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Variations in sensorially-relevant metabolites and indices in PDO wines of common ampelographic background: A case study on commercial Lambrusco wines

Giovanni Luzzini, Riccardo Bicego, Davide Slaghenaufi, Maurizio Ugliano *

University of Verona, Department of Biotechnology, Villa Lebrecht, via della Pieve 70, San Pietro in Cariano, 37029, Italy

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ABSTRACT

PDOs are important regulatory tools with the aim to link specific food products to their geographical origin. Despite the widespread presence of PDOs in wine sector, there is limited understanding as to whether wines of the same PDO exhibit common chemical signatures which can be considered representative of the PDO. Improved understanding of these could help in the development of successful PDO policies. In this context, this study considers 40 wines of three different PDOs of Lambrusco (Salamino, Grasparossa and Sorbara), a sparkling red wine produced in Italy, with the aim to evaluate to which extent a chemical signature reflecting sensorially-relevant metabolites could be identified for each PDO, also considering technological variables such as production technique and vintage. In comparison to other Italian wines, Lambrusco PDOs exhibited common features such as high content of acetate esters, *trans*-3-hexen-1-ol and 1,8-cineole. Lambrusco appellations differed for volatile metabolites such as terpenes, VSCs, C_6 alcohols, TDN. Sorbara was characterized by lower content of phenolic compounds. The study of the impact of aging and production techniques revealed a greater impact of the former on the volatile profile. Young Classico methods were more similar to Charmat wines from the same vintages, rather than to older classic methods.

1. Introduction

Protected Designation of Origins (PDOs) are regulatory tools aiming to support the economic growth of rural areas, aiming to link specific food products to their geographical origin (Barker, 2005). An added value is recognized for products from specific geographical areas, which is reflected by increased willingness to pay for PDOs products (Cross et al., 2011; Cross et al., 2011). Product differentiation is a key aspect of a successful PDO policy, which involves highlighting the unique characteristics that set a PDO product apart from other similar products. In the case of wines, this could include specific grape varieties, distinctive production methods, and unique terroir. PDOs deal therefore also with the concept of chemical identity and ultimately sensory recognizability, based on the fact that the grape variety, its geographical origin, and the way in which wine production take place can impact the metabolic profile of the wine, to an extent that could influence wine sensory characteristics (van Leeuwen et al., 2004; Cadot et al., 2010). Wine PDOs typically highlight an association between a specific grape variety and a defined area of origin, which can occur in different forms. The most obvious one involves a genetically defined variety that is grown in different places, either within a region or in different regions. In the first case, a single PDO exists, with sub-regions being identified and often mentioned in the PDO regulation (Bélis-Bergouignan and Marie-Claude, 2011; Taylor, 2021). In the second case, different PDOs will exist, as in the case, for example, of the iconic red wines Chianti, Brunello and Nobile di Montepulciano, all made with Sangiovese grapes grown in different areas of Tuscany (Vergamini et al., 201), or Verdicchio and Lugana wines, both made with Verdicchio grapes grown in different parts of Italy (Slaghenaufi et al., 2021a). Another less obvious scenario is related to situations in which, for a generic wine name, several PDOs exist, each one of them indicating a specific grape variety, albeit often belonging to the same genetic cluster.

One example of the latter scenario is represented by Lambrusco, an Italian sparkling red wine produced in northern Italy. According to National Catalogue of Vine Varieties the term Lambrusco refers to thirteen different grape varieties employed to produce seven PDOs wines. Among these PDOs, three of the most successful are located in the Modena area, in the north of Italy: “Lambrusco Salamino di Santa Croce”

* Corresponding author.
E-mail address: Maurizio.ugliano@univr.it (M. Ugliano).

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(Salamino), "Lambrusco Grasparossa di Castelvetro" (Grasparossa) and "Lambrusco di Sorbara" (Sorbara). These PDOs foresee the predominant use (at least 85 %) of a single Lambrusco variety from which they derive their names. Within the intricate taxonomy of Lambrusco, these three varieties are reported to be genetically similar, despite Sorbara and Salamino are closer than Grasparossa (Boccacci et al., 2005). A further difference among these three PDOs is associated with the geographic origin of the grapes. Salamino production area is located to the north of Modena province, whereas Grasparossa is located in the south, at the foothills of the Tuscan-Emilian Apennines, and Sorbara lies centrally. In no case do the three areas overlap.

Lambrusco PDOs regulations contemplate, in addition, the possibility of producing wines by secondary fermentation either in tank (Charmat method), or in bottle (Classico method). These two production methods are known to influence wine chemical and sensory characteristics (Hervé and Guilloux-Benatier, 2006; Luzzini et al., 2023) in particular in relation to prolonged contact with yeast lees and the related yeast autolysis process, characteristic of the Classico method (Torresi et al., 2011). Often wines obtained with Classico method are associated with long bottle aging, whereas Charmat wines are typically produced with a target of faster turnaround and are considered less prone to long aging periods.

Many studies on Lambrusco focused on the traceability and authenticity (Durante et al., 2015; Papotti et al., 2013; Salvatore et al., 2013; Durante et al., 2013; Lancellotti et al., 2021) while few studies concern the characterization of the chemical composition in relation to metabolites of enological and sensory relevance. The study of chemical composition, and in particular volatile composition, is relevant for the valorisation of varietal aspects, for the application of precision enology and viticulture techniques, for an improved communication, and therefore to implement a successful PDO policy.

The main aim of this study was to investigate, within a group of PDOs sharing grapes of a common genetic cluster, the existence of profiles of sensory-related metabolites (aromas, polyphenols, colorimetric indices) that could represent the chemical signature of each PDO. Three PDOs based on different Lambrusco varieties, namely *Lambrusco Salamino di Santa Croce, Lambrusco Grasparossa di Castelvetro* and *Lambrusco di Sorbara*, where chosen, each one also being associated with geographically distinct yet adjacent production areas. In consideration of the PDO specifications related to vinification, the influence of production method (Charmat or Classico) and wine age on volatile chemical profile was also considered.

2. Materials and methods

2.1. Reagents

Octan-2-ol (97 %), 1-hexanol (99 %), *cis*-3-hexenol (98 %), *trans*-3-hexenol (97 %), vanillin (99 %), linalool (97 %), terpinen-4-ol (≥95 %), α-terpineol (90 %), geraniol (98 %), linalool oxide (≥97 %), β-citronellol (95 %), p-cymene (99 %), terpinolene (≥85 %), limonene (97 %), 1,8-cineole (99 %), 1,4-cineole(≥98.5 %), β-damascenone (≥98 %), iso-amyl alcohol (98 %), benzyl alcohol (≥99 %), 2-phenylethanol (≥99 %), ethyl acetate (99 %), ethyl butanoate (99 %), ethyl 3-methyl butanoate (≥98 %), isoamyl acetate (≥95 %), ethyl hexanoate (≥95 %), phenyl-ethyl acetate (99 %), n-hexyl acetate (≥98 %), ethyl octanoate (≥98 %), ethyl decanoate (≥98 %), hexanoic acid (≥99 %), octanoic acid (≥98 %), α-phellandrene (95 %), p-menthane-1,8-diol (97 %), 3-methyl-butanoic acid (99 %), 1-butanol (≥99 %), vinyl guaiacol (≥98 %), methyl-vanillate (99 %), ethyl vanillate (99 %), benzaldehyde (≥99 %), methyl salicylate (≥99 %), α-terpinen (≥95 %), β-Myrcene (≥90 %), ethyl 2-hydroxybutanoate (99 %), ethyl 3-hydroxybutanoate (≥98 %), *cis*-2-hexenol (95 %), carbon disulfide (≥99 %), dimethyl sulfide (≥99 %), diethyl sulfide (98 %), dimethyl disulfide (≥98 %), diethyldisulfide (99 %), methionol (98 %) were supplied by Sigma Aldrich (Milan, Italy). 1,1,6-Trimethyl-1.2-dihydronaphtalene (TDN) with 80 % of purity was supplied by Synchem UG & Co (Felsberg, Germany). Dichloromethane (≥99.8 %) and methanol (≥99.8 %), were provided by Honeywell (Seelze, Germany). Sodium chloride (≥99.5 %) was supplied by Sigma Aldrich (Milan, Italy).

