





Available technologies on improving the stability of polyphenols in food processing

Hui Cao¹ | Ozgur Saroglu² | Ayse Karadag² | Zorița Diaconeasa³ | Gianni Zoccatelli⁴ | Carlos Adam Conte-Junior⁵ | Gustavo A. Gonzalez-Aguilar⁶ | Juanying Ou⁷ | Weibin Bai⁷  | Cristina Mara Zamarioli⁸ | Luis Alexandre Pedro de Freitas⁸ | Avi Shpigelman⁹ | Pedro H. Campelo¹⁰ | Esra Capanoglu¹¹  | Ching Lik Hii¹² | Seid Mahdi Jafari¹³ | Yaping Qi¹⁴ | Pan Liao¹⁵ | Mingfu Wang¹⁶ | Liang Zou¹⁷ | Paula Bourke^{18,19} | Jesus Simal-Gandara²⁰  | Jianbo Xiao²⁰ 

¹ College of Food Science and Technology, Guangdong Ocean University, Zhanjiang, Guangdong, China

² Food Engineering Department, Yildiz Technical University, Istanbul, Turkey

³ Faculty of Food Science and Technology, University of Agricultural Science and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania

⁴ Department of Biotechnology, University of Verona, Verona, Italy

⁵ Laboratory of Advanced Analyses in Biochemistry and Molecular Biology (LAABBM), Department of Biochemistry, Institute of Chemistry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

⁶ Coordinación de Tecnología de Alimentos de Origen Vegetal, Centro de Investigación en Alimentación y Desarrollo A. C., Hermosillo, Mexico

⁷ Institute of Food Safety and Nutrition, Jinan University, Guangzhou, China

⁸ Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Núcleo de Pesquisa em Produtos Naturais e Sintéticos – Universidade de São Paulo, Ribeirão Preto, Brazil

⁹ Faculty of Biotechnology and Food Engineering and Russell Berrie Nanotechnology Institute, Technion – Israel Institute of Technology, Haifa, Israel

¹⁰ School of Agrarian Science, Federal University of Amazonas, Manaus, Brazil

¹¹ Department of Food Engineering, Faculty of Chemical and Metallurgical Engineering, İstanbul Technical University, İstanbul, Turkey

¹² Faculty of Science and Engineering, University of Nottingham Malaysia, Semenyih, Malaysia

¹³ Faculty of Food Science and Technology, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran

¹⁴ Purdue Quantum Science and Engineering Institute, Purdue University, West Lafayette, Indiana, USA

¹⁵ Department of Biochemistry, Purdue University, West Lafayette, Indiana, USA

¹⁶ School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong

¹⁷ Key Laboratory of Coarse Cereal Processing, Ministry of Agriculture and Rural Affairs, Chengdu University, Chengdu, China

¹⁸ Plasma Research Group, School of Biosystems and Food Engineering, University College Dublin, Dublin, Ireland

¹⁹ School of Biological Sciences, Institute for Global Food Security, Queens University Belfast, Belfast, UK

²⁰ Nutrition and Bromatology Group, Department of Analytical Chemistry and Food Science, Faculty of Food Science and Technology, University of Vigo – Ourense Campus, Ourense, Spain

Correspondence

Jesus Simal-Gandara and Jianbo Xiao, Nutrition and Bromatology Group, Department of Analytical Chemistry and Food Science, Faculty of Food Science and Technology, University of Vigo – Ourense Campus, E-32004 Ourense, Spain.

Email: jsimal@uvigo.es and jianbo-xiao@yahoo.com

Abstract

Polyphenols are the most important phytochemicals in our diets and have received great attention due to their broad benefits for human health by suppressing oxidative stress and playing a protective role in preventing different pathologies such as

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cardiovascular disease, cancer, diabetes, and obesity. The stability of polyphenols depends on their environments of processing and storage, such as pH and temperature. A wide range of technologies has been developed to stabilize polyphenols during processing. This review will provide an overview of the stability of polyphenols in relation to their structure, the factors impacting the stability of polyphenols, the new products deriving from unstable polyphenols, and the effect of a series of technologies for the stabilization of polyphenols, such as chemical modification, nanotechnology, lyophilization, encapsulation, cold plasma treatment, polyphenol–protein interaction, and emulsion as a means of improving stability. Finally, the effects of cooking and storage on the stability of polyphenols were discussed.

KEYWORDS

drying, emulsion, encapsulation, nanotechnology, polyphenol–protein interaction, polyphenols, stability

1 | INTRODUCTION

Polyphenols are plant secondary metabolites characterized by hydroxylated phenyl moieties, which have received great attention due to their broad bioactivity, acknowledged to promote human health by contrasting oxidative stress, and thus playing a protective role in preventing different pathologies such as cardiovascular disease (Mursu et al., 2008), cancer (Khan et al., 2020), diabetes (Cao et al., 2019; Sun et al., 2020), and obesity (Wang et al., 2014). Polyphenols can be categorized into different groups according to their structure (Figure 1). The group of phenolic acids is constituted by molecules bearing a single phenyl ring and includes essentially derivatives of benzoic acids and cinnamic acids. Flavonoids are based on two aromatic rings linked by an oxygenated heterocyclic ring and are divided into several subgroups, that is, flavonols, flavones, flavanones, isoflavones, flavanols, and anthocyanidins. Further phenolic compounds groups are represented by stilbenoids, lignans, ellagic acids, and polymers of phenolic acids and flavonoids, namely, hydrolysable and condensed tannins.

The benefits toward many pathologic conditions arise in part through the antioxidant properties of polyphenols, being these molecules capable of donating hydrogen atoms and electrons. However, there is consensus that the interaction with target proteins might be the basis of different effects observed *in vitro* and *in vivo*. This mechanism would induce the specific inhibition of key enzymes, the modulation of cell receptors or transcription factors, which can regulate cell functions related to different physiopathologic conditions (Quideau, Deffieux, Douat-Casassus, & Pouységu, 2011; Zhu, Phillipson, Greengrass, Bowery, & Ya, 1997). Despite thousands of studies have been addressed to the characterization of polyphenols and their biological activity, some challenges remain open.

The bioavailability of most polyphenols is very scarce, posing concerns on their real efficacy in promoting health benefits. This could depend on the intrinsic fragility of the molecules, such as anthocyanins (Braga, Murador, de Souza Mesquita, & de Rosso, 2018), but also on their dimension, charge, possible matrix effect, presence of spe-

cific transporters, and so on (Ozdal et al., 2016). Polyphenols undergo extensive metabolism by natural detoxification systems that might lead to the onset of derivatives with different, and in some cases unexplored, biological activity (Liu & Hu, 2007). As a consequence, most polyphenols can reach the colon and interact with microbiota and become deeply modified (Catalkaya et al., 2020). Finally, and most importantly for food industries, the low stability to denaturing conditions catalyzed by heat, pH, light, oxygen, enzymes, and so forth limits the exploitation of polyphenols as functional ingredients, natural antioxidants, and supplements. Depending on the combination of these factors and their intensity, the amount of intact polyphenols that remains in the food that can actually reach the intestine—where their absorption is considered to principally occur—varies greatly, and this then can influence the extent of their bioavailability and further metabolism.

Thermal processes represent the most common way to render foods edible, microbiologically safe, improve their digestibility, and introduce or modulate textures, colors, and flavors. However, it is largely acknowledged that these processes are harmful for polyphenols, which due to their lability tend to easily degrade, although the structural changes of polyphenols during thermal processing are often ignored (Zhao et al., 2019). Nonthermal technologies are regarded with special interest as an alternative to conventional thermal methods, because they can preserve original nutritional and sensory characteristics of fresh foods, but they are sometimes limited by technological or economic reasons, for example, the need for capital and infrastructural investments (Khan et al., 2018).

