79)

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The first three activities of the PhD thesis project are described as follows. In the initial phase of the project, a microbiological isolation campaign was carried out with the aim of quantifying, isolating, identifying, and characterizing native yeast strains present in beehive products, particularly pollen and bee bread, in terms of their probiotic and functional potential. Subsequently, two distinct fermentation trials were conducted: one aimed at producing a low-alcohol mead, with a comparison to traditional alcoholic fermentation; the other carried out in solid-state, initially studying the natural fermentation that occurs within the beehive, and later, through guided fermentation, aiming to obtain a product not only chemically and physically similar to original bee bread, but even improved.

Sviluppo di nuovi alimenti funzionali derivanti dal mondo delle api

Le prime tre attività del progetto di tesi di dottorato sono descritte quanto segue. Nella fase iniziale del Progetto è stata condotta una campagna di isolamenti microbiologici volta alla quantificazione, isolamento, identificazione e caratterizzazione, in termini di potenziale probiotico e funzionale, di ceppi di lievito nativi presenti nei prodotti dell'alveare, in particolare polline e pane d'api al fine di addizionarli poi al polline fresco per aumentarne le proprietà. Successivamente, sono state condotte due prove fermentative distinte: una, finalizzata alla produzione di un idromele a bassa gradazione alcolica, con confronto rispetto alla fermentazione alcolica tradizionale; l'altra in stato solido, studiando in primis la fermentazione che avviene naturalmente all'interno dell'alveare e successivamente, tramite fermentazione guidate, riuscire ad ottenere un prodotto non soltanto simile a livello chimico-fisico al pane d'api originale ma bensì migliore.

Key words: yeasts, probiotic feature, fermentation, honeybee's products, bee pollen, beebread.

1. Introduction

In accordance with the PhD thesis project previously described, this poster reports the main results of the first three activities concerning:

(A1) Yeast biodiversity was higher in bee bread than in pollen. Starmerella strains were found in both matrices, while *Zygosaccharomyces* (fresh bee bread), *Metschnikowia* and *Kodamaea ohmeri* (bee bread), and *Aureobasidium pullulans* (aged bee bread) were isolated selectively.

(A2) *Saccharomyces cerevisiae* strains showed marked functional variability: some displayed strong biofunctional and nutraceutical potential, others were more suitable for supportive roles in mixed fermentations, while certain strains exhibited partial fermentation, valuable for innovative products with residual sugars.

(A3) Spontaneous fermentation best reproduced the natural beehive process, yielding superior organoleptic quality compared to lactic acid bacteria alone. *L. rhamnosus* remained viable, whereas *L. casei Shirota* failed to grow, confirming the essential role of yeasts in the process.

2. Materials and Methods

Twenty-four bee pollen and bee bread samples were collected in central Italy and analyzed for yeast populations. Isolated yeasts were identified, tested for probiotic traits (survival in gastrointestinal-like conditions, adhesion, biofilm formation), functional properties (polyphenol/flavonoid production, antimicrobial and phytase activity), and viability on pollen during 15-day cold storage. For mead, honey water wort was supplemented with nitrogen, pasteurized, inoculated with yeast, and analyzed for sugars, ethanol, polyphenols, antioxidant activity, and pH. Bee bread fermentations used fresh pollen, honey, and water, with *L. casei*, *L. rhamnosus*, or spontaneous fermentation, monitoring microbial growth, pH, sugar/protein content, pollen grain integrity, and bioactive properties.

3. Results and Discussion

3.1 Yeasts isolation, functional features and production of probiotic bee pollen

While most well-characterized probiotics are bacteria (*Bifidobacteria, Lactobacillus*), they have limitations such as high production costs, low viability, and potential transfer of antibiotic resistance. Yeasts, by contrast, tolerate low pH, high salt, heat, and inhibitory compounds, making them promising probiotic candidates. Honeybee products are emerging as a rich source of such yeasts. From 13 isolated strains, *Starmerella* sp. 18BB, 15BB, *Starmerella magnoliae*, and *Metschnikowia viticola* passed all probiotic tests, with 18BB showing the highest biofilm formation. For probiotic pollen, both fresh and freeze-dried formulations were tested, and 18BB maintained excellent viability above 10^6 CFU/g throughout 15 days of storage, outperforming control strains and demonstrating strong adaptation to the pollen substrate.

3.2 Mead production

Mead, one of the oldest alcoholic beverages (8–18% ABV), offers metabolic and physiological benefits, including improved digestion and reduced risk of chronic diseases. Growing consumer demand for low- or non-alcoholic drinks has prompted the development of low-alcohol mead, a product scarcely available on the market. Classical fermentations using 28 S. cerevisiae strains showed variable sugar consumption and ethanol production: strains 487, 452, 424, Maf112, and 406 efficiently consumed sugars, while 373 and 610 left high residual sugar (~24 g/L); strain 410 produced the lowest ethanol, and others ranged 11.7–14° ABV. All meads had lower pH than controls. Non-Saccharomyces strains produced less acetic acid, improving taste, with *Brettanomyces bruxellensis* showing the highest polyphenol content (0.314 g GAE/L) and antioxidant activity (86.64%), outperforming the control.

3.3 bee bread production

Bee pollen, a protein-rich (20–40 g/100 g) pellet collected by honeybees, is unstable due to high moisture content. To preserve it and increase nutrient bioavailability, bees naturally ferment pollen for 1–2 weeks, producing bee bread. This fermentation involves a microbial consortium of lactic acid bacteria, which acidify the substrate and inhibit spoilage, and yeasts, which contribute growth factors and volatile compounds, enhancing aroma. Microbial activity peaks around day six, eliminating molds, then gradually declines until day 19, when yeasts disappear and bacterial counts drop by two log units. Spontaneous fermentation, combining bacteria and yeasts, produces superior organoleptic quality compared to bacteria alone, while preserving pollen structure, polyphenol content, and antioxidant activity, suggesting potential for further optimization of nutraceutical properties.

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Alternative Food Sources as Functional Ingredients for Food Applications (P-II-80)

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With minor adjustments to activities described in the previous abstract, the first two activities of the PhD thesis project involving utilizing alternative food matrices as functional ingredients are outlined. First, Rooibos (*Aspalathus linearis*), which remains underutilized beyond its traditional use as a herbal tea, was tested as a natural alternative antioxidant and functional ingredient for enhancing meat quality and preservation, utilizing biogenic amine quantification and lipid oxidation assays. Second, a comprehensive characterization of commercial and technologically improved borlotti bean lines (*Phaseolus vulgaris*) as novel functional ingredients for bread formulations was performed, covering physicochemical, nutritional, and techno-functional analyses of legume lines.

Fonti alimentari alternative come ingredienti funzionali per applicazioni alimentari

Con lievi modifiche alle attività descritte nel precedente abstract, vengono illustrate le prime due attività del progetto di tesi di dottorato che prevedono l'utilizzo di matrici alimentari alternative come ingredienti funzionali. In primo luogo, il Rooibos (*Aspalathus linearis*), che rimane sottoutilizzato al di là del suo tradizionale utilizzo come tisana, è stato testato come antiossidante naturale alternativo e ingrediente funzionale per migliorare la qualità e la conservazione della carne, utilizzando la quantificazione delle ammine biogene e saggi di ossidazione lipidica. In secondo luogo, è stata condotta una caratterizzazione completa di linee di fagioli borlotti (*Phaseolus vulgaris*) commerciali e tecnologicamente migliorate come nuovi ingredienti funzionali per la formulazione del pane, comprendente analisi fisico-chimiche, nutrizionali e tecnico-funzionali delle linee di legumi.

Key words: *Aspalathus linearis*, biogenic amines, *Phaseolus vulgaris*, functional ingredient, functional food, food quality, preservation, antioxidant

1. Introduction

In accordance with the PhD thesis project previously described with minor adjustments (Darko, 2024), this poster reports the main results of the first two activities concerning:

(A1) Identifying and quantifying biogenic amines and primary oxidation products of rooibos-marinated meat samples.

(A2) A comprehensive characterization of commercial and technologically improved borlotti bean lines (*Phaseolus vulgaris*).

2. Materials and Methods

(A1) **Biogenic Amines Analysis and Peroxide Value Determination**

Biogenic amines analysis was conducted with the protocol described by (Francisco *et al*., 2020). BAs were extracted using salting-out liquid-liquid extraction with dansyl chloride as the derivatization agent. Extraction and analysis of samples were done at intervals of 0, 3, 7, and 13 days. Chromatographic separation and quantification of biogenic amines were carried out using Ultra High-Pressure Liquid Chromatography (UHPLC) equipped with UV and MS detectors. The peroxide value of minced pork meat was determined by the iodometric method for peroxide values, with the protocol described by (Ismaiel *et al*., 2025) with slight modifications. Peroxide value was calculated based on the volume of sodium thiosulfate, the molarity, and the sample weight, and expressed as meqO2/kg of meat.

(A2) **Comprehensive characterization of *Phaseolus vulgaris***

The characterization of commercial borlotti bean (phsl+, lec+, aAI+, leg+) and two technologically improved bean lines; Lady Joy (phsl +, lec -, aAI +, leg+) and 6/4 Olter 2015 (phsl-, lec-, aAI+++, leg++), comprised of the following analyses: Total phenolic content, Crude Protein content, fatty acid profile, and techno-functional properties such as swelling capacity, water and oil holding capacity, foaming capacity and foam stability, determined with protocols described by Badia-Olmos *et al*., (2024); Fanesi *et al*., (2023); Ismaiel *et al*., (2025) and Siwale *et al*., (2023)

3. Results and Discussion

(A1) **Biogenic Amines Analysis**

The most abundant biogenic amines across all treated samples were cadaverine, with average concentrations ranging from 4.89 to 141.90 mg/kg, and tyramine, ranging from 6.05 to 144.46 mg/kg. The least abundant BAs were histamine (1.28 to 3.56 mg/kg), 2-phenylethylamine (0.24 to 3.77 mg/kg), and tryptamine (5.97 to 26.30 mg/kg). Spermidine (6.64 to 13.13 mg/kg) and spermine (3.96 to 12.61 mg/kg) also had lower concentrations. This observation follows a report by (Stadnik & Dolatowski, 2010) stating tyramine and cadaverine as the most prevalent BAs in meat and meat products during storage. The results indicated that, irrespective of the rooibos type, a higher accumulation of biogenic amines was recorded for liquid treatments up to 376.96 mg/kg of meat compared to dry treatments (up to 73.76 mg/kg of meat). Meat samples treated with dry marinade had a better quality than the liquid-treated ones, having a BAI close to 5 mg/kg after 3 days of storage, while the latter recorded a BAI higher than 50 mg/kg within the same timeframe, indicating spoilage.

Peroxide Value Determination

Peroxide values increased over time across all treatment groups. Samples treated with liquid marinade demonstrated lower peroxide values (PV) throughout the storage period than control dry marinade samples due to reduced exposure to atmospheric oxygen. As suggested in previous studies by Gumus *et al*., (2021), food matrices with reduced water activity and a higher exposure to oxygen are often subjected to increased lipid oxidation, which was the case for samples treated under dry conditions. On the other hand, dry extracts specifically green rooibos significantly reduced peroxide values compared to their control counterpart, potentially due to its higher polyphenolic content, which is associated with stronger scavenging activity, confirming the potency of dry extracts to control lipid oxidation in food matrices (Cullere *et al*., 2013). In conclusion, the study indicates that rooibos extracts possess potential as natural preservatives. However, their effectiveness varies significantly depending on the marination method employed. These findings suggest optimizing the use of natural antioxidants in meat preservation systems, offering a step toward sustainable and safe food preservation practices and clean-label trends.