2.2. Wine samples

For this study, forty commercial Lambrusco sparkling red wines were employed. Wines were from three different appellations: "*Lambrusco di Sorbara*" (Sorbara) (18 wines), "Lambrusco *Salamino di Santa Croce"* (Salamino) (8 wines) and *"Lambrusco Grasparossa di Castelvetro"* (Grasparossa) (14 wines). Samples are listed in Table A.1 where are also reported the information about vintages, residual sugar, production method and ethanol percentage. Sorbara samples were chosen so that three different production methods: Charmat (6 wines), Classico (5 wines) and Sur lie (7 wines) were represented. In addition to this set, for comparative purposes, a set of commercial Prosecco (25), Durello (21), as well as samples of red wines, Corvina (7), Primitivo (11), Aglianico (10), Nebbiolo (11), Sangiovese (7), Soave (14), Lugana (20) and Pinot grigio (18) were also included in the study. The characteristics of these wines have been described elsewhere (Slaghenaufi et al., 2022; Luzzini et al., 2023; Slaghenaufi et al., 2021b)

2.3. Analysis of volatile sulfur compounds

Low molecular weight sulfur compounds, were analysed by SPME-GC-MS as described by Slaghenaufi et al. (2021a). In order to prevent compounds volatilization, wine samples were kept at 4°C for 24 h prior to analysis. Samples were prepared by adding 100 μL of DMS-d6 internal standard (2 mg/L in ethanol) to 10 mL of wine placed in a 20 mL glass vial together with 3 g NaCl. Samples were then kept at 4°C until SPME extraction. Prior to SPME extraction samples were equilibrated for 1 min at 40°C, then a polydimethylsiloxane-divinylbenzene fibre (PDMS/DVB) (Supelco, Bellafonte, PA, USA) was exposed to sample headspace for 30 min. VSCs were desorbed in the injector port at 270°C for 2 min in splitless mode. GC-MS analyses were performed as reported previously GC-MS analysis was carried out on an HP 7890 A (Agilent Technologies) gas chromatograph coupled to a 5977B quadrupole mass spectrometer, equipped with a Gerstel MPS3 auto sampler (Müllheim/Ruhr, Germany). Separation was performed using a DB-WAX UI capillary column (30 m × 0.25, 0.25 μm film thickness, Agilent Technologies) and helium (6.0 grade) as carrier gas at 1.2 mL/min of constant flow rate The GC oven was programmed as follows: started at 35 °C for 5 min, increased to 90 °C at 5 °C/min and then to 250 °C at 15 °C/min maintained for 2 min. Mass spectrometer was equipped with an electron impact ionization source (EI) (70 eV). The transfer line, the source and quadrupole temperature were set at 200, 250 °C and 150 °C. Mass spectra were acquired in SIM mode. Samples were analysed in random order. A calibration curve was prepared for each analyte using seven concentration points and three replicate solutions per point in white wines. An amount of 100 μL of DMS-d6 (2 mg/L in ethanol) was added to each calibration solution, which was then submitted to SPME extraction and GC-MS analysis as described for the samples. Calibration curves were obtained using Chemstation software (Agilent Technologies, Inc.) by linear regression, plotting the response ratio (analyte peak area divided by internal standard peak area) against concentration ratio (added analyte concentration divided by internal standard concentration). Linear retention indexes (LRI) are reported in Table A.3.

2.4. Analysis of terpenoids and norisoprenoids

Terpenes, norisoprenoids and methyl salicylate were analysed using SPME extraction coupled with GC-MS analysis as described by Slaghenaufi et al. (2022). Five millilitres of wine were placed into a 20 mL glass vial together with 5 mL of water, 3 g of NaCl and 5 μL of internal standard 2-octanol (4.2 mg/L in ethanol). Samples were equilibrated for

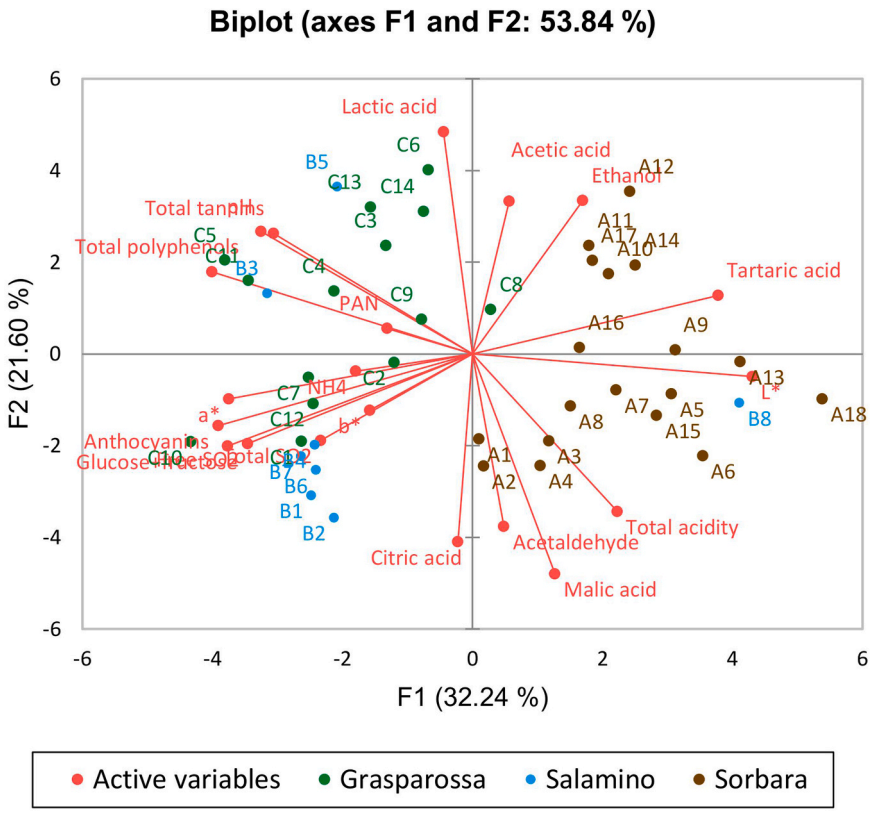

Fig. 1. PCA of all standard enological parameters, phenolic and color indices.

1 min at 40 °C, and then SPME extraction was performed by exposing for 60 min a 50/30 μm divinylbenzene–carboxen–polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco, Bellafonte, PA, USA) into sample headspace. Injection was done in splitless mode by desorbing SPME fibre into the injection port of an HP 7890 A (Agilent Technologies) gas chromatograph coupled to a 5977B quadrupole mass spectrometer, equipped with a Gerstel MPS3 auto sampler (Müllheim/Ruhr, Germany). Separation was performed using a DB-WAX UI capillary column (30 m × 0.25, 0.25 μm film thickness, Agilent Technologies). Helium (6.0 grade) was used as carrier gas in a constant flow rate of 1.2 mL/min. GC oven temperature was initially settled at 40 °C for 3 min, then raised to 230 °C at 4 °C/min and maintained for 20 min. Mass spectrometer was operated in electron ionization (EI) at 70 eV with ion source temperature at 250 °C and quadrupole temperature at 150 °C. Mass spectra were acquired in synchronous Scan (*m/z* 40–200) and SIM mode. Samples were analysed in random order. LRI are reported in Table A.3.

2.5. Analysis of major fermentative volatile compounds and volatile benzenoids

For quantification of alcohols, esters, fatty acids, and benzenoids were extracted using solid phase extraction (SPE) and then analysed by GC-MS following the procedure described by Slaghenaufi et al. (2020). Before extraction 50 mL of wine sample were diluted by adding 50 mL of deionized water, and added with 100 μL of internal standard 2-octanol (4.2 mg/L in ethanol). Samples were loaded onto a BOND ELUT-ENV, SPE cartridge (Agilent Technologies. Santa Clara, CA, USA) previously activated with 20 mL of dichloromethane, 20 mL of methanol and equilibrated with 20 mL of water. After loading, the SPE cartridge was washed with 15 mL of water. A volume of 10 mL of dichloromethane was used to elute volatile compounds. The organic phase was then concentrated under gentle nitrogen stream to 200 μL prior to GC injection. The analysis was carried out using an HP 7890 A (Agilent Technologies) gas chromatograph coupled to a 5977B quadrupole mass spectrometer, equipped with a Gerstel MPS3 auto sampler (Müllheim/Ruhr, Germany). Separation was performed using a DB-WAX UI capillary column (30 m × 0.25, 0.25 μm film thickness, Agilent Technologies). Two μL of sample extract was injected in splitless mode. The injector temperature was set at 250 °C. Helium was used as carrier gas in a constant flow rate of 1.2 mL/min. GC oven was programmed as follows: started at 40 °C for 3 min, raised to 230 °C at 4 °C/min and maintained for 20 min. Mass spectrometer was operated in electron ionization (EI) at 70 eV with ion source temperature at 250 °C and quadrupole temperature at 150 °C. Mass spectra were acquired in SIM mode. Samples were analysed in random order.

A calibration curve was prepared for each analyte using seven concentration points and three replicate solutions per point in model wine (12 % v/v ethanol, 3.5 g/L tartaric acid, pH 3.5) 100 μL of internal standard 2-octanol (4.2 mg/L in ethanol) was added to each calibration solution, which was then submitted to SPE extraction and GC-MS analysis as described for the samples. LRI are reported in Table A.3.

2.6. Standard enological analyses

Acetic acid, malic acid, lactic acid, citric acid, total acidity (expressed as g of tartaric acid), acetaldehyde, glucose + fructose, polyphenols, Primary amino nitrogen (PAN), ammonia, free and bound SO_2 were analysed using a Biosystems Y15 multiparametric analyser (Sinatech, Fermo, Italy). pH was evaluated with a Crison Basic 20 + pHmeter (Barcelona, Spain).

2.7. Polyphenols and colorimetric analysis

Folin-Ciocalteu reagent was used to quantify the total phenolics, according to the procedure described by Singleton and Rossi (1965) (Singleton et al., 1965). Total tannins were determined by methyl cellulose precipitation (Sarneckis et al., 2006). Total anthocyanins were determined using the bisulfite bleaching method.