The enrichment of foods with “protected” polyphenols can represent a more versatile and cost-effective approach. The encapsulation of polyphenols using different shell materials from among polysaccharides, proteins, and lipids can improve their stability to oxidation and thermal degradation, but also introduce new features such as the controlled-release, for example, depending on the environment conditions such as those typical of the gut lumen. A wide range of technologies has been developed to encapsulate polyphenols in the

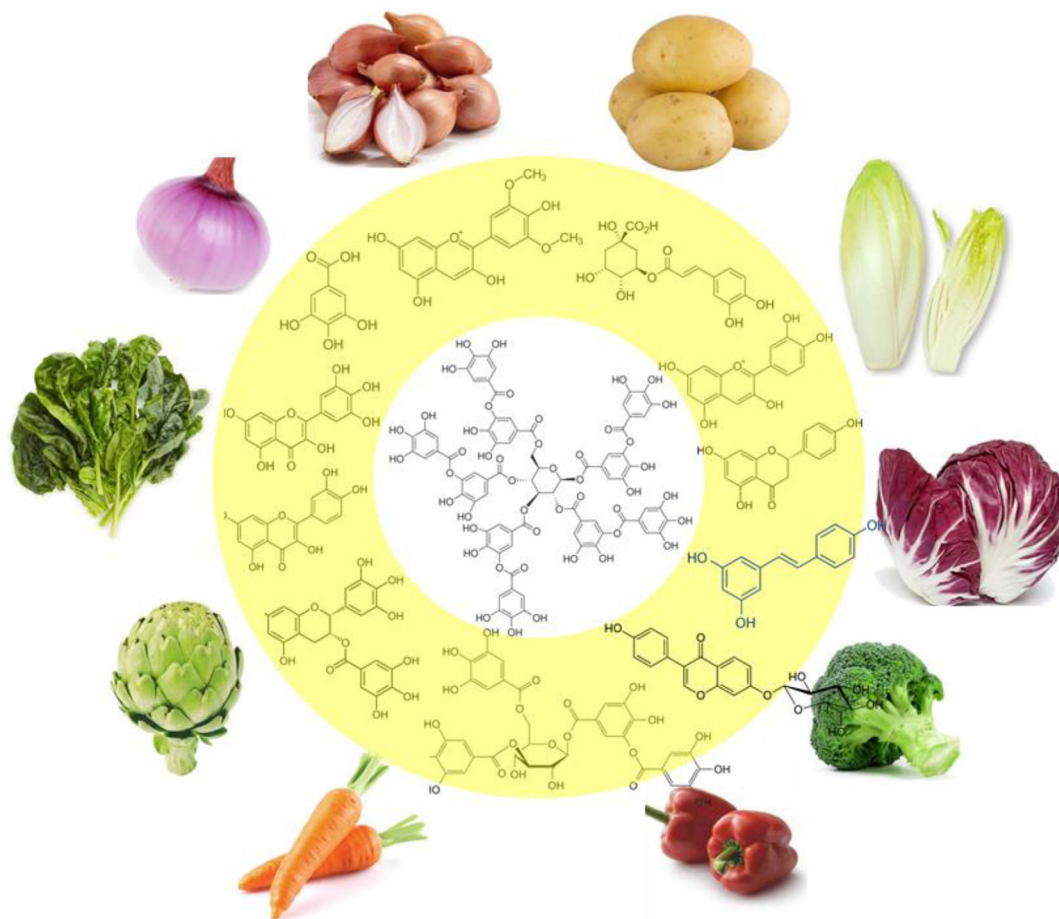


FIGURE 1 Dietary polyphenols in food

micrometer range, such as spray drying, lyophilization, coacervation, fluid bed granulation, and extrusion. Spray drying is probably the most used process due to its relatively simple regulation, limited costs, and possibility to work in continuous mode (Fang & Bhandari, 2010). Other approaches allow reaching particle dimensions in the nano range, for instance by nano emulsification, liposomes, solid lipid nanoparticles, and so on (Fathi, Mozafari, & Mohebbi, 2012, 2014). Besides showing that by these techniques it is possible to increase the stability of the encapsulated polyphenols, it is important to demonstrate also that these molecules are also bioaccessible, meaning that they are liberated during the digestion process and available to be absorbed.

Alternatively, it is possible to formulate foods and ingredients in a way that might protect phenolic compounds via the interaction with specific molecules (e.g., proteins) or with lipids (Liu, Ma, McClements, & Gao, 2016; Lu, Kelly, & Miao, 2016). In these conditions, the binding of polyphenols to these molecules might lead to a series of both positive and negative consequences, at different levels. The association between polyphenols and proteins could mask the antioxidant activity of polyphenols, as described by Arts et al. (2002) for milk proteins and flavonoids, but could also protect polyphenols from oxidation during their passage through the gastrointestinal tract and deliver them to the colon in an intact state (Jakobek, 2015). The binding of polyphenols to

lipids might reduce the absorption of the latter with potential positive health effect (Uchiyama, Taniguchi, Saka, Yoshida, & Yajima, 2011). It is clear that the effects are difficult to predict and depend on the nature of the molecules involved (Jakobek, 2015).

This review will provide an overview of the stability of polyphenols (Figure 2) in relation to their structure, the characterization of new products deriving from unstable polyphenols, and the effect of a series of technologies for the stabilization of polyphenols, such as chemical modification, lyophilization, micro- and nanoencapsulation, and emulsion as a means of improving stability. Finally, the effect of innovative nonthermal processes on polyphenols will be reviewed.

2 | METHODS TO CHARACTERIZE THE STABILITY OF POLYPHENOLS

The stability of polyphenols depends on their environments of processing and storage, such as pH and temperature (Deng, Yang, Capanoglu, Cao, & Xiao, 2018). Therefore, various experimental systems with specific reaction conditions have been established for stability evaluation. In these systems, polyphenols or their extracts are normally incubated or processed under designated conditions, with their stability