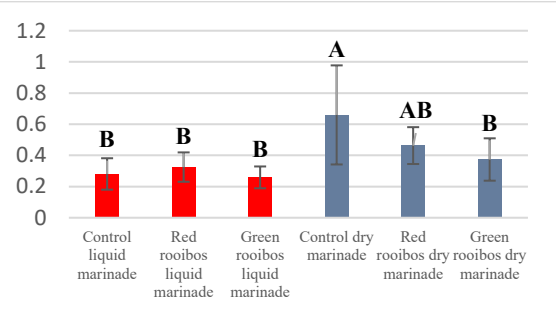


Figure 1: Peroxide value of rooibos-treated samples expressed as meqO2/kg of meat. Mean peroxide value concentrations of treatment groups. Results are presented as means; different letters indicate significant differences within each treatment group (Tukey's HSD for α <= 0.05)

(A2) **Comprehensive Characterization of *Phaseolus vulgaris***

No significant differences were observed among all three bean lines regarding total phenolic content. All three bean lines recorded an average phenolic content of 0.8 mg GAE.g-1, with similar results obtained in a study by (Teixeira-Guedes *et al*., 2019). Six fatty acids were identified in all samples, comprising 2 SFAs, 2 MUFAs, and 2 PUFAs. PUFAs were the most abundant in all three bean lines. Lady Joy and 6/4 Olter 2015 recorded a significantly higher protein content of 24%, yielding similar results, as seen in a study by (Güzel & Sayar, 2012). In terms of techno-functionality, the commercial sample recorded a better swelling capacity, followed by Lady Joy and 6/4 Olter 2015. All samples exhibited comparable oil holding capacity. However, the commercial type recorded the best water holding capacity, followed by Lady Joy and 6/4 Olter 2015. Regarding foam capacity and stability, the new technological lines had better foam capacity than the commercial type, but a lower foam stability. Technological improvements may have altered protein solubility, affecting foam stability. Outcomes suggest these beans as versatile ingredients, and these traits may enhance dough rheology and crumb texture in breads.

4. References

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Microbiological food safety: risk mitigation of foodborne pathogens by applying omics approaches within the food system (P-II-82)

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In the project's initial phase, 5-serotype-sourcer-diverse strains each of *Listeria monocytogenes* and *Salmonella enterica* were selected to compare their individual growth performance parameters. The growth performance similarities among each group allowed to create the cocktail master mix of each set of bacterial species. Subsequently, an enrichment process was conducted using raw minced meat inoculated with the *L. monocytogenes* cocktail mix. Growth curves of the culture enrichment sample, using a 10-fold dilution inoculum, were stablished by counting colonies on selective agar, to accurately determine the cell concentration and growth behaviour of each sample over the 48-hour enrichment period. The viable counts obtained (quantitative data) will be correlated with the sequencing-based approaches, to elucidate the lowest concentration and the earliest enrichment time at which *L. monocytogenes* can be detected when applying NGS methodologies into food safety protocols.

Sicurezza igienico-sanitaria degli alimenti: mitigazione del rischio attraverso utilizzo di metodiche omiche

Nella fase iniziale del progetto, sono stati selezionati 5 ceppi di *L. monocytogenes* e *S. enterica* e sono stati confrontati i loro parametri di crescita. Le somiglianze tra ciascun gruppo hanno permesso di creare il cocktail master mix di ciascun gruppo di specie batteriche. Successivamente, è stato condotto un processo di arricchimento utilizzando carne macinata cruda con la miscela cocktail di *L. monocytogenes* come parte dell'attività secondaria del progetto. Le curve di crescita dei campioni di arricchimento delle colture, utilizzando un inoculo con una diluizione di 10 volte, sono state stabilite contando le colonie su agar selettivo, per determinare con precisione la concentrazione cellulare e il comportamento di crescita di ciascun campione in un periodo di 48 ore. Le conte ottenute (dati quantitativi) saranno utilizzate per confrontare la concentrazione più bassa e il primo tempo in cui è possibile rilevare *L. monocytogenes* quando si integrano gli approcci NGS nei protocolli di sicurezza alimentare.

Key words: *Listeria monocytogenes, Salmonella enterica,* growth performance parameters, ISO selective-enrichment process, Next Generation Sequencing

1. Introduction

In accordance with the PhD thesis project previously described (Jaramillo Zapata, 2024), this poster reports the main results of the conducted activities concerning:

(A1) Selection and growth characterization individually, of both, 5-serotype-source-diverse *L. monocytogenes* and *S. enterica* strains/isolates, in order to compare growth performance parameters to avoid any *bias* when culturing together.

(A2) Selective-enrichment process follow-up of raw mincemeat, comprising the cocktail mix of *L. monocytogenes* at 10-fold dilution inoculum samples, for the purpose of identify the cell concentration and growth behaviour of each sample over a 48-hour period to further comparison with sequencing-based data.

2. Materials and methods

(A1) Preliminary Phase. Strains selection: The study examined different strains of *L. monocytogenes* and *S. enterica.* A clinical reference strain for *L. monocytogenes* was ScottA *(L1),* serotype 4b. Four food isolates: #5 *(L2)* from minced beef (4b), #18 *(L3)* from a dairy product (3a), #36 *(L4)* from pork (1/2a), and #162 *(L5)* from another dairy product (Mataragas *et al.,* 2015). For *S. enterica*, the reference strain was ATCC 14028 *(S1),* serovar *Typhimurium* from animals. The four isolates included #246 *(S2)* from animals (subsp. *enteritidis*), #54398 *(S3)* from packaged meat (*Typhimurium*), #56596/2011 (*S4*) from pig faeces (*Typhimurium*), and #22574 (*S5*) from pig faeces (*Derby*). Individual growth was evaluated in BHI broth and enrichment media, following ISO methods: ISO 11290-1:2017 for *L. monocytogenes* and ISO 6579-1:2017 for *S. enterica*. Sampling at 0, 2, 4, 6, 8, and 24 hours and counting colonies on BHI or TSA plates. Growth performance parameters were calculated: maximum specific growth rate (μmax), generation number (g), and doubling time (G) from the exponential growth phase in BHI broth.

(A2) Primary phase: *L. monocytogenes* isolates (L1–L5) were grown separately in BHI at 37°C for 18 hours, then pooled to create 5 mL master inoculum (LMM) at about 10^8 CFU/mL. Six serial dilutions were prepared and added to stomacher bags with 10 g of minced meat and 90 mL of Half-Fraser (HF) broth, resulting in concentrations from about 10^6 CFU/mL (Dilution Bag D1) to around 10 CFU/mL (D6). A non-inoculated control was also included. The bags were incubated at 30°C for 24 hours, with samples taken at 0, 2, 4, 6, 8, 10,12 and 24 hours. Afterward, 1 mL from each sample was added to 9 mL of Full Fraser (FF) broth for secondary enrichment

and incubated at 37°C for another 24 hours, sampling at 3, 6, and 24 hours. *L. monocytogenes* concentrations were measured by counting colonies on ALOA agar after 48 hours at 37°C. This was conducted in two independent trials. Random colonies were isolated for REP-PCR fingerprinting (data not shown). Additionally, 2 mL aliquots were taken in every time-point sampling for pellet recovery, stored at -20°C for DNA extraction and sequencing integration as part of the next activities.

3. Results and discussion

3.1 **Table 1A** and **Table 2A** detail the growth performance parameters of each set of pathogenic strains/isolates. The *μmax* shows how fast each one grows under the tested conditions. The doubling time(*G*) indicates how quickly the bacterial population doubles, with shorter times indicating faster proliferation. The number of generations(*g*) gives a sense of how many cell divisions occurred to determine the pathogen load. Similarities across all set of strains were confirmed with no significant differences (Tukey-b test, $p >> 0.05$). **Table 1B** and **Table 2B** detail the growth loads achieved after 24 h of incubation in the corresponding media (Englezos *et al*., 2018). **Fig. 1** and **Fig. 2** show the corresponding growth curves on BHI media of individual strains.

Table 5. *Growth performance parameters(A) and counts (Log CFU/ml) (B) of L. monocytogenes strains after 24h of enrichment on BHI broth, Half Fraser (HF) broth, Full Fraser (FF) broth. Values are means± standard deviation of two independent experiments. [a]There are no statistically significant differences in any growth parameter across strains/isolates (Tukey-b test, p >> 0.05)*

A

Growth performance parameters *L. monocytogenes*			
cod	μmax, (h^{-1})[a]	(g)[a]	(G)[a]
L1	**0,83** ± 0,003	**9,58** ± 0,03	**0,83**±0,003
L2	**0,93** ± 0,003	**10,75** ± 0,03	**0,74**± 0,002
L3	**0,75** ± 0,052	**8,64** ± 0,60	**0,93**± 0,065
L4	**0,83** ± 0,061	**9,55** ± 0,71	**0,84**± 0,062
L5	**0,94** ± 0,090	**10,90** ± 1,04	**0,74**± 0,070

B

Log CFU/mL		
BHI	HF	FF
8,96±0,09	**7,94** ±0,47	**7,67**±0,90
8,63±0,39	**7,23**±0,16	**7,50**±0,72
8,15±0,15	**7,33**±0,13	**7,30**±0,52
8,45±0,17	**7,34**±0,21	**6,55**±0,48
8,70±0,36	**7,43**±0,04	**6,76**±0,09

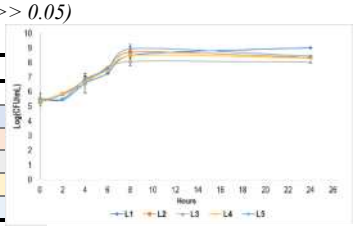


Fig. 1. *Growth curves of L. monocytogenes in BHI*

Table 6. *Growth performance parameters(A) and counts (Log CFU/mL)(B) of S. enterica strain after 24h of enrichment on BHI broth, Buffered Peptone Water (BPW) broth, Rappaport Vassiliadis Soya (RSV) broth. Values are means± standard deviation of two independent experiments. [a]There are no statistically significant differences in any growth parameter across the strains (Tukey-b test, p >> 0.05)*

A

Growth performance parameters *S. enterica*			
cod	(μmax, h^{-1})[a]	(g)[a]	(G)[a]
S1	**1,23** ±0,004	**26,38**±0,02	**0,56**±0,02
S2	**1,04** ± 0,00	**25,37**±0,00	**0,67**±0,01
S3	**1,18** ±0,063	**25,57**±0,03	**0,59**±0,03
S4	**1,10** ± 0,05	**25,31**±0,66	**0,63**±0,03
S5	**1,28**±0,009	**26,44**± 0,03	**0,54**±0,004

B

Log CFU/mL		
BHI	BPW	RSV
9,08±0,006	**8,34**±0,26	**7,36**± 0,1
9,05±0,03	**8,14**± 0,17	**7,11**± 0,4
9,16±0,05	**8,39**± 0,26	**7,12**± 0,4
9,22± 0,02	**8,19**± 0,28	**7,35**± 0,1
8,92±0,02	**8,41**± 0,10	**7,42**± 0,1

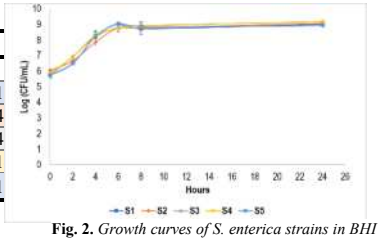
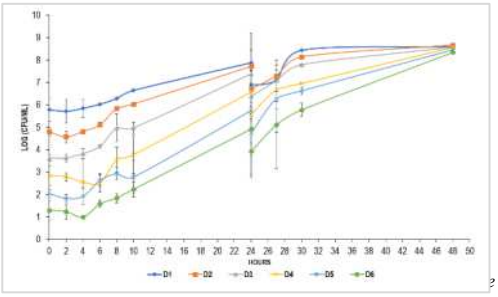


Fig. 2. *Growth curves of S. enterica strains in BHI*

3.2. **Figure 3**. shows the growth curves of LMM established from the different 10-fold dilution inoculum samples (D1-D6) during 48h enrichment process. The growth curves will allow us to correlate quantitative culture data of *L. monocytogenes* concentrations (viable counts) with sequencing-based data (relative abundance) to elucidate the lowest concentration and the earliest time that *L. monocytogenes* can be detected when integrated NGS approaches.



enrichment process of LMM following the ISO standard method. Error bars denoted the standard deviations.