Table 1
Enological parameter of studied Lambrusco.

	All Lambrusco				Grasparossa				Salamino				Sorbara			
	Min	Max	Mean	Sd*	Min	Max	Mean	Sd	Min	Max	Mean	Sd	Min	Max	Mean	Sd	S**
pH	2.69	3.85	3.25	0.23	3.0	3.9	3.4	0.2	2.7	3.6	3.3	0.3	2.7	3.4	3.1	0.2	**YES**
Total acidity (g/L)	7.9	18.2	11.3	2.1	7.9	11.1	10.1	0.9	8.7	17.7	11.1	2.6	8.9	18.2	11.7	2.2	**YES**
Glucose+Fructose (g/L)	0.0	23.4	7.1	5.9	2.1	13.8	7.7	3.3	0	23.4	12.1	7.1	0.1	10.3	2.8	3.5	NO
Total SO_2 (mg/L)	1.0	136.0	65.9	32.1	1	122	61.2	39.1	12	108	64.7	29	15	136	58.8	28.7	NO
Free SO_2 (mg/L)	0.0	24.7	6.5	6.0	0	24.7	7.8	7.3	1.2	20.6	9.2	6.2	0.4	7.9	3.1	2.1	NO
Molecular SO_2 (mg/L)	0	1.37	0.26	0.25	0.00	1.37	0.31	0.36	0.06	0.54	0.34	0.18	0.03	0.44	0.19	0.13	NO
Primary amino nitrogen (PAN) (mg/L)	13.6	257.0	49.2	46.8	18.2	257	63.8	73	15.9	62.2	31.3	15.8	13.6	74.3	38.5	19	NO
NH_4 (mg/L)	0.0	241.5	47.8	51.2	1.7	241.5	52.7	63.7	4.5	97.1	41.2	36.3	0	150	37.9	47.4	NO
Acetic acid (g/L)	0.00	0.74	0.18	0.14	0	0.48	0.15	0.13	0.05	0.28	0.14	0.1	0.02	0.74	0.2	0.17	NO
Lactic acid (g/L)	0.00	3.04	0.85	1.03	0	2.28	0.91	0.84	0.01	3.04	0.62	1.19	0	2.63	0.85	1.14	NO
Acetaldehyde (mg/L)	0.0	60.0	27.6	16.9	0	42.6	18.7	15.25	0	49.1	20.1	16.2	11.4	60	34.3	15.4	**YES**

** Significativity between different denomination according to Kruskal Wallis test (α=0.05)
* Sd is short for standard deviation.

CieLAB parameters were evaluated with a colorimeter Nomasense P100 (Vinventions, Thimister-Clermont, Belgium).

2.8. Statistical analyses

Principal Component Analysis (PCA), Kruskal-Wallis, Correlation analysis (CA) and Hierarchical cluster analysis (HCA) have been performed using XLSTAT 2023 (Addinsoft SARL, Paris, France).

PCA was performed with correlation matrix (Spearman) due to the wide concentration differences among volatile compounds. Kruskal-Wallis (α=0.05) was performed with Dunn multiple pairwise comparison. CA was performed with Spearman correlation (α=0.05). HCA was performed with Ward's agglomeration methods and automatic truncation (entropy).

3. Results and discussion

3.1. Standard enological parameters and phenolic and color indices

A PCA with all the standard enological parameters, phenolic and color indices was performed (Fig. 1). Globally 53.84 % of the total variance was explained with the first two components with PC1 accounting for 32.23 % and PC2 21.6 %. Lambrusco PDOs were well differentiated on the plot with Sorbara showing positive values on the PC1 mainly associated with total acidity and colorimetric parameter L* while Grasparossa and Salamino showing negative values on the PC1. Grasparossa and Salamino were differentiated thanks to the PC2 with Grasparossa showing positive values being associated with pH, total polyphenol, tannins and lactic acid and Salamino showing negative values with two samples scattering in the group of Grasparossa and one in the group of Sorbara. Salamino was mainly associated with anthocyanins, residual glucose fructose free and total SO_2 and the colorimetric parameters a* and b*.

Table 1 shows the values of main enological parameters, color and phenolic indices of all samples. Kruskal-Wallis (α=0.05) analysis revealed that among analysed parameters pH, total acidity, acetaldehyde, total polyphenols, total tannins, anthocyanins and CIELab parameters showed significant differences among the three PDOs.

In general, Lambrusco pH was found in a quite wide range between 2.69 and 3.85 with a mean value of 3.25. Usually, sparkling wines show pH values around or below 3.00 (Jones et al., 2014) while red wines show higher values up to 3.8 (Giacosa et al., 2021). Lambrusco cover the pH ranges of both sparkling and red wines. A wide range of total acidity (mean values between 7.9 and 18.2 g/L) was also found. Sorbara showed significantly lower pH values and higher acetaldehyde content compared to Grasparossa and Salamino, while Grasparossa showed significantly lower total acidity values compared to the other two PDOs. (Fig. 2).

As far as phenolic composition is concerned, in agreement with other studies (Salvatore et al., 2013; Silvestri et al., 2014) significant differences were observed between the three Lambursco PDOs, in our case with Sorbara showing significantly lower contents of all the three parameters than Salamino and Grasparossa, which instead showed similar contents (Fig. 3). In particular, the differences observed in tannin content could indicate differences in perceived astringency and dryness for Sorbara wines, due to their low tannin content (Pavez et al., 2022). It is also worth noting that the higher acetaldehyde content of Sorbara wines is consistent with the lower phenolic content of this PDO. Acetaldehyde is mostly produced by yeast during fermentation and then in part consumed through formation of adducts with tannins and/or anthocyanins [1]. Higher phenolic content will therefore result in lower acetaldehyde levels. A further comparison with total phenolics, tannins and anthocyanins of other Italian red wines (Corvina, Primitivo, Aglianico, Nebbiolo and Sangiovese), showed relatively low levels of phenolic compounds for Lambursco, with values, comparable to Corvina wines, although a large variability exist within the Lambrusco sample set, with

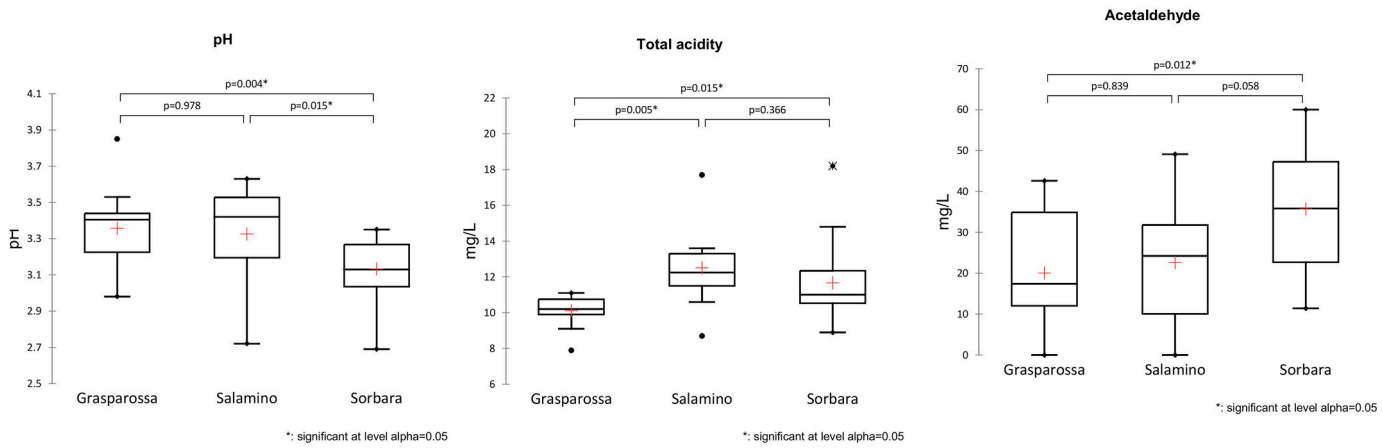

Fig. 2. Comparison of pH, total acidity and acetaldehyde between Lambrusco PDOs.