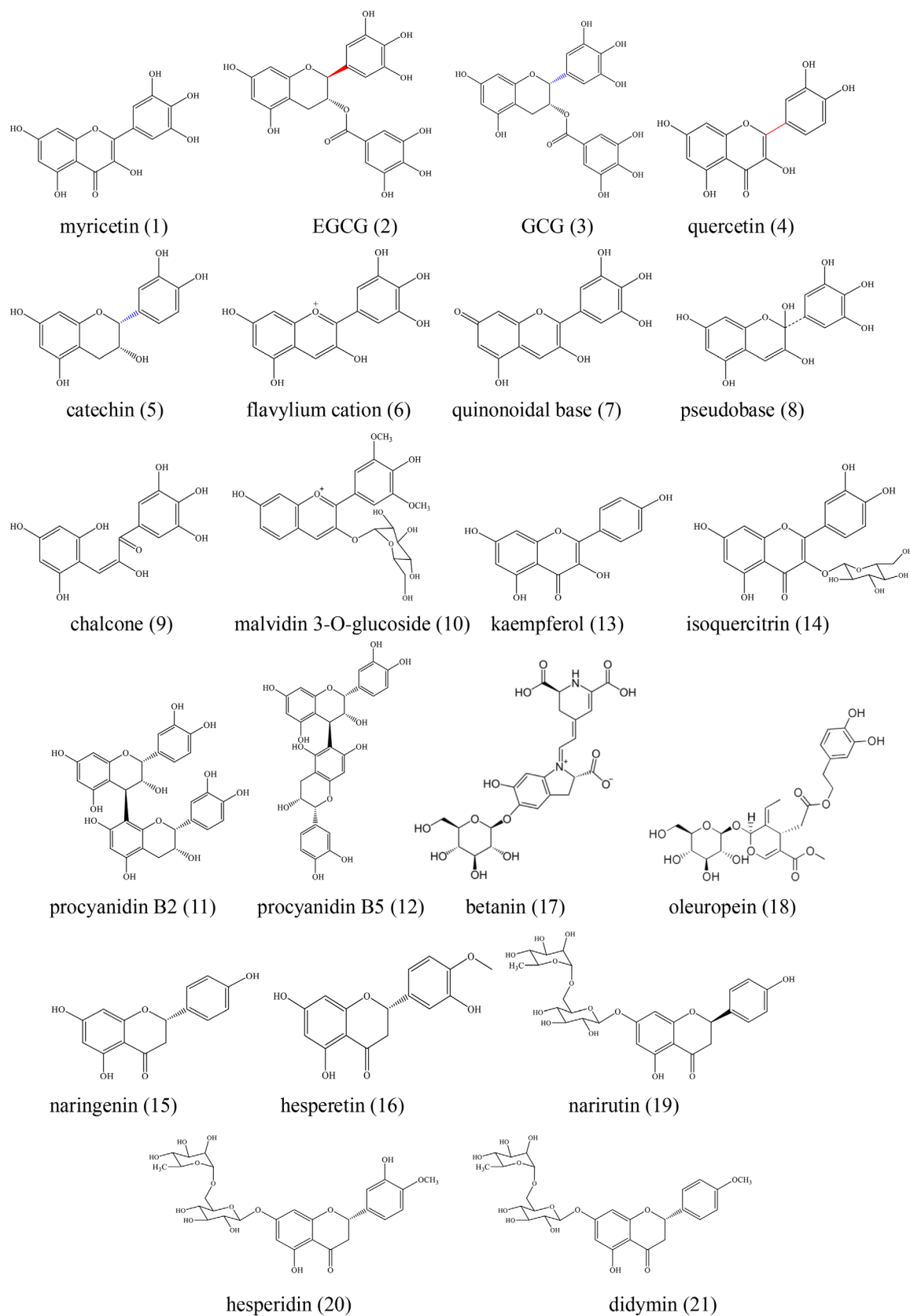


FIGURE 2 The chemical structures of polyphenols involved in current review

subsequently assessed by comparing the change in concentration. Aqueous solution, buffer solution, or juice systems are mostly employed, which are widely applied in models related to humidity, heating, pH, oxygen, light, metal ions, enzymes, high pressure, and in vitro digestive process (Jiang, Lyles, Reynertson, Kronenberg, & Kennelly, 2008; Oliveira & Pintado, 2015; Sang, Lee, Hou, Ho, & Yang, 2005; Sun, Mu, & Xi, 2017; Volf, Ignat, Neamtu, & Popa, 2014; Yu et al., 2014). These models are simple for operation and cost-effective, whereas the evidence they present is fragmentary. Real food processing systems are more complicated, comprising multiple constituents and interactions, in addition to the real digestion environment, which involves further biological activities including intestinal absorption, transportation, and metabolism (Xiao & Högger, 2015; Ou, Teng, El-Nezami, & Wang, 2018). Increasingly, supplementary methods, such as incorporation of polyphenols or their extracts into bakery/meat products, or conducting incubation in cell culture conditions, have been introduced for stability study of polyphenols (Ribas-Agustí et al., 2014; Xiao & Högger, 2015; Ou et al., 2018).

The stability of polyphenols is determined by quantifying the change of content before and after incubation. High-performance liquid chromatography analysis is the most common technique for polyphenol quantification. With the aid of mass spectrometry and nuclear magnetic resonance (NMR) analysis, it can also identify the structure of degradation products (Jiang et al., 2011; Terpinč, Cigić, Polak, Hribar, & Požrl, 2016). Spectrometry is another important technique applied in the stability analysis. By superimposing different absorption spectra measured with ultraviolet (UV) spectrometer, the instability of an individual polyphenol under specific conditions can be directly displayed on the changing spectrum (Friedman & Jürgens, 2000). For the extracts of polyphenols, spectrophotometric assays are usually employed to analyze the differences in total phenolic content (TPC), total flavonoid content (TFC), and total monomeric anthocyanin content. Standardized protocols have been well developed for the content determination and the results are usually expressed in equivalent amount of the standard compounds (Tomas et al., 2015). In addition to the concentration difference, changes of antioxidant activity are also considered to be related to the stability of polyphenols. For this reason, the antioxidant capacity of some polyphenols is measured as well for the stability analysis (Ahmad-Qasem et al., 2016; Yu et al., 2014).

There are several ways to describe the stability of polyphenols, including content change, degradation time, and reaction kinetics. The first one is a common way used, which is always written as a degradation rate to the initial concentration (Volf et al., 2014; Zeng, Ma, Li, & Luo, 2017). Degradation time indicates the required time when the concentration of polyphenol decreases by a certain percentage. It is derived from a time–concentration curve fitted with linear, polynomial, or exponential data regression. For example, early degradation time ($C_{T_{10}}$) and half degradation time ($C_{T_{50}}$, or half-life time) are defined as the time when the concentration decreased to 10% or 50% of the initial concentration (Sang et al., 2005; Xiao & Högger, 2015). Reaction kinetic study demonstrates dynamic impact of environment to the degradation rate of polyphenols, which is carried out by establishing a mathematical model (Cao et al., 2020). On the basis of the reaction rate

constants, the reaction kinetic result is able to recognize the prominent reactions among multiple reactions. What's more, it may provide a prediction for the loss of polyphenols during processing (Wang, Zhou, & Jiang, 2008; Wang, Zhou, & Wen, 2006).

3 | STABILITY OF POLYPHENOLS

Stability of polyphenols is of a great importance when considering food quality and nutritional value. Polyphenols occur ubiquitously in fruits and vegetables having beneficial effects for human health, and their consumption is largely recommended. Food processing and storage conditions can lead to chemical/structural changes in polyphenols, which subsequently lead to instability. The structure of polyphenols consists of one or more aromatic (benzene) rings with at least two hydroxyl groups attached, organic acids (phenolic or aliphatic), sugars, and acylated sugars that are often conjugated to the phenolic primary structure. The conjugated sugars can be mono-, di-, or oligosaccharides (Bravo, 1998). The structure of polyphenols is responsible for the characteristic features such as low to moderate water solubility, antioxidant activity, tendency to deteriorate by oxidation (Rice-Evans, Miller, & Paganga, 1996), and absorbance in both the UV and often in the visible (Vis) spectral regions (Cheyner et al., 2006).

The stability of polyphenols may be dramatically affected by their structural variations without the presence of external factors (temperature, light, pH, and so on) (Xiao & Högger, 2015). The stability of polyphenols can be changed by chemical modification, such as hydroxylation, glycosylation, acylation, and pigmentation. Hydroxylation always reduces the stability of polyphenols, whereas others increase the stability of them (Turturică, Oancea, Râpeanu, & Bahrim, 2015). The hydroxyl groups in polyphenols significantly influenced the stability in the following order resorcinol-type > catechol-type > pyrogallol-type, with the pyrogallol-type being least stable (Xiao & Högger, 2015). Studies have shown that glycosylation can affect the color and improve the stability of anthocyanins in acidic, alkali, and high-temperature environments (Zhang et al., 2014). However, it will reduce the antioxidant capacity of anthocyanins. Acylated anthocyanins show higher stability than their corresponding anthocyanins (Zhao, Wu, Yu, & Chen, 2017). Co-pigmentation occurs by hydrogen bonding between the phenolic groups of anthocyanins and flavonoids (Francis & Markakis, 1989). Generally, more hydroxyl groups provide blue color, whereas methoxyl increases the redness of anthocyanins. The size of the conjugated sugar can affect their overall stability (Levy, Okun, & Shpigelman, 2019). In contrast, any glycosylation of polyphenols can enhance their stability. Myricetin (1) first formed a dimer and then was oxidized, which was finally degraded (Cao et al., 2020) (Figure 3). This process was significantly affected by the temperature, and low temperature is helpful to detect the unstable products.