4. References

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BALOs as biocontrol agent in bivalve shellfish depuration (P-II-83)

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In the first part of the PhD thesis project, Gram-negative species were isolated from seawater to be used as prey for the isolation of autochthonous marine BALOs. BALOs cultures were then purified and subjected to a predatory efficiency screening assay to select the best-performing strain in terms of speed and effectiveness. The most promising predator will be used in a depuration experiment with Mediterranean mussels (*Mytilus galloprovincialis*).

I BALOs come agenti di biocontrollo nella depurazione di molluschi bivalvi

Nella prima parte del progetto di tesi di dottorato, batteri Gram-negativi sono stati isolati dall'acqua di mare con l'obiettivo di impiegarli come prede per l'isolamento di BALOs marini autoctoni. Le colture di BALOs sono state quindi purificate e sottoposte a un test di screening di efficienza predatoria allo scopo di selezionare il ceppo più performante in termini di velocità ed efficacia. Il predatore più promettente verrà utilizzato in un esperimento di depurazione su cozze del Mediterraneo (*Mytilus galloprovincialis*).

Key words: BALOs, predatory bacteria, Gram-negative, predatory efficiency assay, mussels, *Mytilus galloprovincialis*

1. Introduction

Following the PhD thesis project previously described, this poster reports the main results of the first two activities concerning:

(A1) **Isolation of potential Gram-negative preys and autochthonous marine BALOs strains.** Selective and differential media were used to isolate Gram-negative species from seawater and then, the selected strains were used as potential prey for the BALOs isolation;

(A2) **Predatory efficiency screening assay.** The isolated marine BALOs were tested under mussel's depuration condition (pH, temperature) monitoring the prey reduction and the predator growth (PFU/mL) during the time. Both methods, plate count and spectrophotometer readings (600nm) were used.

2. Materials and Methods

Marine water was directly spread on Thiosulfate-Citrate-Bile Sucrose Agar (TCBS) plates and incubated overnight at 32°C. At the same time, marine water was subjected to enrichment according to the protocol proposed by Huq *et al*. (2012). Colonies were randomly selected and purified after repetitive streaking onto TCBS plates. Strains were identified by 16S rDNA sequencing by using primers and PCR conditions described by Weisburg *et al*. (1991).

The enrichment protocol described by Jurkevitch (2006) was followed for marine BALOs isolation. Three potential preys were tested: *Vibrio (V.) mediterranei* and *Citrobacter* (*C.*) *portucalensis* isolated during this study, plus one strain of *Escherichia (E.) coli* isolated from meat during a previous survey. During incubation at 28°C under constant stirring (Orbital shaker 300 rpm), cultures were daily monitored by spectrophotometry (600 nm) and microscope observation (100x). During incubation at 28°C under constant stirring (Orbital shaker 300 rpm), cultures were daily monitored by spectrophotometry (600 nm) and microscope observation. Enrichments were then filtered (Minisart 0.45 μm) and, after decimal serial dilutions in sterile marine water (ASW), plated by the double layer technique according to Jurkevitch (2006). Plates were sealed and incubated at 28°C for 7 days. Plaques growing in diameter were preferentially chosen and purified after several platings by the double layer technique.

For the predatory efficiency assay, a single pure plaque was transferred into an overnight culture of the prey, centrifuged (6500 rpm), and resuspended in 20 mL of ASW (pH 7.8). The prey without the plaque served as control. After 0, 24, 48, and 72 h of incubation at 19°C under constant shaking, tubes were monitored by a spectrophotometer. The prey population level was assessed by the drop method (Collins, 1989) on LB agar plates containing NaCl up to 20g/L. The BALOs population level was assessed by plating each dilution as before described.

3. Results and Discussion

3.1 Isolation of potential Gram-negative preys and autochthonous marine BALOs strains.

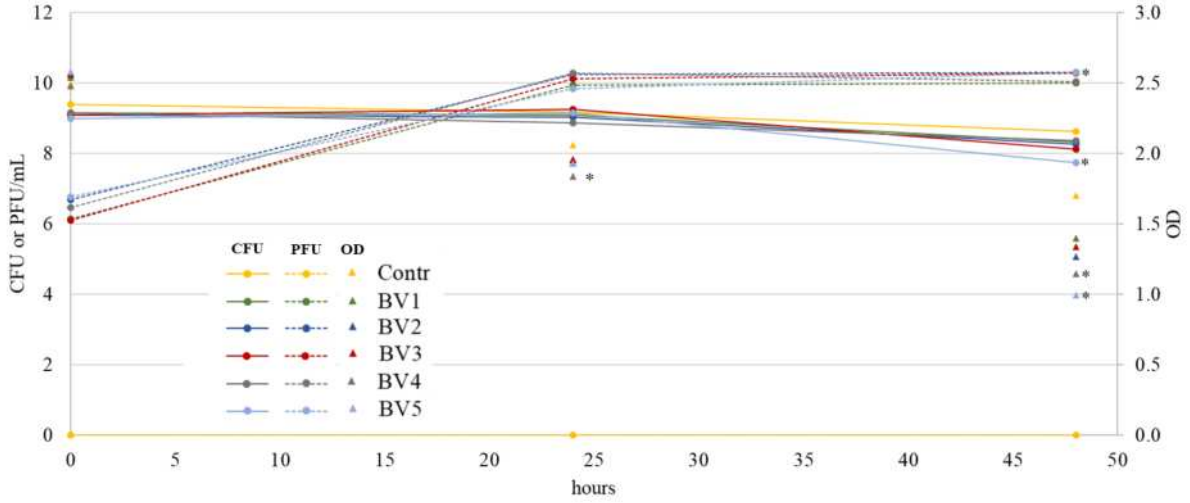
Based on the colonies' appearance on TCBS agar plates seeded with marine water and from plates seeded with the enrichments (n.3), ten colonies in total were chosen. The morphology of colonies that appeared on plates following enrichment was quite comparable, whereas a discernible diversity in the colonies' aspect was observed on plates that were directly seeded with marine water. By 16S rDNA sequencing, one strain could be reported to *V. mediterranei* (99% similarity), 2 to *Photobacterium* spp. (98% in both cases), 2 to *C. portucalensis* (99 and 100%), 3 to *V. harveyi* (98 and 99%), and 2 to *V. chagasii.* The occurrence of *V. harveyi* and *V. mediterranei* during the warm season up to October in the Mediterranean Sea has already been reported (Arias *et al*., 1999).

Upon enrichment with the prey, the faster clarification was noticed only against the unique strain of *V. mediterranei*. The lytic capabilities of BALOs against bacterial hosts may greatly vary, resulting in wildly disparate prey spectrum ranges. While some have large ranges that cover numerous Gram-negative and even some Gram-positive bacteria, others have very narrow ranges, covering only a few species or strains (Najnine *et al*., 2020). Lysis plaques, resulting from the plating of the clarified enrichment, were randomly chosen and purified. A total of 5 BALOs strains - BV_1, BV_2, BV_3, BV_4, BV_5 - were isolated and purified.

3.2 Predatory efficiency screening assay

Predatory efficiency was tested twice in two independent trials using viable counting methods and spectrophotometry. The obtained data perfectly matched. Strain BV4 induced a significant decrease in OD values at 24 and 48 h. Conversely, at 48 h, strain BV5 induced the highest clarification and the greatest decrease in *V. mediterranei* CFU/mL and BALOs PFU/mL (Fig. 1). Based on the results, strain BV5 was selected for further experiments.

Figure 1 *Lytic activity of 5 BALOs strains (BV_1, BV_2, BV_3, BV_4, and BV_5) against V. mediterranei monitored by OD_{600} reduction, CFU/mL of the prey and PFU/mL of the predators at time 0 and after 24 and 48 h of incubation.*



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Mucoadhesive and Mechanical Properties of Prototype Soft-solid Foods Designed for Dysphagia Management (P-II-84)

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The first three activities of the PhD thesis project are described. Firstly, the market analysis of dysphagia-oriented foods currently sold on the Italian market is introduced. Secondly, the techno-functional properties of selected ingredients and the first design of plant-based purées obtained through viscosity modulators are presented. The purées were characterized for their rheological behavior, texture analysis and mucoadhesion on porcine oral tissues. Finally, research on liquid-like formulations for dysphagia management is also reported.

Proprietà Mucoadesive e Meccaniche di Alimenti Soft-solids Prototipi per la Gestione della Disfagia

Le prime tre attività del progetto di tesi di dottorato sono descritte. Viene introdotta l'analisi di mercato dei prodotti per disfagici attualmente venduti sul mercato italiano. Sono poi presentate le proprietà tecno-funzionali degli ingredienti selezionati e la prima progettazione di puree a base vegetale ottenute con modulatori di viscosità. Le puree sono state caratterizzate per il loro comportamento reologico, analisi di texture e mucoadesività su tessuti orali suini. Infine, è riportata la ricerca svolta in merito alle formulazioni liquid-like per la gestione della disfagia.

Key words: Dysphagia, plant-based purées, soft-solids, rheology, ex-vivo mucoadhesion, texture analysis.

1. Introduction

In accordance with the PhD thesis project previously described (Ascrizzi, 2024), this poster reports the main results of the first three activities concerning: (A1) the market analysis of dysphagia-oriented products; (A2) the determination of techno-functional properties of selected ingredients; (A3) the design of prototype vegetable-based purées enriched with upcycled proteins. It is important to note that most of the present research primarily focuses on soft-solid foods; however, effective dysphagia management also demands careful consideration of liquid-like formulations, which play a critical role in patient care. This aspect was explored during the internship abroad, contributing to a more comprehensive approach to the future development of texture-modified foods (TMFs).

2. Materials and Methods

The flaxseed (FS) and hempseed (HS) meals, a by-product from the extraction of oil by cold-pressing of seeds, were sieved to concentrate the protein-rich fractions. Protein and lipid content was determined using Kjeldahl method and Soxhlet extraction, respectively. Dry matter (DM), water holding capacity (WHC) and fat absorption capacity (FAC) were then assessed. The purées were formulated combining the following ingredients: water, zucchini, FS or HS flour, NaCl and viscosity modulator (high acyl gellan gum, GHA; or Pectinase, PECT) to obtain different levels (2-3-4) according to the International Dysphagia Diet Standardization Initiative (IDDSI) framework. Rheological properties of formulated purées were evaluated. Mechanical (back extrusion) and mucoadhesive properties were tested with a texture analyzer, the latter on oral porcine tissues (Tobyn et al. 1992).

Four commercially available gum-based thickeners and contrast agents ($BaSO_4$) for videofluoroscopy (VF) at varying concentrations were used to obtain thickened waters (TWs) and VF boluses corresponding to IDDSI levels 1-2-3. The IDDSI flow test was conducted using a 10 mL syringe: each sample flowed for 10 seconds then stopped, and the remaining volume was measured. This method enables for the classification of liquid-like TMFs according to IDDSI framework. Subsequently, density (ρ) and rheological behavior of TW and VF boluses were assessed.

3. Results and Discussion

3.1 Market analysis of dysphagia-oriented products

The first study (Ascrizzi et al., 2024) assessed the nutritional composition of dysphagia-oriented products available on the Italian online market and compared energy, nutrient, and salt content per 100 g and per serving. Among 70 products analyzed, nutritional profiles varied widely, highlighting inconsistent formulation standards. Salt content was notably high, with 68% of products classified as medium or high content, conflicting with WHO guidelines. Critically, most labels also omitted the IDDSI level, hindering safe and appropriate use by patients and clinicians.