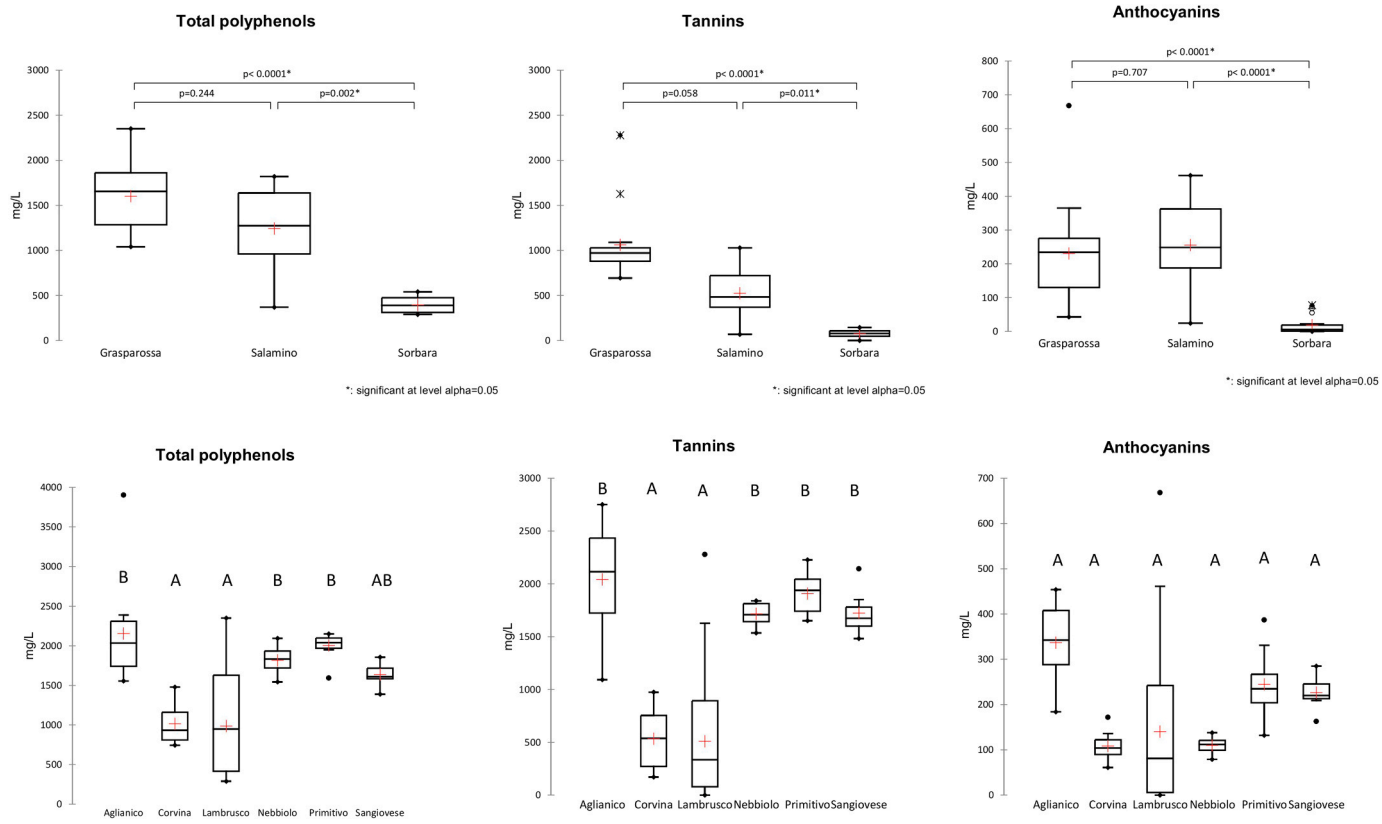

Fig. 3. Comparison of total phenolics, tannins and anthocyanins contents between Lambrusco and Corvina (7), Primitivo (7), Aglianico (10), Nebbiolo (11) and Sangiovese (7). Comparison of total phenolics, tannins and anthocyanins contents between Lambrusco Grasparossa, Salamino and Sorbara.

certain samples falling in the range of Sangiovese for total polyphenols.

CIELab values of different Lambrusco are reported in Fig. 4, attesting their chromatic characteristics. Lambrusco di Sorbara showed significant higher values for the parameter L*(clarity) and a*(red-green) while no differences were found for b*(blue-yellow) parameter. In Fig. 4 are plotted L* and a* values, where is possible to notice how Lambrusco Grasparossa and Salamino were overlapping while Sorbara was separated, with the only exception of a Salamino sample which falls within the Sorbara space. Since the colour parameters are closely correlated with anthocyanin content, possible correlations with the colorimetric parameters were explored. Correlation of anthocyanin with CIELab a* parameter, was significant and positive (ρ=0.59), while with the parameter L*, the correlation was significant and negative (ρ=-0.75). In general, both in terms of phenolic content and colorimetry, Sorbara was found to be very different from the other two PDOs.

3.2. *Volatile chemical profile*

A total of 61 volatile compounds have been identified and quantified in wine samples, including 6 alcohols, 4 C_6 alcohols, 12 esters, 3 acids, 11 benzenoids, 13 terpenes, 7 norisoprenoids, 5 low molecular weight volatile sulfur compounds (LMWSCs) (Table A.3).

3.2.1. *Main characteristics of Lambrusco volatile chemical profile*

A comparison of Lambrusco volatile profile with samples of sparkling white wines such as Prosecco, and Durello, as well as some red and white

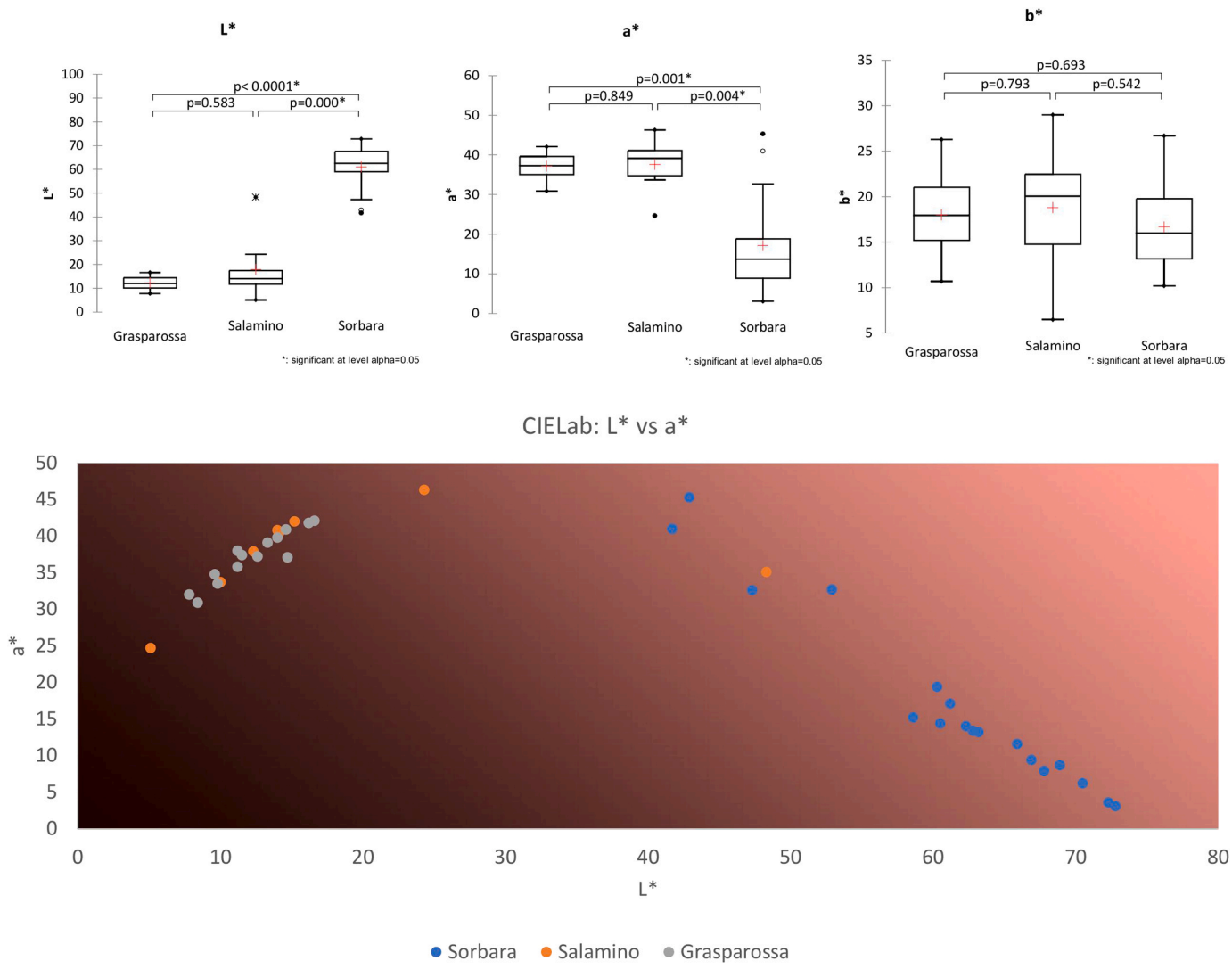

Fig. 4. L*, a* and b* parameters with significative difference according to Kruskal Wallis test (α=0.05) for the three different Lambrusco. Scatter plot of CIELab parameters L*vs a* for each wine considered. The background color is meant as an estimation of the wine color.

wines was carried out in order to identify key compositional features of Lambrusco. PCA analysis performed with significantly different compounds between varieties (Kruskall-Wallis) showed 32.66 % of the total variance was explained with the first two components (Fig. 5). The samples were differentiated according to wine types, with sparkling wines, still white wines, and still red winesdistinctly separated. The three sparkling wines (Lambrusco, Prosecco and Durello) showed negative values on the PC2, being differentiated from still white wines which showed positive values both on PC1 and PC2, and from red wines which showed negative values on the PC1 and positive on the PC2. Interestingly in the group of sparkling wines, red sparkling wines were closer to still red wine while white sparkling wines to still white wines. Sparkling wines were characterized by bicyclic terpenes, some acetate esters, cis-3-hexen-1-ol and β-myrcene. Still white wines were instead characterized by fermentative compounds, including ethyl and acetate esters, fatty acids and alcohols, and cyclic terpene. Finally, still red wines were mainly associated with benzenoids, terpenes and C_6 alcohols.