Various chemical reactions may affect the stability of polyphenols. Modification reactions that involve hydroxyl groups, such as esterification, alkylation, carboxymethylation, carbamate formation, dealkylation, chelate formation, and so on, play an important role in the stability of polyphenols. Moreover, other reactions related to the aromatic

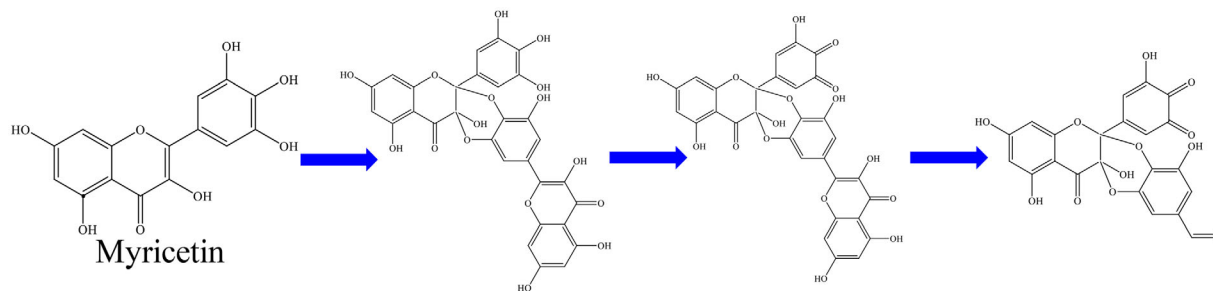


FIGURE 3 Chemical change of myricetin (1) in DMEM at 4°C (Cao et al., 2020)

rings, such as alkylation, acetylation, methylation/condensation, halogenation, sulfonation/sulfitation, amination, nitration, and so on (García-Viguera & Zafrilla, 2001), can dramatically modify polyphenols structure and subsequently, their stability.

It was suggested that hydroxyl moiety on the B ring of catechins may play a key role in the epimerization of catechin (Suzuki et al., 2003). Epimerization is one of the most important mechanisms that lead to the instability of polyphenols and it occurs due to temperature, pH, or metal ions variations in a food matrix. These variations are common, for instance, during tea processing, such as pasteurization. (–)-Epigallocatechin gallate (EGCG) (2), the most abundant polyphenols in green tea, was changed to (–)-gallocatechin gallate (GCG) (3) that undergoes epimerization (Ishino, Yanase, & Nakatsuka, 2010). The only difference between these two compounds is the conversion of 2,3-cis bond in EGCG (2) to 2,3-trans bond in GCG (3).

Another main mechanism that leads to instability of polyphenols is autoxidation. Polyphenols may undergo autoxidation in the presence of oxygen, resulting peroxides and hydroperoxides. The highly debated antioxidant activity of polyphenols is directly associated to their molecular structure, more precisely to their number of hydroxyl groups that are directly involved in polyphenols autoxidation, and the free radical scavenging capabilities by donating hydrogen atoms. Briefly, the more hydroxyl groups they contain, the more instability they exhibit (Wang et al., 2008), because hydroxyl groups are the ones that can react to result in the reduced stability of these compounds. Autoxidation can lead to a decrease in polyphenols content, following the oxidative polymerization and degradation that finally leads to a lower bioactivity (Sang et al., 2005).

Polyphenols are highly unstable molecules and rapidly transformed into various reaction products during postharvesting and processing. What happens in fact is that the membrane of cells breaks down due to various factors within food processing, and the compounds undergo a series of enzymatic and chemical reactions with other food components, resulting in the complexity of dietary polyphenol composition. Polyphenols are highly reactive compounds and good substrates for various enzymes, including polyphenol oxidases (PPO), peroxidases, glycosidases, and esterases. Therefore, polyphenol composition of plant-derived foods and beverages depends not only on the raw material used but also on the extraction methods and subsequent biochemical and chemical reactions of plant polyphenols. The result-

ing products account for a large part of the polyphenol contents in some foods and beverages and some of the new compounds formed in these processes may show particular properties that differ from those of their precursors.

The most important biochemical process is enzymatic oxidation. This process is very common in plant-based foods and it occurs immediately after cell lysis. The result of this process is browning, which can be detrimental for some foods (decreasing food quality), but beneficial for others, such as coffee, tea, cocoa, and raisins. The resulting products of polyphenols via biochemical and chemical reactions have been studied, mostly in wine and tea. The conversion of genuine anthocyanins to other molecules during food processing results in either loss or stabilization of color and increases the range of available hues. Most anthocyanin derivatives have an orange color (pyranoanthocyanins), whereas some are from purple (ethyl-linked species and tannin–anthocyanin adducts) to azure (portisins) (Figure 4). Flavanol dimers resulting from catechin oxidation have shown the ability to inhibit enzymes similar to those of their procyanidin isomers, whereas yellow products obtained after another oxidation step were more active (Plumb, De Pascual-Teresa, Santos-Buelga, Cheyner, & Williamson, 1998).

The properties of polyphenols are also affected by their interactions with other constituents of food matrix. For example, the phenomenon of co-pigmentation in anthocyanins, which arise from the interactions of anthocyanins with other compounds, results in color intensification. The bioactivities of polyphenols have been widely studied. Some reports suggested that the epimerized polyphenols have higher bioactivities (John, Mandal, & Natarajan, 2012), such as stronger scavenging properties (Guo et al., 1999). On the other hand, other reports have shown the contrary (Hu, Zhou, & Chen, 2009). Derivatives of polyphenols are formed in food systems, and the concentration of polyphenol decreases, which partly accounts for the instability of polyphenols.

Polyphenols have great antioxidant properties due to their molecular structures and the nature of substitutions on the aromatic rings. For instance, quercetin (4) and catechin (5) have a similar number of hydroxyl groups, at the same positions. However, quercetin differs by containing a 2,3-double bond in the ring C and the 4-oxo function (Rice-Evans et al., 1996), which provides this compound higher antioxidant activity in comparison with catechin that has a saturated heterocyclic ring.

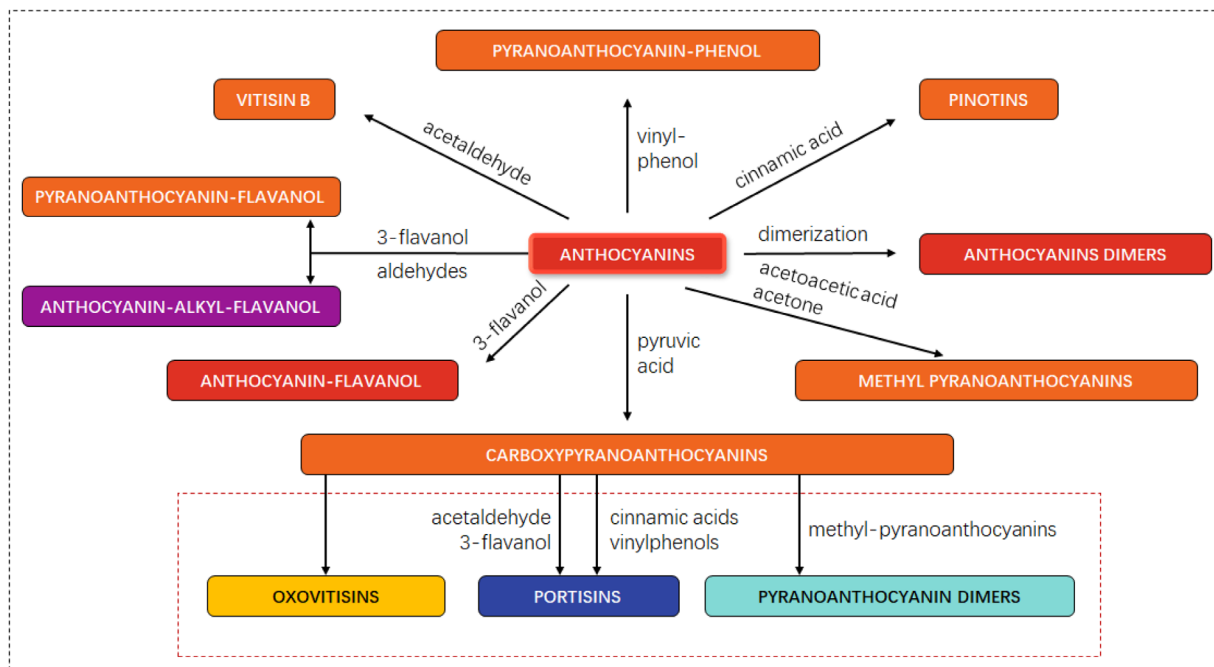


FIGURE 4 The colors of anthocyanins derivatives

4 | FACTORS IMPACTING THE STABILITY OF POLYPHENOLS

The stability of polyphenols is affected by structural changes that occur due to various biochemical and chemical reactions. These reactions take place when environmental or extrinsic factors, such as light, temperature, pH, or interactions with other food constituents, occur and interfere with the polyphenol stability and thus functionality.