3.2 Determination of techno-functional properties of selected ingredients

Flour characterization (**Table 1**) revealed that FS had significantly higher protein content and WHC, which is an important factor for purée texture and stability. Both FS and HS displayed similar lipid content, DM, and FAC.

Table 1 *Characterization of FS and HS flours. For each column, different letters indicate a significant difference.*

Flour	Proteins	Lipids	DM	WHC	FAC

	(g/100g)	(g/100g)	(g/100g)	(g/100g)	(g/100g)
FS	33.7 $_{a}$	9.5 $_{a}$	92.2 $_{a}$	0.63 $_{a}$	0.15 $_{a}$
HS	26.4 $_{b}$	9.5 $_{a}$	92.9 $_{a}$	0.17 $_{b}$	0.13 $_{a}$

3.3 Design of prototype vegetable-based purées enriched with upcycled proteins

An optimized base recipe (PLAIN) made of zucchini, water, NaCl and FS or HS flour was developed. Viscosity-modified purées were then formulated using either GHA or PECT. Data of shear-imposed flow curves were modelled using Herschel-Bulkley equation (1):

$$\sigma = \sigma_0 + k \cdot \dot{\gamma}^{n} \qquad (1)$$

where σ is the shear stress (Pa), σ_0 is the yield stress (Pa), k is the consistency index (Pa·s^n), n is the flow index, and $\dot{\gamma}$ the shear rate (s^{-1}). Oral-relevant viscosities, used to classify TMFs according to their corresponding IDDSI levels, were extrapolated from flow curves at 50 s^{-1}. Back extrusion was used to assess the deformation resistance and structural integrity of samples. Mucoadhesive test was used to evaluate food adhesion to mucosal tissue (Cook et al., 2017), a critical factor: excessive adhesion may hinder bolus transit, while insufficient adhesion may increase aspiration risk. The key results from rheological, mechanical, and mucoadhesive tests are reported in **Table 2**.

Table 2 *Rheological, mechanical, mucoadhesive behavior (here presented on porcine tongue), and retrieved IDDSI level at 50 s^{-1} of formulated purées. For each column, different letters indicate a significant difference.*

		Rheology			Back extrusion			Mucoadhesion		IDDSI level
	Purée	σ_0 (Pa)	k (Pa·s^n)	n (-)	Positive peak (N)	Positive area (N·mm)	Negative area (N·mm)	Work of adhesion (N·mm)	Peak force (N)	
FS based	PLAIN	4.51	41.15	0.17	1.73±0.08 $_{b}$	11.60±0.68 $_{b}$	9.66±0.81 $_{a}$	0.56±0.07 $_{a;b}$	0.39±0.04 $_{a;b}$	3
	GHA	11.69	79.17	0.16	2.10±0.16 $_{a}$	13.59±1.64 $_{a}$	11.52±0.48 $_{a}$	0.69±0.04 $_{a}$	0.41±0.02 $_{a}$	4
	PECT	1.43	17.20	0.18	0.66±0.01 $_{c}$	5.39±0.08 $_{c}$	2.89±0.22 $_{c}$	0.50±0.04 $_{b}$	0.37±0.02 $_{b}$	2
HS based	PLAIN	5.93	45.61	0.18	0.86±0.03 $_{c}$	6.63±0.34 $_{c}$	3.23±0.21 $_{c}$	0.30±0.03 $_{c}$	0.26±0.01 $_{c}$	3
	GHA	17.74	70.65	0.16	1.84±0.37 $_{b}$	12.85±1.11 $_{b}$	6.33±0.83 $_{b}$	0.49±0.16 $_{b}$	0.37±0.07 $_{b}$	4
	PECT	4.52	26.97	0.22	0.26±0.01 $_{d}$	2.62±0.09 $_{d}$	0.73±0.03 $_{d}$	0.13±0.07 $_{d}$	0.12±0.06 $_{d}$	2

Results revealed significant differences in physical properties depending on the structuring of the soft material. Viscosity values, aligned with IDDSI levels, offer consumer-friendly guidance. However, mechanical cohesiveness and mucoadhesion offer deeper insights than flowability alone for the characterization of TMFs.

3.4 Exploration of liquid-like formulations to broaden the background of dysphagia-oriented foods

Data of stress-imposed flow curves of TWs and VF boluses were modelled using Ostwald-de Waele equation (2):

$$\eta(\dot{\gamma}) = K \cdot \dot{\gamma}^{n-1} \qquad (2)$$

where η is the apparent viscosity (Pa·s), K is the consistency (Pa·s^n), n is the flow index, and $\dot{\gamma}$ the shear rate (s^{-1}). A rheological map (**Figure 1**) was developed to investigate the influence of rheological parameters (K, n) and ρ on the IDDSI level predicted by the syringe test. As previously observed, η varied widely within the same level, further underscoring the relevance of rheological properties and ρ in characterizing TMFs.

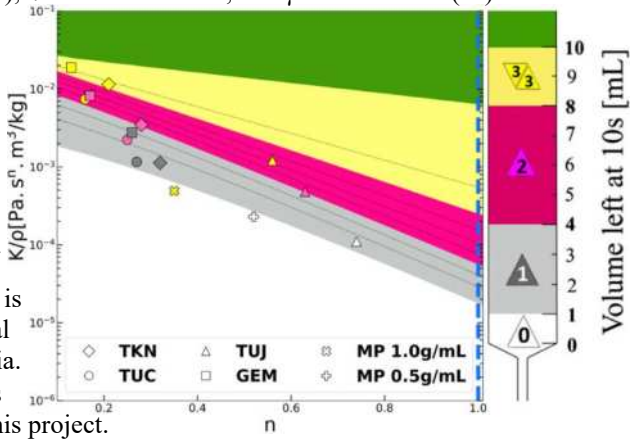


Figure 1 *Rheological map of TW (TKN, TUC, TUJ, GEM) and VF bolus (MP). Each colour (white, grey, pink, yellow, green) corresponds to the respective IDDSI level (0-1-2-3-4). Map derived from Lecanu et al., 2024.*

Monitoring mucoadhesive, mechanical, and rheological properties is essential for designing TMFs with optimized texture and functional performance, ensuring safer swallowing for patients with dysphagia. Gaining deeper insight into both soft-solid and liquid-like TMFs is key to advancing future formulations and flow modelling within this project.

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Optimizing the dehydration of tuna by-products as a strategy to develop innovative nutritious fish powder (P-II-85)

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In line with the PhD project's goal of valorising fish by-products through different technological and food reformulation strategies, the drying kinetics of tuna by-products were mathematically described and optimized. The study aimed to obtain a stabilised powder by lowering water activity (a_w) while preserving key quality attributes such as colour and polyunsaturated fatty acids content, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Four drying temperatures (35 °C, 45 °C, 55 °C, 65 °C) were tested. In addition, the dehydration process was mathematically modelled, and the best operative conditions were validated. Drying at 65 °C proved the most effective in preserving quality and minimising drying time and was thus selected for the development of tuna powder to be used on an industrial scale or for gastronomic applications.

Ottimizzazione del processo di disidratazione per sviluppare un ingrediente nutrizionalmente valido dai sottoprodotti della lavorazione del tonno

In linea con l'obiettivo del progetto di dottorato volto alla valorizzazione dei sottoprodotti ittici mediante tecnologie alimentari, sono state descritte e modellate, per la prima volta, le cinetiche di essiccazione del muscolo di tonno derivante dalla lavorazione industriale. L'obiettivo è stato quello di ottenere una polvere stabilizzata tramite riduzione dell'attività dell'acqua (a_w), preservando colore e contenuto di acidi grassi polinsaturi. Sono state testate quattro temperature (35 °C, 45 °C, 55 °C e 65 °C) ed è stata effettuata la descrizione matematica del processo, la stima dei tempi di disidratazione e la validazione dei parametri stimati. L'essiccazione a 65 °C è risultata la più efficace nel preservare la qualità e ridurre i tempi di trattamento, ed è stata pertanto selezionata per la produzione di un ingrediente a uso gastronomico.

Key words: Sustainability, fish by-products, food technologies, process modelling, nutritional quality.

1. Introduction

Following the doctoral thesis, this poster reports the main results of the first two research activities concerning:

(A1) the mathematical description of the dehydration kinetics of tuna by-product performed at different temperatures;

(A2) study of the kinetic dehydration of tuna by-products and definition of the best conditions to obtain shelf stable tuna powder protecting their main quality parameters;

2. Materials and Methods

SkipJack Tuna (*Katsuwonus pelamis*) a processed by-product, was received from a local fish processing company (Zarotti S.p.A, Campania, Italy), transported under frozen conditions, and stored at −20 °C until use. Samples were dehydrated at four different temperatures using a domestic convection dryer. Samples were periodically analysed during the dehydration experiments for their water activity and moisture content. Fatty acids were determined by gas chromatography after lipid extraction and transesterification to fatty acid methyl esters (FAMEs). Colour was measured using a colorimeter. Mathematical description of the dehydration kinetics was performed using a Weibullian model (Corradini et al., 2006).

3. Results and Discussion

3.1 *Tuna dehydration kinetics during drying*

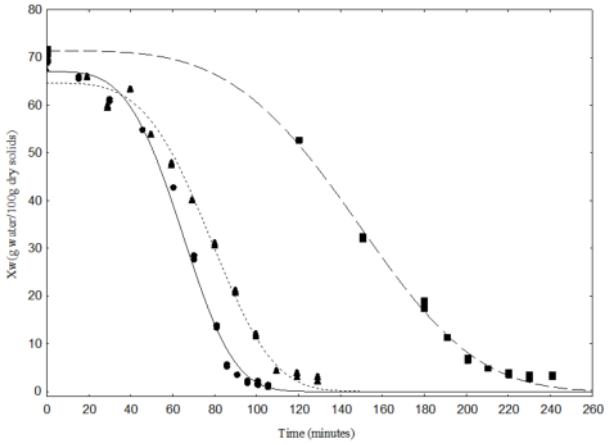
Drying kinetics were effectively modelled by using the Weibull equation (Eq. 1):

$$Xw(t) = Xw_0(t) * (-kt^n) \quad (1)$$

where *Xw(t)* is the moisture content (g H_2O/ 100 g dry solids) at time *t*, Xw_0 is the moisture content of the raw sample, *k* is the dehydration rate (g H_20/ 100g dry solids*min^{-1}), and *n* is a shape factor (Corradini et al., 2006). During the initial fitting procedures, the parameter *n* was left free to vary to enhance model accuracy. A second series of fittings was then performed using a fixed *n* value of 3.72, calculated as the mean of the previously

estimated values (Fig. 1). The high regression coefficients (R^2 >0.99) demonstrated the high goodness of fitting of the Weibullian model.

Figure 1 *Fitting of the dehydration kinetics of tuna at 45°C (—■– –), 55°C (– –▲– –), and 65°C (—●—).*



As expected, the drying rate increase significantly as a function of the dehydration temperature with value of $5.91*10^{-9}$, $6.37*10^{-8}$, and $1.29*10^{-7}$min-1, respectively at 45 °C, 55 °C, and 65 °C.

In addition, the relationship between the moisture content and water activity (a_w) was graphically expressed as moisture isotherm curves (Tejada-Ortigoza et al., 2017) while the mathematical description was performed by using the Guggenheim-Anderson-De Boer (GAB) equation (Eq. 2):

$$Xw = \frac{m*C*K*aw}{(1-K*aw)*(1-K*aw+C*K*aw)} \qquad (2)$$

Where Xw and m are the equilibrium and monolayer moisture content (g water/100 g d.b.), aw is the activity water, C and K are fitting dimensionless parameters.