Occurrence of acetate esters in Lambrusco wines was quantitatively relevant (Fig. 6), especially isoamyl acetate and 2-phenethyl acetate. Isoamyl acetate showed an average content of about 2 mg/L, but in some cases, up to 9 mg/L. Similarly, 2-phenethyl acetate, showed a mean content of 200 μg/L, but certain samples reached 2 mg/L. Lambrusco showed content of acetate esters more similar to sparkling white wines (Prosecco, Soave, Lugana and Pinot grigio) than to red wines. Acetate esters are synthesized from higher alcohols and acetyl-CoA mediated by acetyltransferases (Lambrechts and Pretorius 2000; Verstrepen et al., 2003). The production of acetate esters is modulated by different fermentation variables, compositional and process such as fermentation temperature and pH, skin contact, second fermentation, grape maturity, sugar, grape varieties, as well as intrinsic lipid composition and nitrogen content and composition (Houtman et al., 1980; Saerens et al., 2008; Caliari et al., 2015). Fermentation of white wines is carried out without grape skin contact and at low temperature, which are known to promote the production of esters (Deed et al., 2017). Lambrusco is often produced using both pre-fermentation cold maceration which is also known to increase the ester content (Cai et al., 2014), and fermenting entirely or partially in contact with the grape skins, often at relatively low temperature compared to red wines. The combination of these technologies may have led to the high content of acetate esters as well as the observed high variability. Given the high residual nitrogen values of some samples, the possibility that the highest values of acetate esters (up to 9 mg/L of isoamyl acetate) were due to high PAN values in the grapes was also explored, but no association was observed (Table 1). Lambrusco was also found to be rich in trans-3-hexen-1-ol, a C6 alcohol, with concentration similar to Prosecco wines. C6 alcohols are

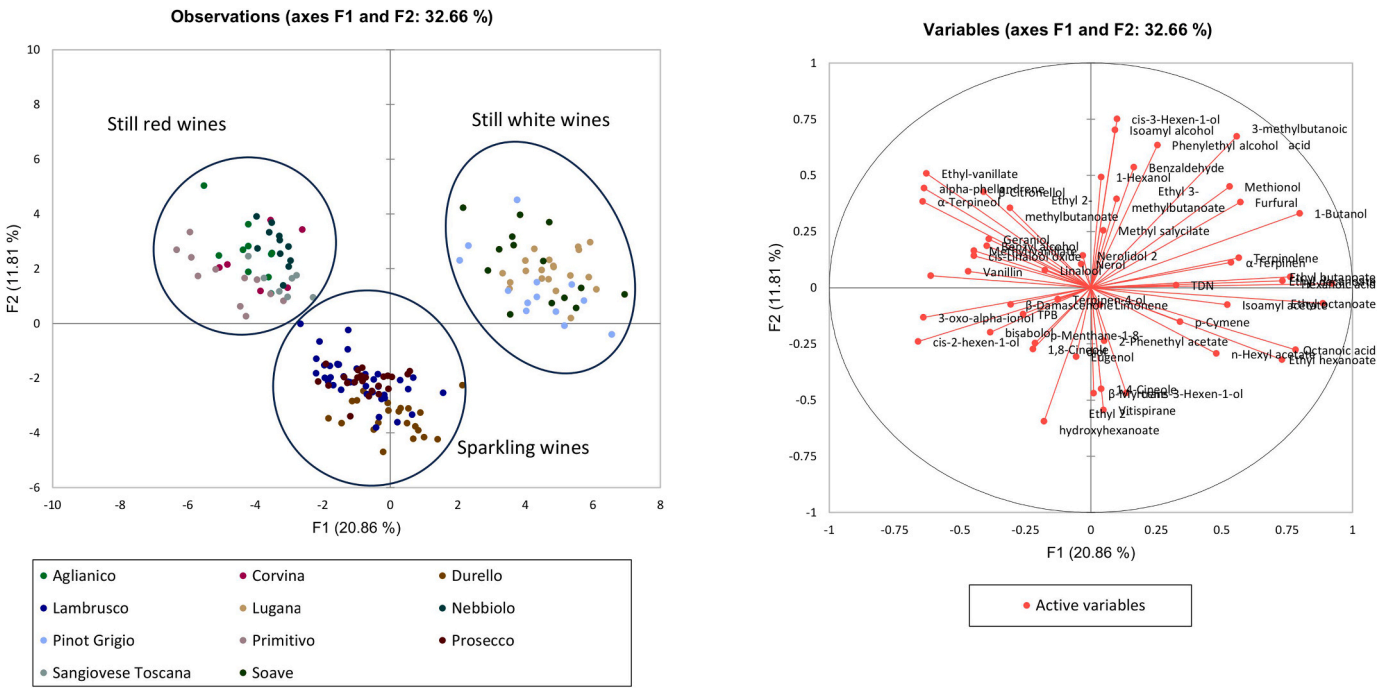

Fig. 5. PCA performed with volatile compounds of Aglianico (10), Corvina (7), Durello (21), Lambrusco (40), Nebbiolo (11), Primitivo (7), Prosecco (25), Sangiovese (7), Soave (14), Lugana (20) and Pinot grigio (18). Circles around the samples are not the result of statistical processing but are for better interpretation of the plot.

compounds with green and grassy notes, formed from enzymatic oxidation of fatty acids, α-linolenic and α-linoleic, during grape crushing in pre-fermentative stage (Benkwitz et al., 2012; Ugliano et al., 2009). C6 alcohols content is often linked to grape variety since their content depends on musts precursors levels but also on grape enzymatic activities (Xu et al., 2017).

Finally, Lambrusco showed significantly higher content of 1,8-cineole a bicyclic terpene with eucalyptus odor (Fariña et al., 2005). 1,8-Cineole is usually linked with wine aging and it is associated to monoterpenes alcohols through acid-catalysed cyclisation, in particular to linalool (Slaghenaufi and Ugliano, 2018).

In general, an aspect to consider is that despite the levels of these compounds being significantly higher in Lambrusco wines compared to other wine types, a wide variability has been observed for all volatile compounds (Fig. 6 and Table A.3). This could be due to the effect of Lambrusco varieties which are different event if genetically similar, different grape area of origin, different aging period and production methods.

3.2.2. *Volatile chemical composition of different Lambrusco PDOs*

PCA of the volatile compounds quantified in the different Lambrusco PDOs allowed to explore the existence of volatile chemical patterns specific of individual PDOs (Fig. 7). Total explained variance with the first two PCs was 37.23 % with the first component explaining 22.7 % and the second component 14.46 %. First component was associated with intra appellation variability mainly related to the production methods and vintages/aging period. Older vintages and Classico method were in fact associated with positive values, and therefore higher content of LMWSC, branched chain esters, non-megastigmane norisoprenoids and benzenoids. Young Charmat Lambrusco wines were associated with negative values on the PC1 and were richer in acetate and ethyl esters and terpenes. A good distinction of the three PDOs was showed on the PC2 with Grasparossa showing positive values, associated with acetate esters and cyclic terpenes Sorbara showing negative values associated with non-megastigmane norisoprenoids, vanillates and C_6 alcohol and Salamino showing intermediate values with some samples more similar to Sorbara wines. This observation highlights the difficulty in identifying specific PDO-driven volatile markers when variations in important technological parameters (e.g. Classico and Charmat method) are allowed within the appellation. We have recently reported similar observations for Durello sparkling wines (Luzzini et al., 2023).

In consideration of the large variability induced by production methods and vintages, subsets of samples were isolated from the initial sample set in order to address individually the influence of different variables.

With regard to the effect of the PDO, Lambrusco samples of the three PDO but of the same vintage (2021) and production method (Charmat) were compared (Kruskal-Wallis, α=0.05). PDOs mainly differ in grape variety, although genetically similar, and in geographical origin, although close to each other. In this section, the combined effect of these two variables underlying the different PDOs will be evaluated. Significantly different compounds between the PDOs are reported in Table 2, which included volatile sulfur compounds, benzenoids, C_6 alcohols, terpenes and norisoprenoids. This result highlighted that varietal compounds played a major role in discriminating between different Lambrusco types, while fermentation-derived compounds had a negligible impact, mostly limited to volatile sulfur compounds, some of which are also controlled by variety, as in the case of dimethyl sulfide (Segurel et al., 2005)

Terpenes are varietal compounds typical of aromatic varieties such as Muscat or Gewürztraminer. These metabolites are well known for varietal discrimination (Mateo and Jimenez, 2000) but also to be strongly influenced by grape area of origin (Luzzini et al., 2021; Wen et al., 2015). Interestingly, while the three appellations showed a rather low and very similar total terpene content (around 15 μg/L), individual terpenes showed significant differences between appellations, as shown on the PCA plot of terpene compounds alone (Fig. 8). Grasparossa showed positive values on the PC1, Salamino negative on the PC2 and Sorbara positive values on the PC2. Only one sample of Grasparossa was close to Salamino samples. Salamino and Sorbara showed higher content of most linear terpenes such as linalool and β-myrcene, with Salamino also showing higher content of β-citronellol, while Grasparossa showed higher content of cyclic terpenes α-terpineol, terpinen-4-ol, terpinolene, α-terpinen and p-cymene and the bicyclic terpene 1,4-cineole.