4.1 | pH

The main factor that affects the stability of polyphenols in fruits and vegetables is the pH. Generally, polyphenols are more stable as the pH value of the solution is lower. For example, catechins in tea are very stable in aqueous solution at a pH below 4, and become unstable in solutions with pH over 6 (Ananingsih, Sharma, & Zhou, 2013). It has been reported that tea polyphenols are stable at lower pH and the pH value of the tea solutions plays a crucial role in the stability of the aqueous tea sample (Chen, Zhu, Tsang, & Huang, 2001). The study was conducted on tea polyphenols and revealed that at pH 5, catechins and theaflavins showed no sign of degradation. However, the degradation rate increased once the pH value was higher.

The variations of pH value could influence the stability of polyphenols by changing their chemical structure, which is also responsible for color variation of polyphenols. For instance, anthocyanins, a class of polyphenols, are abundant in colored fruits and vegetables and they have a wide variation in stability, with some being very unstable. Depending on pH level, anthocyanins can be found in different chemical forms. In aqueous solution, anthocyanins undergo structural rear-

rangements forming four molecular structures: the flavylium cation, the quinonoidal base, carbinol, and the chalcone. At pH 1.0, the predominant species is the flavylium cation (6) (red color), being most stable in acidic solutions. With increasing pH values, quinonoidal base (7) (colorless) is predominant due to the fast loss of the proton. Pseudobase (8) and chalcone (9) (colorless) can be observed at pH between 5 and 6, whereas at a pH value higher than 6, anthocyanins are degraded (Castañeda-Ovando, de Lourdes Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009). Therefore, anthocyanins alter their color to pink, green, blue, and yellow with the change of pH due to structural changes. The color of malvidin 3-O-glucoside (10) is red at pH 1.0, and it changes to ash gray at pH 7.0 and dark blue at pH 11.0 (Figure 5). A study incorporated anthocyanins into ultrafine zein fibers in order to develop pH-sensitive indicator membranes. The pH-sensitive membrane exhibited color changes from pink to green, when exposed to acidic and alkaline buffers, respectively (Prietto et al., 2018).

4.2 | Temperature

Temperature is another factor that strongly influences the polyphenols stability. Therefore, the temperature during food processing and storage should be strictly controlled to maintain the stability of polyphenols and their quality functionality (antioxidant activity). Processing and storage at low temperature are preferable. Studies have shown that polyphenols could undergo epimerization at high temperature. For example, the epimerization of procyanidin B2 (11) and procyanidin B5 (12) in cocoa bean happened when the roasting temperature was higher than 140°C (Kothe, Zimmermann, & Galensa, 2013).

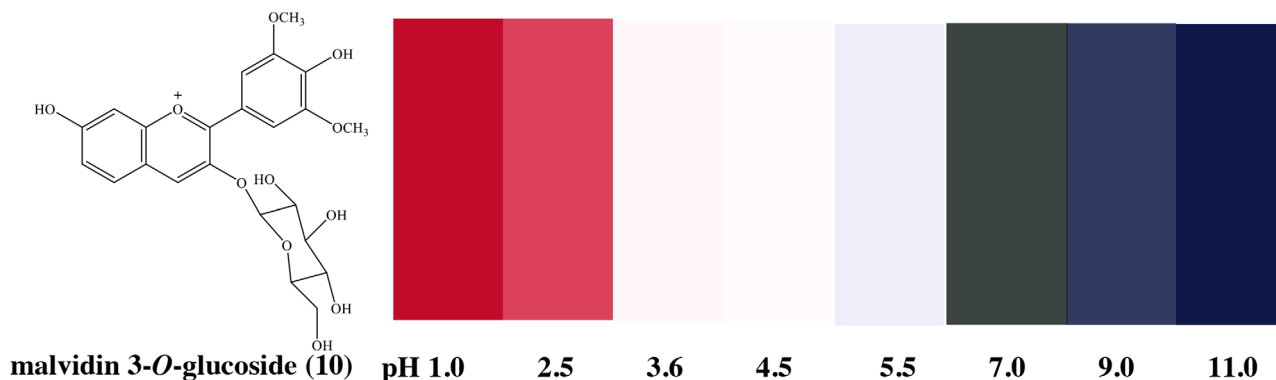


FIGURE 5 The colors of malvidin 3-O-glucoside (10) in NaH_2PO_4 – Na_2HPO_4 buffer (0.1 mM) with different pH

Storage conditions can affect the polyphenol content, mainly due to hydrolysis, oxidations, and complications with storage at higher temperatures, which negatively influences the stability of polyphenols (Zafrilla et al., 2003). At low temperatures (4°C), the stability of polyphenols is rather good, and the structures are relatively stable for longer periods, which may be related to the inhibition of phenol oxidase activity at low temperatures, lowering the oxidation and hence condensation and degradation (Cao et al., 2020).

The stability of anthocyanins has a significant effect on product appearance due to the characteristic color and health-promoting properties. Anthocyanin degradation is a highly debated and studied topic due to their wide industrial applications within the food and cosmetic industries. Anthocyanin degradation was reported to be strongly influenced by temperature. Compared to anthocyanins, the phenolic acids and flavonoid O-glycosides/C-glycosides are much more stable and less susceptible to degradation at higher temperature (Teleszko, Nowicka, & Wojdyło, 2016).

4.3 | Interactions with other food components

In plant-derived foods and beverages, polyphenols are a part of multicomponent systems, with interactions that can affect their stability, activity, and bioavailability. Ascorbic acid and sugars, for instance, are common components naturally encountered and often added externally during food processing, which are suggested to affect polyphenol stability (Kopjar, Tiban, Pilizota, & Babic, 2009). On the other hand, some reports suggested the protective effect of different sugars (fructose, sucrose, glucose, mannose, and galactose) on EGCG (2), due to a combination of several mechanisms: decreased oxygen solubility, chelation of transition metal ions and scavenging of reactive oxygen species (Shkolnikov, Belochvostov, Okun, & Shpigelman, 2020). Another study suggested that ascorbic acid improves catechins stability in green tea. It was suggested that ascorbic acid possibly serves as a reductant that can also protect these catechins and recycle their free radical form (Chen, Zhu, Wong, Zhang, & Chung, 1998). Moreover, ascorbic acid was reported to increase anthocyanins degradation rate, whereas the flavonols quercetin (4) and kaempferol (13) and the fla-

vanol catechin protected anthocyanins from degradation in the presence of ascorbic acid (Poei-Langston & Wrolstad, 1981).

4.4 | Light and oxygen

It is well-known that exposure to light has detrimental effects on polyphenols. Anthocyanins for instance are highly sensitive molecules to light, and many studies have demonstrated that polyphenol-rich food should be stored in the dark. On the contrary, a study investigating the effect of light on fresh-cut broccoli demonstrated that the presence of light during storage prevented enzymatic browning and preserved nutritional quality.