By combining the results obtained through the GAB and Weibull models, it was possible to estimate the dehydration time required to achieve a water activity consistently below 0.3. The estimated times were 211.48 min, 110.34 min, and 90.51 min at temperatures of 45°C, 55°C, and 65°C, respectively. A second series of dehydration experiments allowed to validate the estimated moisture content with an overall difference of less than 0.73%.

3.2 Determination of quality properties

No significant changes in colour were observed when the samples reached the moisture content levels estimated for each drying temperature. Mean values of L* = 70.25 ± 2.25; a* = 3.61 ± 1.00; and b* = 26.92 ± 1.82 were observed. Table 1 shows the effects of the different dehydration temperatures on fatty acid composition. Even though the sum of EPA and DHA (g/100g of dry basis) showed some increase with drying temperature, from 0.17 g/100g at 45°C to 0.22 g/100g at 65°C no statistical differences were revealed. These observations are consistent with Ortiz et al. (2013), who reported that PUFA oxidation is more strongly influenced by drying time than by temperature.

Table 1 *Effects of the different dehydration temperatures on fatty acid composition.*

Sample	Saturated fatty acids (% of total fatty acids)	Monounsaturated fatty acids (% of total fatty acids)	Polyunsaturated fatty acids (% of total fatty acids	EPA (% of total fatty acids)	DHA(% of total fatty acids)	EPA+DHA (g/ 100 g d.b.)
45°C	68.02 ± 1.16	23.50 ±1.44	8.49 ±0.66	0.69 ± 0.17^{ab}	4.10 ± 0.78^{a}	0.17 ± 0.03
55°C	64.46 ± 3.36	26.93 ±3.03	8.61 ±1.19	0.56 ± 0.13^{ab}	3.17 ± 0.39^{ab}	0.15 ± 0.02
65°C	55.96 ± 1.27	34.00 ±1.17	10.05 ±1.22	0.73 ± 0.16^{a}	3.92 ± 0.68^{a}	0.22 ± 0.03

Data are expressed as average ± standard deviation in three replicates. Values in the same column having the same letter (a and b) for each parameter are not significantly different at a confidence level of 95%.

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Enhancement and stability of Sicilian olive oils: accelerated shelf-life study (P-II-86)

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This experimental PhD thesis project aims to characterize the evolution of the chemical-physical and nutritional properties of Sicilian extra virgin olive oils during storage, through an accelerated shelf-life model, easily transferable to the industrial context. Particular attention will be focused on the stability and interaction of bioactive compounds, which characterize olive oils, as well as their beneficial effects on human health. The study also includes the evaluation of secondary shelf-life, analyzing the impact of post-opening oxygen exposure on oxidative dynamics, in order to optimize packaging and storage strategies with the aim of maintaining oil quality longer.

Valorizzazione e stabilità di oli di oliva Siciliani: studio accelerato della shelf life

Questo progetto di tesi di dottorato sperimentale ha l'obiettivo di caratterizzare l'evoluzione delle proprietà chimico-fisiche e nutrizionali di oli extravergini di oliva siciliani durante la conservazione, mediante un modello di shelf-life accelerata, facilmente trasferibile al contesto industriale. Particolare attenzione sarà rivolta alla stabilità e all'interazione dei composti bioattivi, caratterizzanti gli oli di oliva, nonché ai loro effetti benefici sulla salute umana. Lo studio include anche la valutazione della shelf-life secondaria, analizzando l'impatto dell'esposizione all'ossigeno post-apertura sulle dinamiche ossidative, al fine di ottimizzare strategie di confezionamento e conservazione con l'obiettivo di mantenere la qualità dell'olio più a lungo.

Key words: Extra virgin olive oil, accelerated shelf-life test, oxidative stability, phenolic compounds, secondary shelf-life.

1. Introduction

According to recent literature, in the olive oil sector, the development of an efficient shelf-life prediction model is extremely important to avoid the risk of downgrading the product to the virgin oil category. As reported by (Calligaris et al., 2022), an accelerated approach (ASLT) is often proposed to speed up the shelf life assessment process. This procedure, if applied appropriately, allows the shelf life of the product stored under normal conditions to be estimated. This would allow the company to carry out assessments throughout the production process, focusing on each stage. As a result, the olive oil industry would be able to maintain the shelf life of the product for longer. The aim of the PhD thesis project is to develop a predictive model to estimate the primary and secondary shelf life of extra virgin olive oils, using non-destructive analytical techniques (such as NMR, fluorescence spectroscopy, or Raman resonance spectroscopy, among others) with low environmental impact. These techniques enable rapid, economical, and reliable screening for the qualitative assessment of olive oil. This experimental work reports the main results of the ASLT for a more rapid determination of chemical parameters (which, according to (Commission Regulation, 2022), must comply with the mandatory limits for the marketing of olive oil) using the Arrhenius model as described by (Calligaris et al., 2022). This accelerated approach was achieved by using high temperatures (30 °C, 60 °C). Temperature, is certainly the most commonly used factor for accelerating reactions in accelerated shelf-life studies, but it should be noted that the use of temperatures above 60 °C is questionable because it could lead to oxidative reactions and the development of rancid off-flavours that would not indicate an accurate prediction of the product's shelf life (Conte et al., 2020).

2. Materials and Methods

Different samples of olive oils were collected from Sicily. Each olive oil sample was poured into 50 mL amber glass bottles and stored closed, in dark, in separate ovens Heraeus (typ. FVT 420-K) at controlled temperatures of 30 °C, and 60°C, respectively. The samples were taken out of the oven at regular monthly intervals. All experiments and analytical determinations were carried out at least in duplicate. Quality chemical indices were measured following IOC methods. These inlcude free acidity (FA), expressed as % of oleic acid in the mass percentage of oil (g/100 g) (IOC, 2017a), peroxide value (PV) given as milliequivalents of active oxygen per kilogram of oil (mEq O_2/kg oil) (IOC, 2017b), UV absorption coefficients at λ= 232 (K_{232}) and λ= 270 nm (K_{270}) as well as ΔK were determined.

3. Results and Discussion

As expected, all the samples complied with the quality indexes reported by IOC (IOC, 2017a; IOC, 2017b) and EU regulation No. 2022/2104 (Commission Regulation, 2022), as reported in Table 1.

Table 1 *Initial values of official quality parameters of samples used for the development of shelf-life predictive model and its validation.*

Quality indices	Biancolilla	Cerasuola	Nocellara
Free Acidity %	0.42±0.01	0.35±0.09	0.42±0.01
Peroxide Value(mEq_2/kg)	9.45±0.01	9.91±0.01	10.57±0.01
K_{232}	1.65±0.07	1.56±0.06	1.61±0.06
K_{270}	0.12±0.02	0.13±0.01	0.15±0.03
ΔK	-0.0092	-0.002	-0.0043

From the average of the three different olive oils, it was found that the reaction order for K_{270} parameter was zero, as shown in Fig. 1. The reaction order was determinate by visual inspection of the plots of *I*, *ln (I)* and *1/I* against storage time.

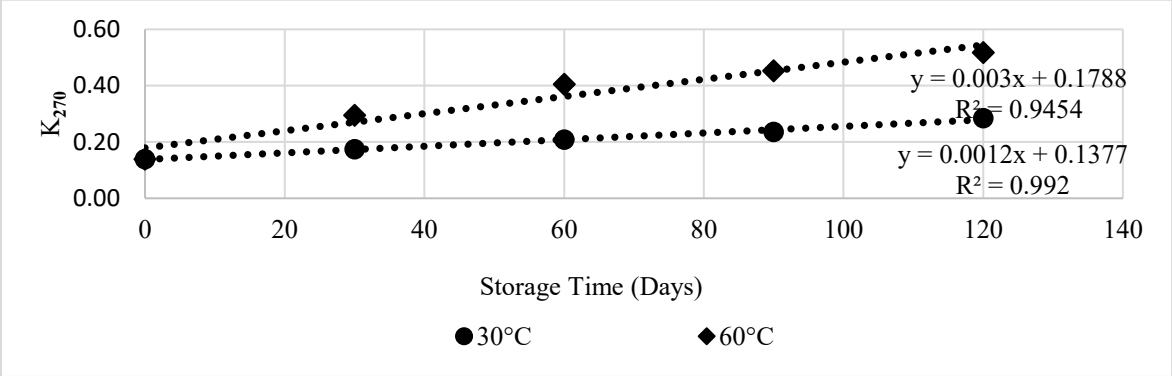


Figure 1 *Evolution of K_{270} of EVOOs subjected to accelerated storage conditions*

This experimental study confirmed that the ASLT method is effective in predicting EVOO shelf life, using K_{270} as the most reliable quality indicator for estimating the best before date. Further research is needed to explore non-destructive techniques for shelf-life estimation and to understand how oxidation rates vary with temperature and lipid composition of the different oils.

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Study of the association between phenolic metabotypes and cardiometabolic health in response to chronic intake of (poly)phenols (P-II-87)

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This PhD thesis research project aims at exploring the association between aggregate phenolic metabotypes and cardiometabolic health in response to the chronic consumption of personalised, plant-based, (poly)phenol-rich diets, by conducting a randomized controlled trial, the PRE-CARE-DIET study. Personalised diets will increase (poly)phenol intake by at least 50%, focusing on foods like coffee, cocoa powder, green tea, whole grains, legumes, and olive oil. This research aims to demonstrate the potential of precision nutrition on the prevention and management of cardiometabolic diseases. The primary outcome is the 10-year cardiovascular risk (SCORE2/SCORE2-OP), with secondary outcomes covering biomarkers, inflammatory status, and gut microbiota composition.

Studio dell'associazione tra metabotipi fenolici e salute cardiometabolica in risposta all'assunzione cronica di (poli)fenoli

Questo progetto di tesi di dottorato mira a esplorare l'associazione tra i metabotipi fenolici aggregati e la salute cardiometabolica in risposta al consumo cronico di diete personalizzate, a base vegetale e ricche di (poli)fenoli, conducendo uno studio randomizzato controllato, PRE-CARE-DIET. Le diete aumenteranno l'assunzione di (poli)fenoli di almeno il 50%, attraverso alimenti come caffè, cacao, tè verde, cereali integrali, legumi e olio d'oliva. Si mira a dimostrare il potenziale della nutrizione di precisione nella prevenzione e gestione delle malattie cardiometaboliche. Gli outcomes sono il rischio cardiovascolare a 10 anni, oltre ai biomarcatori, lo stato infiammatorio e la composizione del microbiota intestinale.

Key words: Personalised nutrition, (poly)phenol-rich diets, cardiometabolic health.

1. Introduction

In accordance with the PhD thesis project, this poster reports the preliminary results of the first three activities concerning:

(A1) **Targeted recruitment** of 330 subjects into one of the two phenolic metabotypes through a screening interview and their metabolic profiling, if deemed eligible.

(A2) **Observational period** consisting of collecting data on the diet and lifestyle (7-day food diary, food frequency and physical activity questionnaires) of the volunteers, as well as prescribing a personalised diet (intervention group) or general dietary recommendations (control group).

(A3) **Intervention period**: initial dietary prescription and monitoring every 8 weeks; collection of biological, anthropometric and cardiometabolic data, and diet and lifestyle assessment at every visit.

2. Materials and Methods

Within the framework of the PRE-CARE-DIET study, a chronic nutritional intervention study, which is divided into three phases - targeted recruitment, observational, and intervention period - volunteers between 40-80 years old, with at least a cardiometabolic risk factor, are recruited and initially profiled according to their phenolic metabotype. Once enrolled, various anthropometric, dietary, lifestyle, and biological data are collected. Their eating habits are studied during the observational phase, and they are given energy-adjusted dietary recommendations to follow during the 16-week experimental phase, during which volunteers in the intervention group (IG) are prescribed a personalised diet plan with an increase of at least 50% in the amount of (poly)phenols in the diet, while volunteers in the control group (CG) receive dietary recommendations based on the national dietary guidelines. The dietary and lifestyle data, as well as the preparation of the dietary plans, are done using the *RicercAlimentare* web application, developed by the research group. Various food products are provided to the volunteers to increase their adherence to the prescribed dietary recommendations. Dietary adherence and changes in (poly)phenol intake are assessed and associated with health outcomes, specifically the SCORE2/SCORE2-OP risk scores.