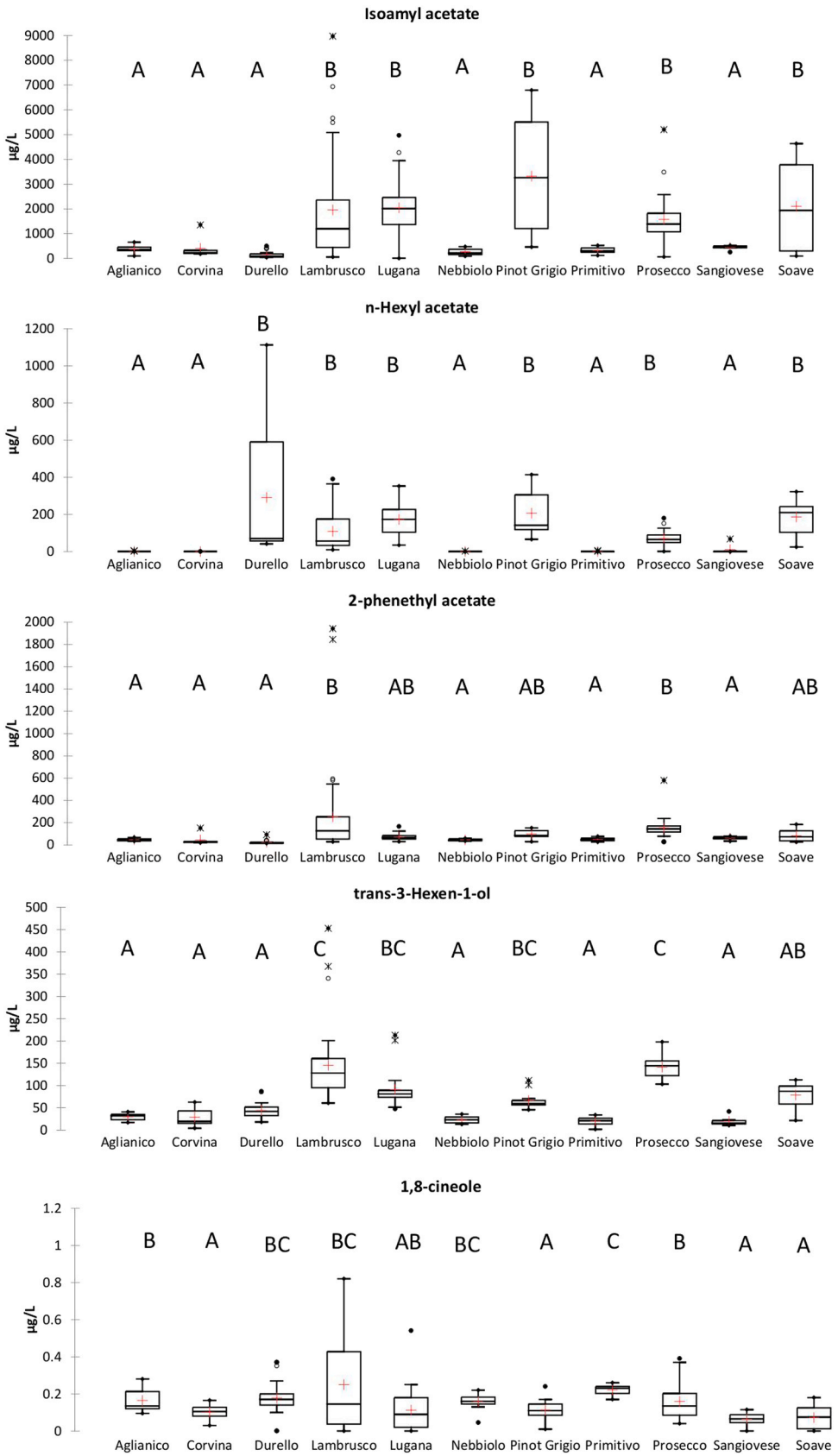

Fig. 6. Concentration of isoamyl acetate, 2-phenetyl acetate, n-hexyl acetate, trans-3-hexen-1-ol and 1,8-cineole in Lambrusco (40), Prosecco (25), Durello (21), Aglianico (10), Corvina (7), Nebbiolo (11), Primitivo (7), Sangiovese (7), Soave (14), Lugana (20) and Pinot grigio (18) wines. Letters refers to Kruskal-Wallis test (α=0.05).

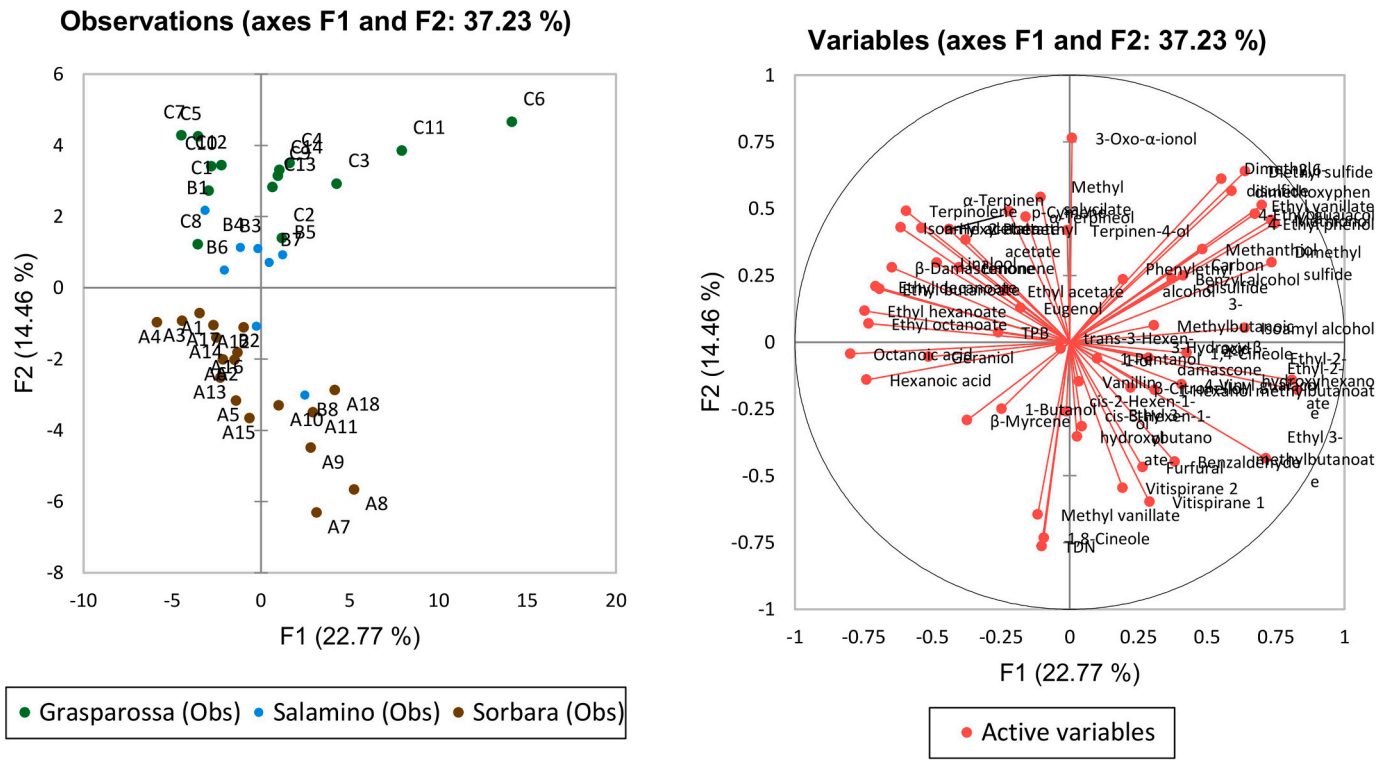

Fig. 7. PCA of Lambrusco wines performed with all volatile compounds. Samples are plotted as the mean value of the replicates.

Table 2
Significantly different compounds between Lambrusco appellations (Kruskal-Wallis, α=0.05).