Another important factor that plays a key role in the stability of polyphenols is the presence or absence of oxygen. The oxidation of polyphenols by PPO into quinones leads to enzymatic browning and subsequently to a decrease in polyphenols content (Figure 6). Enzymatic browning occurs when there is an imbalance between oxidative and reductive processes due to the presence of oxygen. Briefly, polyphenol oxidation is proportional to the oxygen concentration.

4.5 | Metal ions

The antioxidant features of polyphenols depend on the different metal ions that are bound to them. They can act on two antioxidant pathways: direct reaction with free radicals and chelating of metal ions involved in production of reactive oxygen species (Grazul & Budzisz, 2009). Catechins reacting with Cu^{2+} and Mn^{2+} increase polyphenols antioxidant activity, whereas with Fe^{2+} they reduce it (Komatsu et al., 1993). However, polyphenols exhibit pro-oxidant activity as well (Perron & Brumaghim, 2009). Moreover, catechins can alternatively switch to a pro-oxidant action in the presence of transition metal (Azam, Hadi, Khan, & Hadi, 2004). Another factor influencing the stability of polyphenols is the added nitrite salt. Nitrite salt can induce modifications in polyphenols stability, depending on their structure and concentration. Nitrite salt is often included in food products and it is known to decrease the stability of polyphenols Other components that

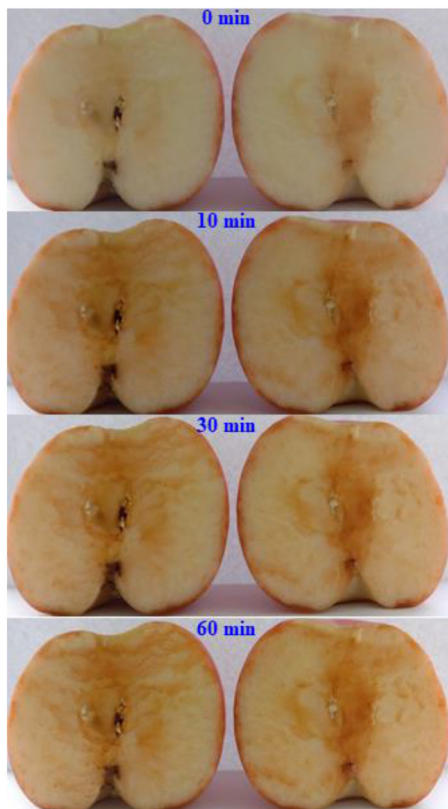


FIGURE 6 The apple polyphenols are easily oxidized by polyphenol oxidase in air

may affect the stability of polyphenols are sulfur dioxide or different enzymes.

5 | CHEMICAL MODIFICATION IMPROVING THE STABILITY

To increase its food commercializing applications, polyphenols can be stabilized through various techniques including several types of chemical reactions, complex formation, and co-pigmentations. As a promising strategy to improving polyphenols in baked meat products during processing and storage, extractable polyphenols in mulberry juice-enriched dried minced pork slices were reacted with β -cyclodextrin (β -CD) (combined with thermal treatments) and presented good thermal-chemical stability (Cheng, Liu, Zhang, Chen, & Wang, 2018, 2019).

The anthocyanins' stability is influenced by the hydroxylation and methoxylation on ring B (Vargas, Cortez, Duch, Lizama, & Méndez, 2013), which increase blueness and redness, respectively, and acylation and glucosylation (Lee et al., 2017), which decrease the stability of aglycones in neutral pH medium. Several flavonols and flavanones (e.g., isoquercitrin [14], naringenin [15], quercetin [4], and hesperetin [16]) were modified via subsequent transglucosylation by CD glucosyltransferase from *Bacillus macerans* to generate their glucosides, in which the oligoglucosylation improved the resistance of the aglycones to oxidative degradation by the Cu^{2+} ions. The potential of saponin and

polyphenols for preventing color loss of anthocyanins in model beverages was reported in a study, which reported that the green tea extract contained groups that may interact with anthocyanins, by hydrophobic interactions, thereby increasing stability (Chung, Rojanasasithara, Mutilangi, & McClements, 2016b). Hydrogen bonding and electrostatic interactions contribute to the stability of pectin-anthocyanins interactions at acid medium and promote stability under gastrointestinal simulation (Koh, Xu, & Wicker, 2020). Because acylation improves the color and pigment stability, the sugar moieties may be acylated with aromatic acids to avoid the degradation of unstable intermediaries into phenolic acids (Zhang et al., 2020). The addition of co-pigments can prevent the color loss by the protection of C-2 flavylum chromophore from the nucleophilic attack of water, which can occur by (i) intramolecular interactions or (ii) intermolecularly by van der Waals forces between the polarizable nuclei of the anthocyanin with phenolics. The intermolecular co-pigmentation with gallic acid was used in blueberry juice to enhance the stability and colors of anthocyanins (Zhang et al., 2020). Another stabilization method includes complex formation: hydroxycinnamic acid derivatives were found to impact the color expression of anthocyanins and anthocyanins-metal chelates, mainly when covalently attached. Food-grade biopolymers such as gum arabic and whey protein isolate improved the physical and chemical stability of model beverages containing anthocyanins, in the presence of ascorbic acid where the supramolecular formation through hydrogen bonding was suggested for enhancing color stability in the presence of ascorbic acid (Chung, Rojanasasithara, Mutilangi, & McClements, 2016a).

Among the several manners of stabilization of betalains, complex formation and co-pigmentation are presented as promising strategies. The effect of inorganic metals on the stability of betalains in *Rivina humilis* L. berry juice was studied in presence of metal and AA and showed enhanced pigment stability and regeneration of pigments after thermal processing and storage probably, due to synergistic action of AA and selenium. β -CD can absorb free water, and thus it was studied to suitably enhance the stability of betanin (17), improving the pigment retention. Probably, the β -CD effects are due to the formation of a 1:1 inclusion complex (Tutunchi, Roufegarinejad, Hamishehkar, & Alizadeh, 2019). For betacyanins, it was observed that acylation with aliphatic acids, by a bathochromic shift of absorption from 543 to 550 nm (Heuer, Wray, Metzger, & Strack, 1992), stabilizes them through co-pigmentation such as intramolecular interactions.

Besides stabilization, attention has also been given to encapsulation techniques. Food-grade biopolymers were studied to form supramolecular complexes of β -carotene and canthaxanthin/arabinogalactan with increased photostability with the aim to improve the stability of carotenoids in food processing (Polyakov et al., 2009). Similarly, complexes of lutein and zeaxanthin with glycyrrhizic acid were also studied, with increased stability in both aqueous and nonaqueous environments and oxidation resistance (Apanasenko et al., 2015). A study of caseins isolated from caprine and bovine milk combined with arabinogalactan showed increased lutein water solubility and chemical stability from 48 to 96 h of storage, under accelerated photo-oxidation conditions, at room temperature. The result was better for the caprine

TABLE 1 Chemical modifications strategies used in polyphenols improving stability