3. Results and Discussion

3.1 Targeted recruitment

To date, 1075 potential volunteers have contacted the project team, of whom 297 have been called to a screening visit to assess their eligibility for the study. Of these, 191 were recruited and 23 are still awaiting the nutritional challenge Oral Polyphenol Challenge Test (OPCT), to determine their phenolic metabotype. Of the 82 volunteers who have completed the experimental phase of the study, 37 (45.1 %) belong to the C1 metabotype (low producers)

and 45 (54.9 %) to the C2 metabotype (high producers).

3.2 Observational period

At the moment, 124 volunteers have taken part in the observational phase: 78 belong to the IG and 46 to the CG. Using a workflow developed for prescribing personalised, sustainable, plant-based, (poly)phenol-enriched diets, diet plans are prescribed, including foods rich in these compounds, such as green tea, coffee, cocoa powder, and specific fruits and vegetables. **Figure 1a** presents the comparison in (poly)phenol content between the 7-day food diary and the personalised diets/dietary guidelines given to the volunteers (115 who have already started the experimental phase) in IG (n=72) and CG (n=43), showing a 76.6% ($p<0.001$) increase in recommended consumption in the IG.

3.3 Intervention period

To date, 82 volunteers have completed the 16-week experimental phase of the study (50 IG and 32 CG). Considering some missing data regarding the dietary and/or blood data of two volunteers, **Figure 1b** and **Table 1** present the results of 80 volunteers (48 IG and 32 CG), comparing (poly)phenol consumption, as well as anthropometric, blood pressure, cholesterol, and triglyceride changes during the experimental phase. The volunteers in the IG reported a 64.5% ($p<0.001$) increase in (poly)phenol consumption.

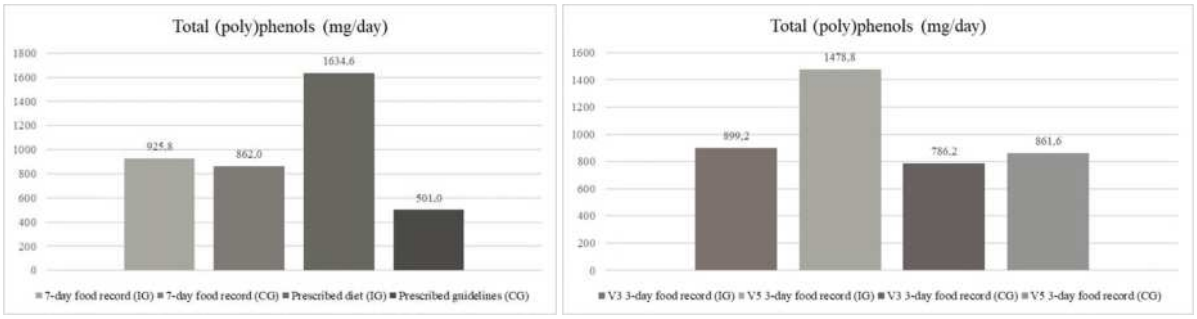


Figure 1a *Comparison of the (poly)phenol content reported in the 7-day food diary and that prescribed in the diets (IG) and guidelines (CG).* **1b** *Comparison of the (poly)phenol content reported in the 3-day food diary at the initial (V3) and final (V5) visits of the experimental phase of the study.*

Table 1 *Variation in anthropometric parameters, blood pressure, cholesterol, and triglyceride levels during the experimental phase (from visits 3 to 5) in the intervention and control groups.*

Δ V3-V5	**Intervention group (n=48)**		**Control group (n=32)**	
	Mean ± SD	p value	Mean ± SD	p value
Body Mass Index (kg/m^2)	-0.3 ± 0.8	**0.007**	-0.1 ± 0.7	0.431
Fat Mass (%)	-0.2 ± 1.6	0.358	0.1 ± 1.5	0.855
Fat Free Mass (%)	0.2 ± 1.7	0.382	-0.3 ± 1.8	0.346
Systolic Blood Pressure (mmHg)	0.1 ± 13.8	0.964	0.5 ± 9.9	0.769
Diastolic Blood Pressure (mmHg)	-2.2 ± 6.7	**0.031**	-0.6 ± 6.8	0.641
Heart Rate (bpm)	-3.7 ± 7.7	**0.002**	0.4 ± 5.4	0.697
Total Cholesterol (mg/dL)	-11.7 ± 23.1	**0.001**	1.8 ± 25.6	0.702
HDL-Cholesterol (mg/dL)	-1.5 ± 8.2	0.203	3.3 ± 7.1	**0.013**
LDL-Cholesterol (mg/dL)	-8.3 ± 18.2	**0.003**	-1.6 ± 19.6	0.657
Triglycerides (mg/dL)	-9.3 ± 48.1	0.189	-0.2 ± 32.6	0.974

Thus, the experimental results agree with the expected ones, and the original PhD thesis project can proceed without any substantial amendment (apart from the possible extension of the volunteer enrolment period in order to reach the required number). The enrolment of the volunteers is expected to be completed by the end of the year and, therefore, the collection of all the data related to the visits in the experimental phase are expected to finish by the middle of the final year of the PhD. This will allow an intensive analysis of the samples/data and the writing of the thesis during the second half of the final year, in accordance with the planned timetable.

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Predictive models for the effect of foods containing (poly)phenols: a personalized nutritional approach (P-II-88)

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This PhD research project aims at developing machine learning models to predict the effects of (poly)phenol-rich foods in subjects at cardiometabolic risk, using a personalized nutritional approach within the PRE-CARE-DIET study, a targeted-recruitment, randomized controlled intervention. The activities carried out during this second year of the PhD program are part of the PRE-CARE-DIET intervention study. In particular, the study implementation continued, along with the development of a predictive model aimed at profiling the study participants.

Modelli predittivi per l'effetto degli alimenti contenenti (poli)fenoli: un approccio nutrizionale personalizzato

Questo progetto di dottorato si svolge nell'ambito del PRE-CARE-DIET, uno studio di intervento randomizzato controllato, volto a sviluppare modelli di machine learning per predire gli effetti degli alimenti ricchi di (poli)fenoli in soggetti a rischio cardiometabolico, utilizzando un approccio nutrizionale personalizzato. Le attività svolte durante questo secondo anno di dottorato si inseriscono nell'ambito dello studio di intervento PRE-CARE-DIET. In particolare, è proseguita la conduzione dello studio e lo sviluppo del modello predittivo finalizzato alla profilazione dei soggetti partecipanti.

Key words: (poly)phenols, cardiometabolic health, phenolic metabotype, precision nutrition, predictive model.

1. Introduction

In line with the timeline of the PhD project, this work presents the main results related to the first two activities:

1) **Management of volunteers' enrolment and multiple visits:** The study is being carried out and consists of 5 different visits for each volunteer, spread over about 5/6 months. During the visits, dietary and lifestyle information, anthropometric measurements, clinical data, and biological samples (blood, urine, faeces, saliva) are collected.
2) **Metabotyping:** Development of a predictive model to cluster volunteers based on their capacity to metabolize (poly)phenols (PP) following a nutritional challenge, the Oral (Poly)phenol Challenge Test (OPCT), consisting of the consumption of 3 different tablets rich in PP. The OPCT is administered at visits 1, 3, 4, and 5.

2. Materials and Methods

A targeted, randomized controlled intervention study is being carried out in subjects (40-80 years old, BMI 18.5-35 kg/m^2) at cardiometabolic risk. A total of 500 volunteers is profiled according to their ability to metabolize phenolic compounds following the OPCT. Based on their assigned metabotype, 2/3 of participants are enrolled and randomly allocated to an intervention or control group (2:1) for a 16-week trial. The intervention group receives a personalized diet designed to increase PP intake by at least 50%, while the control group follows general healthy eating guidelines. Out of >200 phenolic metabolites identified in the OPCT study, 25 key metabolites were selected using machine learning (ML) techniques to develop the PRE-CARE-DIET clustering predictive model for subject profiling. Among several ML algorithms tested, Random Forest demonstrated the best performance, allowing volunteers to be clustered into two distinct metabotypes: Low Producers (C1) and High Producers (C2). Given this model, volunteers are continuously profiled through the analysis of 24-h spot urine samples by UHPLC-QqQ-MS/MS. The experimental phase includes three follow-up visits spread over the 16-week period. A deep-phenotyping of the volunteers is carried out in each visit. Cardiometabolic health is assessed through blood pressure measurements, anthropometrics, and blood-based biomarkers (lipid profile, glucose levels, HOMA indices, etc.). In addition, validated algorithms are used to calculate cardiometabolic risk scores, including SCORE2/2-OP and QDiabetes.

3. Results and Discussion

3.1 Study Population and Recruitment Status

To date, over 1000 individuals have contacted the research team expressing interest in participating in the study. Following the application of inclusion and exclusion criteria, a subset of eligible volunteers has been enrolled and included in the ongoing protocol. The progress of the study and recruitment status are illustrated in Figure 1. Currently, 82 participants have successfully completed the last visit (V5). The mean age of the population is 55±1 years, with a predominance of females (65%) over males (35%). Regarding group allocation, 61% of the participants have been assigned to the intervention group, while 39% belong to the control group.

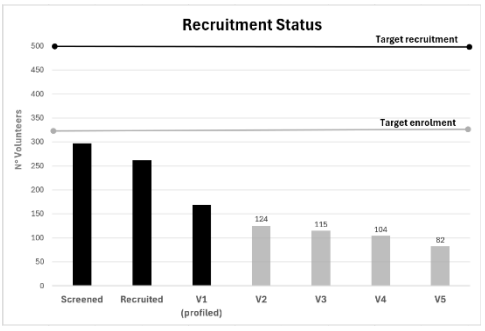


Figure 1. PRE-CARE-DIET recruitment status.

3.2 Metabotyping

Metabotyping has been performed on urine samples collected at Visit 1 for 150 participants, prior to the intervention phase. Volunteer classification into two phenolic metabotypes, Cluster 1 (C1) – *Low producers* and Cluster 2 (C2) – *High producers*, using 25 selected phenolic metabolites is evidenced Figure 2 using Principal Component Analysis (PCA). To date, 43% of volunteers are classified as C1 and 57% as C2. Future analyses will explore the temporal robustness of phenolic metabotypes by reassessing cluster stability across Visit 3, 4, and 5.

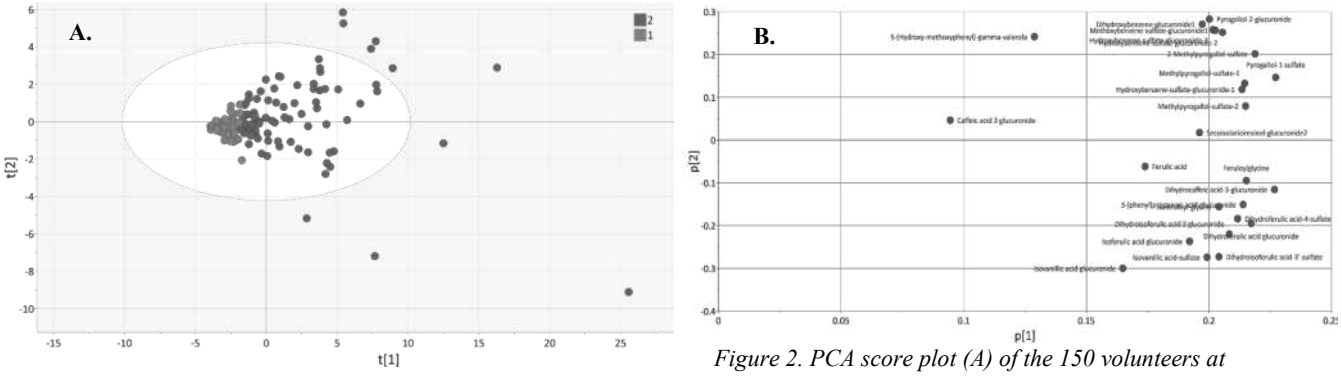


Figure 2. PCA score plot (A) of the 150 volunteers at visit 1 and loading plot (B) of the 25 phenolic metabolites.