		Grasparossa			Salamino			Sorbara		
	p-value	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean
LMWSC										
Carbon disulfide	**0.001**	3.62	6.46	5.08	3.53	6.67	4.23	2.86	3.43	3.05
Methanthiol	**0.016**	0.60	2.37	1.34	1.22	2.19	1.58	0.70	1.19	0.92
Dimethyl sulfide	**0.050**	1.19	6.57	2.98	1.64	4.15	2.48	0.89	3.09	1.51
Diethyl sulfide	**0.001**	0.28	0.99	0.48	0.24	0.50	0.31	0.12	0.21	0.16
Dimethyl disulfide	**0.001**	0.03	0.11	0.06	0.02	0.05	0.03	0.00	0.03	0.01
C_6 alcohols										
trans−3-Hexen−1-ol	**0.025**	61.6	131.8	92.8	92.0	452.6	211.0	69.1	367.2	166.9
cis−2-Hexen−1-ol	**0.001**	10.6	13.9	12.1	14.1	23.5	17.1	9.9	15.2	13.3
Benzenoids										
Methyl vanillate	**0.001**	4.31	7.36	6.29	6.15	11.6	8.99	10.6	20.6	16.1
Ethyl vanillate	**0.010**	4.43	47.1	18.9	3.51	8.99	5.49	2.84	5.99	4.96
Benzyl alcohol	**0.003**	78.2	308	124.9	29.0	134	65.7	22.1	75.4	37.1
4-Ethyl phenol	**0.023**	4.54	1053	200	4.47	26.0	9.56	3.84	4.96	4.35
Terpenes										
α-Terpinen	**0.001**	1.06	3.36	2.35	1.26	2.22	1.72	1.10	3.47	1.90
β-Myrcene	**0.001**	0.39	3.74	1.45	0.56	3.95	1.96	0.69	1.96	1.16
1,4-Cineole	**0.027**	0.18	0.56	0.36	0.18	0.26	0.22	0.21	0.28	0.25
β -Citronellol	**0.02**	0.39	3.74	1.25	0.56	3.95	1.96	0.60	1.96	1.05
1,8-Cineole	**0.002**	<LOQ	0.12	0.05	<LOQ	0.21	0.09	0.15	0.72	0.43
p-Cymene	**0.001**	0.21	0.71	0.36	0.04	0.10	0.07	0.14	0.27	0.20
Linalool	**0.017**	0.37	2.47	1.10	1.94	4.54	2.62	0.65	2.85	2.01
Terpinen−4-ol	**0.031**	0.48	1.97	1.19	0.37	0.78	0.60	0.42	1.31	0.83
Norisoprenoids										
TDN	**0.002**	0.36	3.15	1.75	0.57	2.30	1.43	3.91	9.74	6.02

Conversely Sorbara showed higher level of the bicyclic terpene 1, 8-cineole and limonene, two compound that are linked through acid catalysed reactions (Fariña et al., 2005). While linear and some cyclic terpenes characterize young wines from different varieties already, bicyclic terpenes are usually not present in grapes and young wines and are formed during wine aging thanks to acid catalysed cyclization reactions of linear terpenes (Slaghenaufi and Ugliano, 2018), especially linalool. Although the concentrations of 1,4- and 1,8-cineole did not reach odor thresholds in 2021 wines (0.63 μg/L and 1.3 μg/L), their concentrations were not far apart. Due to the synergistic effect that these two terpenoids have in solution, their aromatic contribution should not be excluded. Noteworty, the pattern of bicyclic terpenes differed in the Lambrusco types, with Sorbara showing higher 1,8-cineole and Grasparossa higher 1,4-cineole. In this case Sorbara this might also reflect lower pH compared (Fig. 2), as an influence of pH on the yield of 1, 8-cineole but not on 1,4-cineole was previously reported (Luzzini

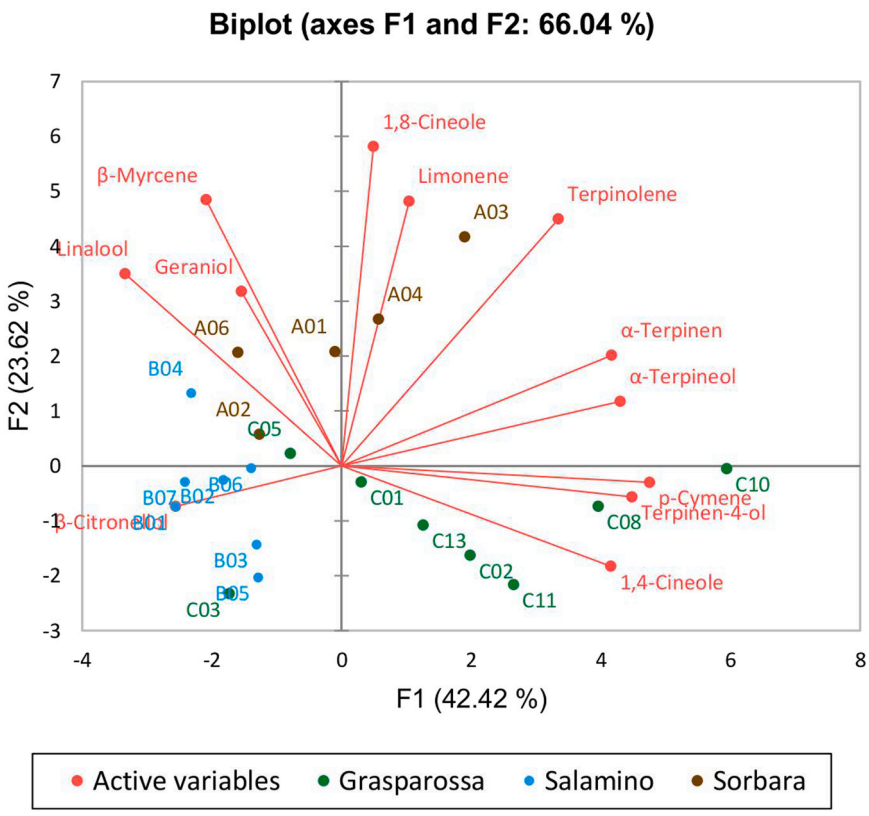

Fig. 8. PCA of Lambrusco appellations performed with terpenes only.

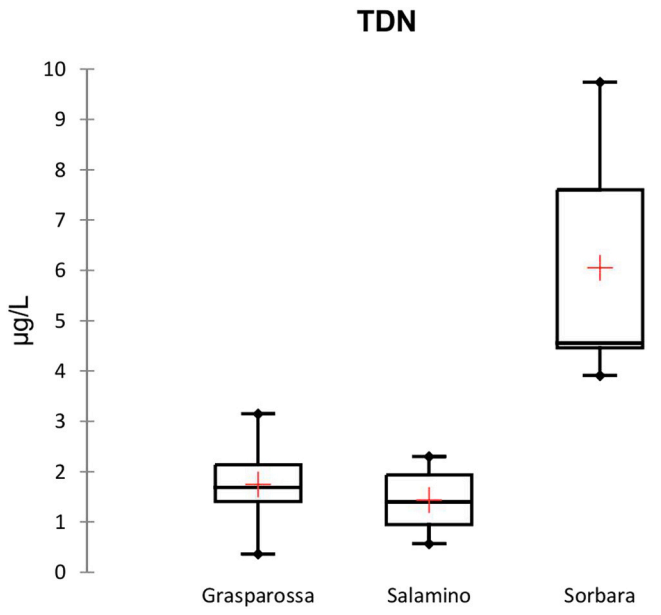

Fig. 9. TDN concentration in Lambrusco wines of different PDOs.

et al., 2022).

Sorbara was also richer in 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) a non-megastigmane norisoprenoid, breakdown products of carotenoids, well known to be strongly affected by aging treatment, despite in this case all the wines were in young version of the same vintage (Fig. 9). TDN is known to be influenced by grape variety, and in particular to be an aroma marker of Riesling wines, where it contributes to the typical kerosene notes (Sacks et al., 2012; Simpson, 1979; Rapp et al., 1986; Winterhalter et al., 2001), but it is also influenced by grape area of origin (Sabon et al., 2002; Slaghenaufi et al., 2021b).

Concerning volatile sulfur compounds, Sorbara showed significantly lower content of all the compounds compared to Grasparossa and Salamino which in turn showed similar levels. DMS apart, volatile sulfur compounds are usually considered off flavour because of the unpleasant onion and rotten egg smells (Mestres et al.), but in some cases at low concentrations they can contribute positively (Segurel et al., 2005). DMS at concentrations in the range of 25 µg/L contributes to the fruity attributes of the wine (Escudero et al., 2007). The occurrence of DMS is linked to the degradation over time of s-methyl methionine. (Samaniego Solis et al., 2024; Segurel et al., 2005). In this case, despite being young wines, no correlation has been found between the YAN content and DMS.

C6 alcohols are compounds with green and grassy notes, formed from enzymatic oxidation of fatty acids, α-linolenic and α-linoleic, during grape crushing in pre-fermentative stage (Waterhouse et al., 2016) and can be considered unaffected by the metabolic activity of yeasts (Herraiz et al., 1990). C6 alcohols are well known to be strongly affected by grape varieties and grape area of origin. In this case Salamino showed higher content of *cis*-2-hexenol and *trans*-3-hexenol. The latter also differentiated Sorbara and Grasparossa.