Product	Polyphenol	Treatment	Pathways	Improved properties	Type of stability	Reference
Mulberry juice-enriched pork slice	Phenolics; flavonoids	β -CD addition	Inclusion complex formation	Heat and chemical resistance	In hot processing	Cheng et al., 2019
Plant food: bioflavonoids	Flavonoid aglycones	Enzymatic hydrolysis	Glucosylation	Oxidation resistance	During processing	Ee et al., 2019
	AC	SPE	Acidified methanol	Color loss resistance	During storage	Vargas et al., 2013
Dragon fruit skin	AC	Acyl-gellan gum bonds	Acylation	Heat resistance	During processing	
Model beverage in AA		Polyphenols addition	Hydrophobic interaction	Inhibiting color fading	During storage	Chung et al., 2016b
Blueberry powder and extract	AC	Blueberry pectin and AC standards and extraction	H-bonding; electrostatic interactions	Preventing degradation	Under in vitro digestion	Koh et al., 2020
Fresh red cabbage	Cyanidin	Free and covalently bound phenolic acids	Complex formation; aromatic diacylation	Blue color expression	Processing and storage	Sigurdson, Robbins, Collins, & Giusti, 2017
Blueberry juice	AC	Copigmentation with gallic acid	Complex formation	Degradation delayed; less color fading	During storage	Zhang et al., 2020
Model beverages	AC	Whey protein and arabic gum	Complex formation by H-bonding	Color stability	During storage	Chung et al., 2015; 2016a
Berry juice	Betalains	Selenium and AA addition	Complex formation	Protection and pigment regeneration	After processing and storage	Khan & Giridhar, 2014
Red beet extract	Betanin and phenolics	Ultrasound-assisted β -CD addition	Inclusion Complex formation	Pigment retention	During storage	Tutunchi et al., 2019
Model beverage	Betanin	Sequestrants addition	Metal chelating	Half-life degradation resistance	During processing and storage	Pasch & Elbe, 1979
Standard powder	β -Carotene, canthaxanthin	AG	Supramolecular complexes	Photostability	During food processing	Polyakov et al., 2009
Model beverage	Lutein, zeaxanthin	Glycyrrhizin and its disodium salts; AG		Oxidation Stability; water solubility		Apanasenko et al., 2015
Corn oil	Lutein	Bovine and caprine casein; AG	Binding supporting complex formation	Water solubility chemical stability	During storage	Mora-Gutierrez et al., 2018

Abbreviations: β -CD, beta-cyclodextrin; AA, ascorbic acid; AG, arabinogalactan; AC, anthocyanins; OSA, octenyl succinic anhydride; MD, maltodextrin.

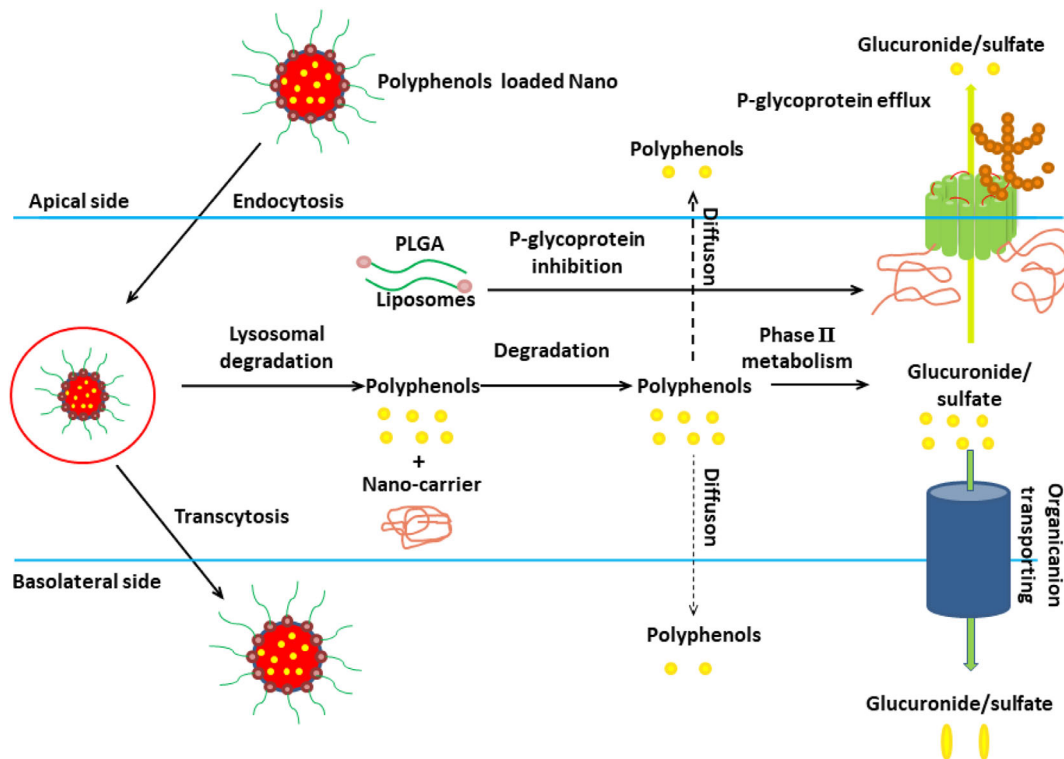


FIGURE 7 Proposed polyphenols-grafted nanoformulation for enhanced stability and absorption of polyphenols

casein type and was attributed to a binding supporting complex formation by hydrophobic interactions (Mora-Gutierrez et al., 2018). Table 1 summarizes the main chemical modifications commonly used to improve the stability of polyphenols in food.

6 | NANOTECHNOLOGY IMPROVING THE STABILITY

It has been observed that the benefit of polyphenols is not only dependent on their intake but also on their bioavailability. After absorption, phenolics reaching the blood and tissues are biotransformed and are different from those present in food, thus the evaluation of their biological activity might represent a big challenge (Velderrain-Rodríguez et al., 2014). Only small amounts are available and significant alterations of their redox potential can occur, limiting their bioactivity and reducing health benefits (Pacheco-Ordaz, Antunes-Ricardo, Gutiérrez-Urbe, & González-Aguilar, 2018). On the other hand, the absorption, pharmacokinetics, and systemic metabolism of polyphenols and their biotransformation by the GI microbiota, as well as the interaction with macromolecules such as lipids, polysaccharides, and proteins, have been extensively studied in the last decade (González-Aguilar, Blancas-Benítez, & Sáyago-Ayerdi, 2017). One of the approaches to improve the bioavailability of polyphenols is by incorporating them into nanoparticles. In recent years, nanotechnology has been rapidly expanding in the food and pharmaceutical industries, especially with the application of nanoencapsulation of bioactive compounds for biological purposes (Figure 7) (Rahim et al., 2019).

Nanoscale approaches to encapsulate phenolic compounds allow protecting them from various factors, which promote their chemical degradation, thereby increasing potential applications. Dicastillo et al. (2019a) extracted Maqui berry phenolics (consisting mostly of anthocyanins) and encapsulated them using an electrospray methodology and hydroxypropyl- β -cyclodextrin as encapsulating material. Authors report increased thermotolerance under autoclaving (121°C, 15 min) and baking (180–185°C, 25 min) conditions, according to minimal losses of phenolics when heated, as compared to nonencapsulated compounds. Increased thermotolerance has also been reported in açai (*Euterpe oleracea* Mart.) anthocyanins encapsulated in electrospray particles, with zein as encapsulating material (Dicastillo et al., 2019b). Treated samples were heated to the same autoclaving and baking conditions mentioned above, where a protective effect was evident; further experiments also showed increased stability under simulated digestion conditions.

Others have shown significant increases in half-life of encapsulated phenolic compounds during storage. For example, Guldiken, Gibis, Boyacioglu, Capanoglu, and Weiss (2018) extracted black carrot anthocyanins and incorporated them into nanoscale liposomes, which remained stable during a 21-day storage period. However, authors cautioned that the stability was dependent on the percentage of fatty acids present in the system, that is, phenolic oxidation could be induced by oxidized fatty acids under nonideal ratios.

Jeong, Lee, and Lee (2020) evaluated different combinations of fruit (açai, aronia, blackberry, cranberry, wild berry, raspberry, blueberry, and red grape) and vegetable (spinach and cabbage) concentrates, when encapsulated in chitosan/gum arabic or chitosan/carrageenan

nanoparticles. Authors determined that, during *in vitro* digestion, chitosan/gum Arabic particles were not stable, due to their increased polydispersity index. In contrast, the chitosan/carrageenan combination was more stable under conditions evaluated. The protective effects of encapsulation were also evident when antioxidant activity was evaluated, where ORAC values for free compounds decreased almost completely, as compared to a retention of 25%–50% for encapsulated compounds. Similarly, TPC decreased to approximately 25% for free compounds, whereas encapsulation maintained a higher value of approximately 60%. This evidence suggests that a proper selection of encapsulating material is crucial to yield successful results, when treating phenolic compounds.