3.3 Cardiometabolic status at baseline and end of experimental phase

Cardiometabolic parameters are assessed at Visit 3 (V3) - baseline of the intervention - and at Visit 5 (V5), the end of the 16-week experimental phase. Table 1 below summarizes the main findings for both the intervention and control groups (data expressed as mean ± SEM). All anthropometric, clinical, and biochemical outcomes reported here are based on data from 81 individuals, due to a single missing case. This will be addressed in future analyses using appropriate statistical imputation strategies, if needed.

	Intervention Group		**Control Group**		***p*-value**
	V3 (mean±SEM)	**V5** (mean±SEM)	**V3** (mean±SEM)	**V5** (mean±SEM)	**Δ V3-V5**
BMI (kg/m^2)	27±1	27±1	28±1	28±1	0.218
Systolic pressure (mmHg)	122±2	121±2	124±3	125±3	0.773
Diastolic pressure (mmHg)	82±1	80±2	83±2	82±2	0.247
Heart rate (bpm)	72±1	68±1	70±1	71±2	**0.007**
Total-C (mg/dL)	218±4	207±5	205±5	207±5	**0.016**
HDL-C (mg/dL)	66±3	64±2	63±2	67±3	**0.006**
LDL-C (mg/dL)	130±3	121±4	122±5	121±4	0.107
TG (mg/dL)	116±9	108±9	98±7	98±5	0.378
Glucose (mg/dL)	86±1	85±1	86±1	87±1	0.325
Insulin (uUI/mL)	11±1	12±1	11±1	11±1	0.590
HOMA-IR	1.3±0.1	1.03±0.1	1.2±0.1	1.2±0.1	0.871
HOMA-β (%)	149±13	287±28	141±14	241±24	0.213
Score2/2OP	3.8±0.5	3.6±0.5	3.4±06	3.2±0.6	0.868
QDiabetes	3.1±0.7	2.8±0.4	5.5±1.6	6.1±1.6	0.349

Table 1. Anthropometric, clinical, and biochemical outcomes reported for both intervention and control group at visit 3 and visit 5.

3.4 Preliminary considerations

At this preliminary stage, some trends are observed. While BMI remained stable in both groups, the intervention group shows a slight reduction in systolic and diastolic blood pressure, and a notable decrease in heart rate, possibly indicating improved cardiovascular regulation. Lipid profile improvements are more evident in the intervention group, with reductions in LDL-C (−9 mg/dL), triglycerides (−8 mg/dL), and total cholesterol (−9 mg/dL). Glycemic control indicators, although relatively stable overall, showed a reduction in HOMA-IR (1.3±0.1 to 1.0±0.1) and a marked increase in HOMA-β (149% to 287%), pointing to enhanced insulin sensitivity and β-cell responsiveness in the intervention group. Cardiometabolic risk scores showed small but favorable changes: QDiabetes decreased slightly in the intervention group (3.1±0.7 to 2.8±0.4), in contrast to an increase observed in controls.

In summary, these preliminary findings suggest early beneficial effects of the personalized (poly)phenol-rich dietary intervention on cardiometabolic health, particularly on lipid metabolism, insulin function, and cardiovascular parameters.

Development of the Nutritional Inflammatory Index: cell culture- and artificial intelligence-based methods to evaluate the Inflammatory Modulation of Foods and Recipes (P-II-89)

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The first two activities of the PhD thesis project are described. At first, they were selected several foods and recipes representing different categories, taking into account their nutritional and technological characteristics and the frequency with which they are consumed in Italy. In the second place, foods and recipes were digested *in vitro* using the INFOGEST protocol. Finally, a triculture intestinal cell culture model was developed for the analysis of cytokine modulation.

Sviluppo dell'indice infiammatorio nutrizionale: metodi basati su colture cellulari e intelligenza artificiale per valutare la modulazione infiammatoria di alimenti e ricette

Vengono descritte le prime attività del progetto di tesi di dottorato. In primo luogo, è stata eseguita la selezione di diversi alimenti e ricette rappresentativi di diverse categorie, considerando le caratteristiche nutrizionali e della frequenza di consumo in Italia. Successivamente, gli alimenti e ricette selezionate sono stati digeriti di maniera *in vitro* utilizzando il protocollo Infogest. Infine, è stato sviluppato un modello intestinale di tricoltura per l'analisi della modulazione delle citochine.

1. Introduction

This poster presents the main findings of the project:

- The selection and *in vitro* digestion of recipes representative of traditional Italian cuisine, as well dishes created by important stakeholders.
- The assessment of cytokine production and modulation induced by these digested foods, using Ella™ Automated ELISA.

2. Materials and Methods

2.1. Food Selection and *In Vitro* Digestion

Two Italian dishes representative of the culinary tradition, carbonara and pasta with tomato sauce, were selected and analyzed, including their ingredients and preparation methods. Additionally, three recipes cooked by a three-star Chef, Heinz Beck, including the ingredients (cooking procedure not available). Among the recipes proposed by Chef Heinz Beck, there are three dishes representing different food categories: meat-based (HB-MB), fish-based (HB-FB) and vegetable-based (HB-PB).

To simulate digestion under physiologically relevant conditions, the INFOGEST protocol was adopted an internationally recognized and validated *in vitro* digestion cell culture model (Brodkorb et al., 2019).This protocol mimics the oral, gastric, and intestinal phases of human digestion, resulting in a final digested fraction suitable for cellular applications. After in vitro digestion, four different concentrations of digesta were used (D4, D3, D2, D1), corresponding to varying concentrations of food digesta based on the dry matter content for each food and their respective concentrations used in the experiment (Table 1). For each digested sample, these concentrations were obtained by combining decreasing volumes of digesta with culture medium as follows: D4 (400 µL of digesta + 600 µL of medium), D3 (200 µL + 800 µL), D2 (100 µL + 900 µL), and D1 (50 µL + 950 µL).

2.2. Development an intestinal human tri-cellular *In Vitro* model

An *in vitro* tri-culture cell culture model (3C) was established by co-culturing human CaCo-2 enterocyte-like cells and HT29-MTX-E12 goblet-like mucus-producing cells on transwell inserts. Human THP-1 monocytes were subsequently added to the basolateral compartment to mimic immune cell interaction (Ramal-Sanchez et al., 2025). Inflammation was induced by treating the model with 100 nM phorbol-12-myristate-13-acetate (PMA) for 20 hours. Following induction, food digests prepared as described above were applied to the apical compartment at four different concentrations and incubated for 4 hours.

Supernatants were collected from the basolateral compartment and analyzed for pro-inflammatory cytokine production (IL-6, TNF-α, IL-12p70, IL-18, and IL-8) using the Ella™ Automated ELISA. The anti-inflammatory drug Prednisolone was included as a positive control.

3. Results

3.1 Digesta concentrations

Table 1 shows the concentrations of digested foods tested on the 3C model. Initial concentrations are expressed in grams of dry matter per liter (g d.m./L).

DIGESTA	Acronym	Concentration (g d.m./L)	Concentration (g d.m./L)			
			D4	D3	D2	D1

Carbonara	CbD	72.11	28.84	14.42	7.21	3.61
Pasta with tomato sauce	PsD	52.11	20.84	10.42	5.21	2.61
Heinz Beck – Meat based	HB-MB	36.53	14.61	7.31	3.65	1.83
Heinz Beck – Fish based	HB-FB	40.58	16.23	8.12	4.06	2.03
Heinz Beck – Plant based	HB-PB	45.36	18.14	9.07	4.54	2.27

Table 1. Digesta analyzed to date their corresponding concentrations effectively tested on the 3C model (D4, D3, D2, D1).

3.2. Testing the digested foods in a cellular model of inflammation.

3.2.1. Traditional recipes

Analyzing pasta with tomato sauce and carbonara results show the values obtained for every concentration of carbonara and pasta with tomato sauce, in terms of cytokine production for the 4 cytokines analyzed (IL-18, IL-6, TNF-α, IL-8). The cytokine shifts for the two recipes, carbonara and pasta with tomato sauce, were normalized for the control group and compared to the effect obtained with 5 µM prednisolone (Figure 1.A). While PsD shows a decrease in TNF-α levels for the highest concentration with respect to prednisolone, CbD shows an increase in IL-6, TNF-α and IL-8 levels with respect to prednisolone for some of the digesta concentrations (Figure 1.B).

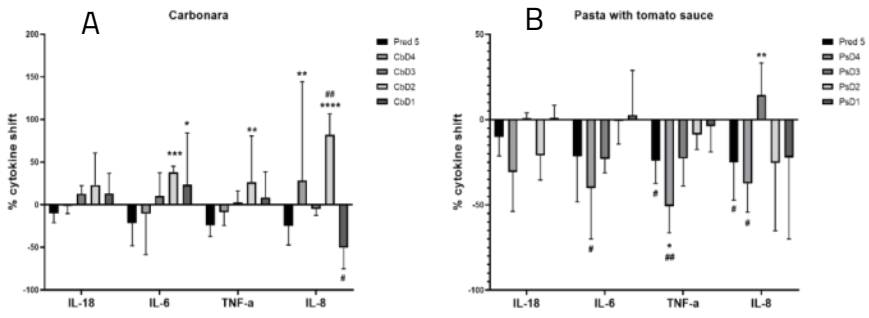


Figure 1. Cytokine shift after carbonara and pasta with tomato sauce testing on the 3C model. Different concentrations (D4, D3, D2, D1) of carbonara (CbD) and pasta with tomato sauce (PsD) were tested on the 3C model, and the effects were compared to 5 µM prednisolone (Pred 5). Results are expressed as % of cytokine shift, showing mean ± SD of the % of cytokine shift with respect to the control group. N=3; *p<0.05; **p<0.01, with respect to Pred 5 µM.

3.2.2 Chef recipes:

Three recipes selected and cooked by a three-star Chef have been analyzed. The cytokine shifts for the three recipes, HB-MB, HB-FB, and HB-PB, were normalized for the control group and compared to the effect obtained with 5 µM prednisolone. HB-MB shows an increase in IL-6, TNF-α and IL-8 cytokines after the treatment with D3, D2 and D4 concentrations, respectively (Figure 2.A), while HB-FB showed a slight increase in IL-6 after the addition of D4 (Figure 2.B). HB-PB showed the most significant results, with a high increase in IL-18, IL-6, TNF-α and IL-8 cytokines after the addition of most of the concentrations (Figure 2.C).