With regard to benzenoids, compounds, with spicy, dried fig, tobacco, and chocolate aromas (Francis et al., 1998; Francis et al., 1998.). Many of them are associated with extraction during barrel aging, but are also naturally present in the grapes and are influenced by grape varieties and area of origin (Slaghenaufi et al., 2019). Sorbara was richer in methyl vanillate, while Grasparossa in ethyl vanillate. Benzyl alcohol showed different level in all the wine types, with Grasparossa showing higher levels, Salamino intermediate and Sorbara lower. Additionally, in some samples of Grasparossa a high content of the spoilage compound 4-ethyl phenol (4-EP) was observed, potentially contributing to leather and barnyard attributes (Robinson et al., 2014) (Robinson et al., 2014). 4-EP is produced by *Brettanomyces* sp. and *Dekkera* by reduction of vinyl

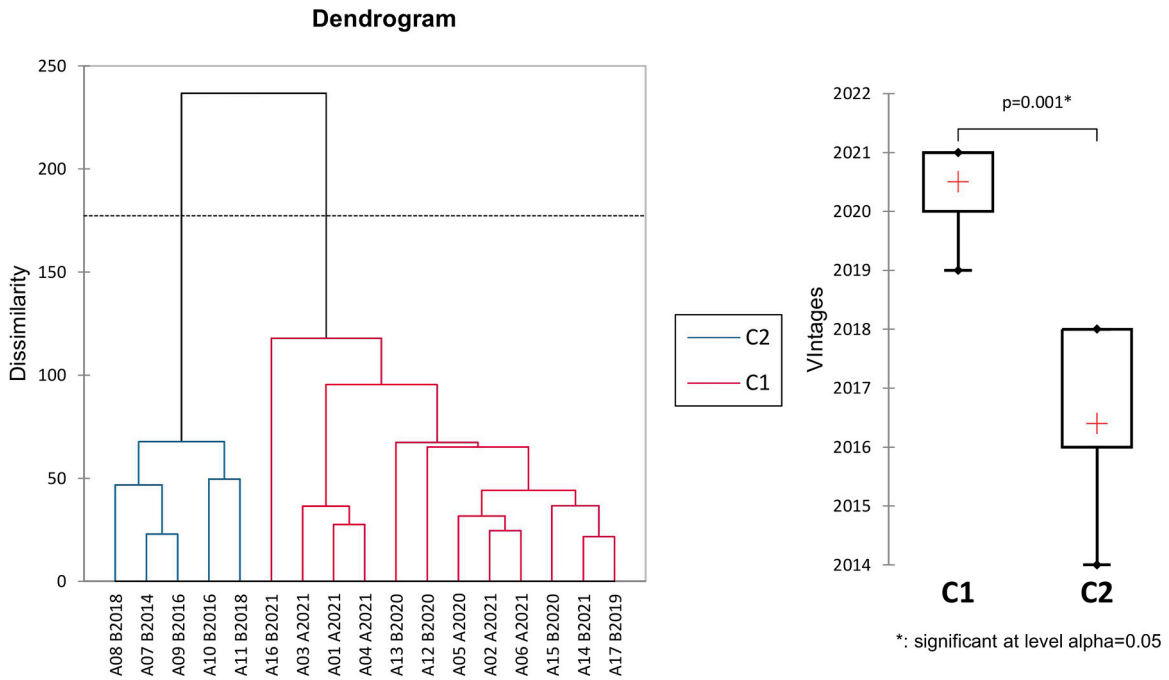

Fig. 10. HCA analysis of volatile compounds of Sorbara wines. Codes of samples are referred to sample id, production techniques (A= Charmat, B=Classico method) and vintages (2014–2021).

Table 3
Different compounds between the clusters.

Compounds	p-Value	C1	C2
Ethyl butanoate	0.045	266.0	178.7
Ethyl hexanoate	0.003	688.2	349.1
Ethyl octanoate	0.002	480.8	146.3
Ethyl decanoate	0.002	75.33	5.99
Ethyl 2-hydroxyhexanoate	0.004	0.92	1.90
Ethyl 2-methylbutanoate	0.002	8.20	32.81
Ethyl 3-methylbutanoate	0.003	13.66	47.69
Isoamyl acetate	0.002	1904	115.8
2-Phenethyl acetate	0.011	158.92	35.80
Hexanoic acid	0.006	5041	3023
Octanoic acid	0.002	8473	5259
Furfural	0.002	36.28	255
2,6-Dimethoxy phenol	0.008	4.42	5.72
1,4-Cineole	0.018	0.28	0.44
p-Cymene	0.005	0.18	0.09
Terpinolene	0.004	0.13	0.04
Linalool	0.002	1.92	0.21
Terpinen−4-ol	0.015	0.92	0.50
α-Terpineol	0.020	2.25	1.30
Nerolidol 2	0.006	0.39	0.00
β-Damascenone	0.002	2.23	0.98
Vitispirane 1	0.027	4.25	6.15

phenols to ethyl phenols (Robinson et al., 2014) (Robinson et al., 2014). Usually the occurrence of this compound is associated with contamination of winery environments or equipment and insufficient molecular SO_2 levels (Chatonnet et al., 1992). In this case almost all the wines showed low content of molecular SO_2, below 0.8 mg/L.

3.2.3. *Influence of production methods and aging*

A subset of samples of Sorbara alone was used to investigate the influence of production method and aging. This sample set included 6 Charmat samples from 2020 and 2021 vintages (A1-A6) and 12 Classico samples from vintages ranging from 2014 to 2021 (A7-A18) (Table A.1). To have an insight on the chemical patterns and to highlight similarities among the samples, HCA analysis of the Sorbara data set was performed with all volatile compounds. Two main clusters can be identified, corresponding to two different groups, C1 and C2 (Fig. 10).

C1 was a mixed group consisting of 12 samples, 6 Charmat and 6 Classico, while C2 was formed only by 5 Classico methods. Interestingly the Classico methods in C2 are all from older vintages (2014–2018) while the Classico methods in C1 are from younger vintages (2019–2021). More in general, as reported in Fig. 10, the two clusters differed for production vintages. Variability within the Sorbara wines was therefore primarily associated with the aging period rather than the production method. Kruskal Wallis test (α=0.05) was employed to identify significant different compounds between the clusters (Table 3).

Contextually the compounds that differentiated the two clusters were associated with aging treatment. C1, which includes significantly younger wines was richer in ethyl and acetate esters, while C2 was richer in branched chain ethyl esters and fatty acids in accordance with the literature (Antalick et al., 2014; Ramey et al., 1980). C1 was also richer in terpenes, with the only exception of 1,4-cineole, a bicyclic terpene with hay odor linked to aging treatment through acid catalysed reactions (Slaghenaufi and Ugliano, 2018; Luzzini et al., 2022). Concerning norisoprenoids, C2 was found richer in vitispirane a non-megastigmane compound which have been reported as marker of aged sparkling wines (Francioli et al., 2003).,while C1 in β-damascenone a megastigmane norisoprenoids. C1 was richer in furfural a product of sugar enolization and dehydration through acid-catalysed reactions related to long aging time (Waterhouse et al., 2016). These results suggest that the variations associated with different production methods are small when not supported by a long period of aging in contact with lees. Yeast autolysis phenomena are in fact long processes, and in this case the young classico method, was clustered together with the Charmat method from same vintages.

4. Conclusions

In the present study sensory-relevant metabolites underlying the chemical signature of different Lambrusco PDO wines were identified, demonstrating the existence of chemical markers attesting the uniqueness of PDO wines. However, our data also indicate that, when appellations contemplate categories related to specific vinification techniques, their influence on wine composition is not systematic, which

could generate some confusion at the level of consumers. In the case of the Lambrusco PDOs studied, the type of secondary fermentation (Classico or Charmat method) is central to the expression of specific chemical signatures and should be better addressed.

The identification of markers of individual PDOs confirmed the existence of metabolic diversity among them and thus of the existence of chemical bases that support the establishment of three distinct appellations. Lambrusco PDOs distinctions were mostly linked to varietal compound such as norisoprenoids, benzenoids, C_6 alcohols, low molecular weight sulfur compounds and above all terpenes. Grasparossa was indeed characterized by cyclic terpenes and 1,4-cineole and Salamino by C_6 compounds and linear terpenes, especially β-citronellol. Conversely, fermentation-derived compounds played a minor role in distinguishing Lambrusco PDOs, although esters can be considered key markers of the entire Lambrusco category in comparison to other sparkling and red wines. In terms of phenolic compounds, Sorbara showed significantly lower content of total polyphenols, tannins and anthocyanins compared to Salamino and Grasparossa.

It is worth mentioning that, as the present study was focused on commercial wines, limited availability on metadata related to the production process, particularly concerning the second fermentation were available. This constraint restricted the analysis to the only available technological data: PDOs, production methodology and aging period.

The data concerning the influence of secondary fermentation type suggest that, in the context of Lambrusco PDOs, additional care should be devoted to the category of Classico method samples, where a minimum period of bottle aging could be needed to support the expression of characters typical of this type of vinification.

Ethics approval and consent to participate

Not applicable.

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CRediT authorship contribution statement

Luzzini Giovanni: Writing – original draft, Formal analysis, Data curation. **Bicego Riccardo:** Formal analysis. **Slaghenaufi Davide:** Writing – original draft, Methodology. **Ugliano Maurizio:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Nothing to declare If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2025.107300.

Data availability

No data was used for the research described in the article.

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