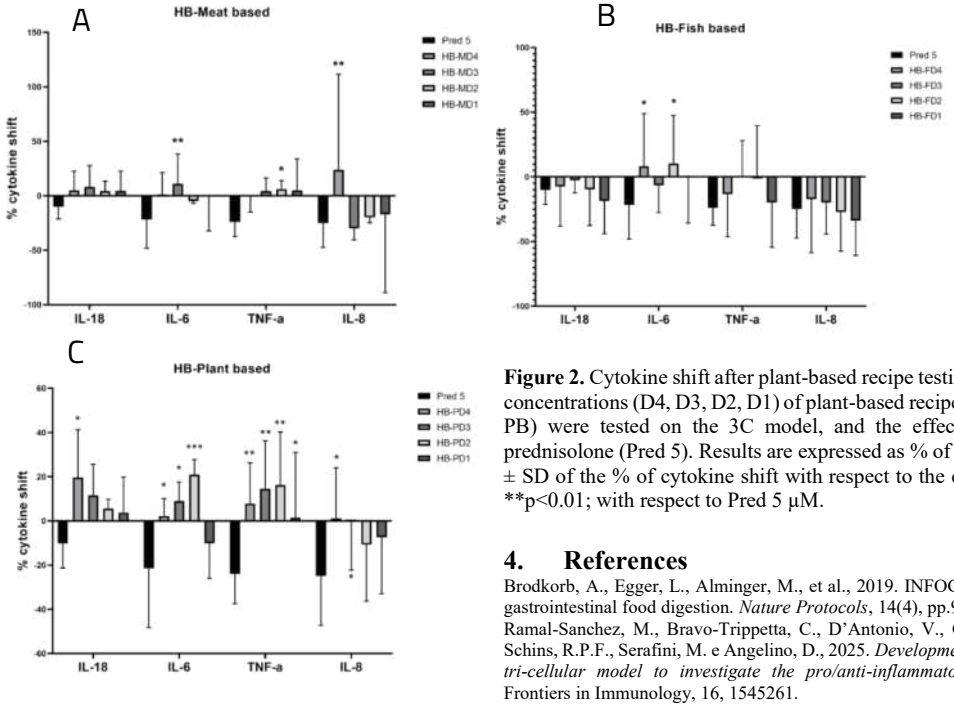


Figure 2. Cytokine shift after plant-based recipe testing on the 3C model. Different concentrations (D4, D3, D2, D1) of plant-based recipe (HB-MD, HB-FB AND HB-PB) were tested on the 3C model, and the effects were compared to 5 µM prednisolone (Pred 5). Results are expressed as % of cytokine shift, showing mean ± SD of the % of cytokine shift with respect to the control group. N=3; *p<0.05; **p<0.01; with respect to Pred 5 µM.

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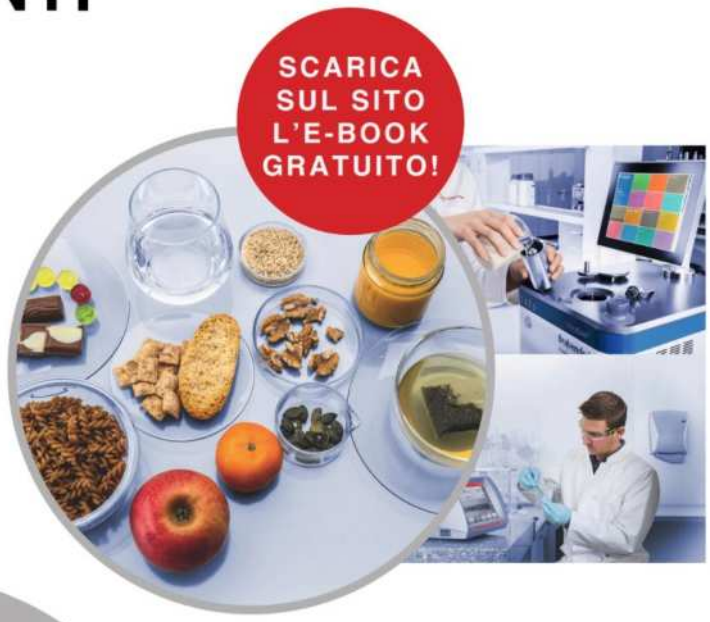
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Accelerating answers to your important questions
Our company's heritage dates back to 1935, when Dr. Arnold Beckman founded National Technical Laboratories in California. That year, he sold 87 of his recently patented pH meters, a groundbreaking technology that could be used for everything from water quality testing to laboratory research.

Our mission today, which has changed little since 1935, is to empower those who are seeking answers to life's important scientific and healthcare questions. This mission embodies all our efforts to help customers get the data and answers they need, when they need them. These answers may be important for expediting development of a new, life-saving biopharmaceutical, to help exonerate an innocent person based on DNA evidence, or to help monitor patients with chronic diseases.

If your life's calling demands answers for these (or dozens of other) important endeavors, you need to know Beckman Coulter Life Sciences. No matter the type or scope of questions you have, we can help you get the answers you need—often faster than you thought possible.

In 2012 Danaher purchased Beckman Coulter and divided the company into two operating companies, Diagnostics (HQ - Brea, CA) and Life Sciences (HQ – Indianapolis, IN). Beckman Coulter Life Sciences is a leader in scientific research instruments primarily serving the academia and pharma markets. The company also serves the clinical research market as well as applied markets such as agricultural, food and beverage, gas and oil, aerospace, among several others.

Our products line: **Centrifugation:** Instruments, Rotors, Tubes & Bottles, Parts & Accessories. **Flow Cytometry:** Instruments, Reagents, Software, CytoFLEX Platform Upgrades, Parts & Accessories. **Genomics:** Reagents, Instruments, Labware & Accessories. **Liquid Handling Automation:** Instruments, Tips, Parts & Accessories.
Counters & Analyzers: Cell Counters & Analyzers, Particle Size Analyzers, TOC Analyzers, Air Particle Counters, Liquid Particle Counters, Reagents & Controls, Parts & Accessories.
Microbioreactors: Instruments, Modules, Plates & Covers.

DALTON
BIOTECNOLOGIE

Dalton è una società specializzata nel settore delle Biotecnologie.

Ricerca, sviluppa e produce colture microbiche di interesse alimentare e probiotico.

Fin dalla sua fondazione, nel 1974, l'azienda è impegnata nel miglioramento degli standard di prodotto e di processo al fine di costruire, con i propri clienti, rapporti di partnership basati sulla fiducia e sull'affidabilità.

È fornitore sia di grandi gruppi internazionali sia di piccoli produttori che si dimostrano scrupolosamente attenti alle esigenze dei consumatori. Ponendosi a sostegno dei sistemi di produzione già all'inizio della filiera, Dalton è garante della sicurezza dell'intero processo e dell'alta qualità richiesta nei settori alimentare e farmaceutico.

Una caratteristica distintiva di Dalton è il profondo impegno nella ricerca scientifica, condotta con un approccio etico e sostenibile, volta all'ottimizzazione delle risorse energetiche e alla riduzione dei rifiuti di produzione. Questo approccio si arricchisce grazie alla collaborazione con enti di ricerca e università, creando sinergie che consolidano l'innovazione applicata nel settore alimentare.

Dalton opera attraverso tre principali divisioni tecnico-scientifiche:



1. **Microbiologia casearia** – Produzione di fermenti lattici e colture personalizzate per l'industria lattiero–casearia.
2. **Microbiologia enologica** – Produzione di colture di lieviti e batteri per supportare i processi fermentativi e le esigenze produttive delle cantine vitivinicole.
3. **Microbiologia probiotica e wellness** – Soluzioni dedicate alla nutrizione e al benessere, con colture di interesse probiotico e attenzione alle dinamiche salutistiche.

Grazie alle sue diverse competenze, Dalton riesce ad offrire non solo prodotti, ma anche un approccio scientifico integrato, fondato sulla ricerca collaborativa, che unisce know-how industriale e approfondimento accademico. Ricerca, sostenibilità e collaborazione scientifica si integrano per fornire alle aziende partner i migliori servizi specializzati nei settori della microbiologia enologica, casearia e probiotica.

ISTITUTO DANONE

Nato nel 1991 e operante come Fondazione dal 2011, l'Istituto Danone si dedica da sempre all'approfondimento e alla diffusione delle conoscenze scientifiche nel campo della nutrizione e della salute, collaborando con società scientifiche, mondo accademico e istituzioni.

Radicato nel principio del "conoscere e far conoscere", è focalizzato sui *turning points*, cioè quei momenti cruciali che impattano sullo stile di vita e sulle abitudini alimentari, quindi fondamentali per la salute. Da sempre ha un ruolo attivo nel contrastare *fake news* e disinformazione, unendo rigore scientifico e capacità divulgativa.

Tra le iniziative di punta vi è la collana "Items", pubblicazioni scientifiche che hanno festeggiato i 30 anni di vita e, da quest'anno 2025, il Premio Andrea Ghiselli dedicato alle migliori tesi di dottorato sull'impatto della nutrizione sulla salute, con particolare attenzione all'invecchiamento in salute.

DANONE
ONE PLANET. ONE HEALTH

ISTITUTO DANONE
ITALY



Food Hub SRL SB is an innovative startup and *Benefit Company* founded in 2021 by **Fabio D'Elia** – PhD in Food Science and Technology from **DISTAL – University of Bologna** – together with **Francesco De Carolis**.

Born within the academic ecosystem, Food Hub was created to accelerate the transfer of knowledge and foster collaboration between universities, research centers, startups, corporates, and professionals in the agri-food sector. For researchers and professors, Food Hub offers tailored services that enhance the visibility, dissemination, and exploitation of scientific results.

Our activities include:

- The **design and implementation of dissemination and communication strategies**
- The **creation of technical and popular science content** (articles, newsletters, podcasts, videos)
- **Stakeholder engagement workshops** with industry and policymakers
- **Market intelligence and technology scouting** to connect academic outcomes with industrial applications.

Through its community of **over 40,000 agri-food stakeholders** and an observatory of more than **800 innovative startups and SMEs**, Food Hub ensures that research results reach the right audiences, creating opportunities for partnerships, technology transfer, and impact beyond academia.

As a *Benefit Company*, Food Hub is committed not only to supporting the competitiveness of the agri-food sector but also to promoting sustainable development, knowledge sharing, and the growth of young talents. This dual mission makes Food Hub a unique player in Italy: a bridge between scientific research and industry, able to transform innovation into concrete value for society.

BACCALÀ MONTI

Dal 1903 **Baccalà Monti** porta avanti una tradizione familiare che unisce esperienza, ricerca e innovazione tecnologica al servizio di un prodotto autentico e naturalmente sano.

Con sede a **Corropoli (TE)**, **Foods Import dei F.lli Monti S.p.A.** è oggi una delle realtà italiane di riferimento nella lavorazione del **baccalà ammollato**, grazie a una filiera diretta con il più importante produttore norvegese e a processi certificati che garantiscono qualità, tracciabilità e sicurezza alimentare.

Cuore dell'attività è la **ricerca e sviluppo**, motore strategico che guida l'evoluzione dell'azienda nel rendere il baccalà un alimento moderno: altamente proteico, sostenibile, conveniente e pronto all'uso.

Grazie all'applicazione della **tecnologia HPP (High Pressure Processing)**, i prodotti Monti offrono una shelf life prolungata senza additivi né conservanti, mantenendo intatti gusto, valori nutrizionali e purezza delle carni. L'ammollo e la pulizia, tradizionalmente impegnativi, sono oggi completamente gestiti in azienda: il consumatore può così godere di un baccalà **già pronto, pulito, senza scarti e perfetto sia da cuocere che da gustare crudo come carpaccio**.
A completare l'offerta, **Baccalà Monti gestisce anche una selezione di referenze di baccalà ammollato e surgelato**, pensate per soddisfare le esigenze di chi ama la praticità dei prodotti congelati senza rinunciare alla qualità e al gusto autentico del mare.

Questo approccio rende il baccalà un alimento perfettamente in linea con le esigenze di chi cerca **una dieta sana, equilibrata e pratica**, senza rinunciare alla qualità.
Un prodotto che coniuga **valore nutrizionale e convenienza**, grazie a un **rapporto costo/porzione vantaggioso** e a una **semplicità di utilizzo** che lo avvicina anche ai consumatori più giovani, attenti al benessere ma con poco tempo da dedicare alla cucina.

In **Baccalà Monti**, la tradizione incontra la tecnologia per trasformare un prodotto naturale e ricco di valori in una **proposta alimentare contemporanea**, che unisce cultura gastronomica, benessere e responsabilità verso il futuro.

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Food Hub

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