Savaghebi, Barzegar, and Mozafari (2020) encapsulated phenolic compounds of algal origin in nanoliposomes, after optimizing the process. Under ideal conditions, they report that thermotolerance of the compounds increased, because oxidation of free compounds began at 132.3°C, whereas for compounds that were encapsulated oxidation began at 157.1°C, according to differential scanning calorimetry. Findings also suggest that antioxidant capacity remained acceptable, which led the authors to propose the use of nanoliposome-encapsulated algal phenolic compounds as antioxidants in lipid-based foods.

The increased stability of encapsulated phenolic compounds can yield other advantages related to their bioactivities. For example, Pereira et al. (2018) extracted phenolic compounds from guabiroba fruit (*Campomanesia xanthocarpa* O. Berg.) and encapsulated them with poly(D,L-lactic-co-glycolic) acid (PLGA) as encapsulating material. Authors evaluated their bioactivity as inhibitors of *Listeria innocua* (as a nonpathogenic surrogate for *L. monocytogenes*). Data showed a significant threefold lower dose required to inhibit *L. innocua* when the extract was administered encapsulated, as compared to free. These data suggest that encapsulating phenolic compounds increases not only their stability, but potentiates their bioactivity against some microorganisms.

Encapsulating phenolics can also serve to protect them when extracted from fruit by-products, for example, Oliveira, Angonese, Ferreira, and Gomes (2017) used PLGA to encapsulate phenolic compounds from passion fruit by-products. A controlled release was observed, with a kinetic profile similar to that of a bacterial growth curve, which could be mathematically modeled. Stability and bioactivity of the compounds were also measured after encapsulating, because these properties enhanced the antibacterial effects of the extracts, according to an approximately 20-fold lower dose required to inhibit *L. innocua* and *Escherichia coli* growth. Nevertheless, these results varied according to materials used to encapsulate them, as well as their ratios.

Practical applications of encapsulated phenolics include masking their bitter flavor, protecting them against processing conditions and changes during storage, among others. Tavakoli, Hosseini, Jafari, and Katouzian (2018) produced an olive leaf extract rich in oleuropein (18) and encapsulated it within nanoliposomes, which were then added to yogurt in order to improve its functionality. Authors performed numerous analyses and report that enriched yogurt had an increased antioxidant activity, due to oleuropein and other phenolics added. Encapsulating the extracts had the added benefit of avoiding changes

to sensorial attributes, which were apparent when yogurt was supplemented with free extract. Stability of the particles varied significantly, requiring an initial optimization process; this prerequisite has been reported by others.

7 | ENCAPSULATION IMPROVING THE STABILITY

The vast spectrum of polyphenol biological activities can be impaired by their low stability, unpleasant flavor, and low bioavailability (Fang & Bhandari, 2010; Munin & Edwards-Lévy, 2011). The applicability of these compounds for human benefits requires efforts to overcome their drawbacks by encapsulating and protecting them from environmental factors such as pH, oxygen, light, and temperature (Fang & Bhandari, 2010; Munin & Edwards-Lévy, 2011; Pimentel-Moral et al., 2018). There are several methods of encapsulation or microencapsulation that can be applied to natural polyphenols, but the method of choice must base on polyphenol characteristics such as chemical structure, solubility, compatibility to coating/encapsulating agent, thermo-physical stability, and others (Liang et al., 2017; Martins, Pereira, Siqueira, Salomão, & Freitas, 2013; Sawale, Patil, Hussain, Singh, & Singh, 2017). Besides, the coating/encapsulating agent characteristics and target properties of final products must also be considered, such as particle size and morphology (Farrag, El-Messery, El-Said, Soliman, & El-Din, 2018).

The mechanical methods, such as extrusion/spheronization (Silva et al., 2018), coating or granulation (Andrade, Martins, & Freitas, 2015; Freitas, 2019; Oliveira et al., 2015), and hot melt (Guimarães et al., 2017), are usually applied to large-size particles or products (Zamarioli, Martins, Carvalho, & Freitas, 2015). In general, smaller particles can be obtained by physicochemical methods such as spray drying (Martins et al., 2013; Nosari, Lima, Serra, & Freitas, 2015; Rocha et al., 2011), spray cooling (Martins, Siqueira, Fonseca, & Freitas, 2014; Pereira, Colombo, Martins, & Pedro de Freitas, 2014), freeze (Ballesteros, Ramirez, Orrego, Teixeira, & Mussatto, 2017) and spray freeze drying (Teixeira et al., 2017), ionic gelation (Cutrim, Alvim, & Cortez, 2019; Pasukamonset, Kwon, & Adisakwattana, 2016; Stoica, Şomoghi, & Ion, 2013; Zam, Bashour, Abdelwahed, & Khayata, 2014), emulsion evaporation, co-precipitation, and supercritical fluid technology (Munin & Edwards-Lévy, 2011). The chemical methods include the interfacial polycondensation, *in situ* polymerization, interfacial polymerization, interfacial crosslinking, and their variations (Munin & Edwards-Lévy, 2011; Bartosz & Irene, 2016).

Recently, the encapsulation of polyphenols has been reviewed demonstrating its importance to improve polyphenols properties and make their industrial application feasible (Munin & Edwards-Lévy, 2011; Bartosz & Irene, 2016). The most used technique for natural compounds encapsulation is spray drying (Freitas et al., 2019) due to its simplicity, easy scale-up, and low cost as compared to physicochemical methods, for example (Martins et al., 2013). Spray drying results in micrometric capsules that can be shell-and-core, multiple core, or even matrix types, depending on process conditions and formulation (Martins et al., 2013). Before spray drying, mixtures of polyphenols are

formulated with carriers in solution or suspension and then atomized in a hot air stream (Wang et al., 2020).

Ballesteros et al. (2017) applied spray drying to encapsulate phenolic compounds and flavonoids extracted from spent coffee grounds using maltodextrin, gum arabic, or their combination as wall material. However, the authors compared the products obtained by spray and freeze drying and obtained better retention of both phenolic compounds and flavonoid (62% and 73%) when applying freeze drying, besides a higher antioxidant activity (73% and 86%). Interestingly spray-dried particles had lower phenolic and flavonoid content but their antioxidant activity was superior than the freeze-dried capsules. Furthermore, spray-dried particles resulted in spherical particles, whereas freeze-dried showed an irregular morphology (Ballesteros et al., 2017). A mixture of gum arabic and maltodextrin DE 16.5-19 was also used to encapsulate EGCG from green tea, *Camellia sinensis* sp. leaves, using spray drying (Rocha et al., 2011) and resulted in encapsulation efficiencies of $85\% \pm 3\%$ and particle size of 120 ± 28 nm. The authors claimed that the encapsulated bioactive is more stable and noncytotoxic to Du145 prostate cells (Rocha et al., 2011). Green tea polyphenols were also encapsulated in liposomes (Upputuri & Mandal, 2017) using 20:1 phosphatidylcholine and cholesterol with 77.7% encapsulation efficiency and particle sizes around 250 nm, with release kinetics very close to zero-order model (Upputuri & Mandal, 2017).

Polyphenolic antioxidant extracts of *Rosmarinus officinalis* L. leaves were prepared by their double emulsification with polyglycerol polyricinoleate (4%) and whey protein isolates (2% and 4%) and then spray dried using maltodextrins (MDE 10 and 21) as a drying aid (Bušić et al., 2018). Microparticles were obtained with encapsulation efficiencies from 27.08% to 42.83% and the polyphenol stability showed to be correlated to powder size and maltodextrin with a lower dextrose-equivalent number, MDE 10, and 4% WPI (Bušić et al., 2018). The encapsulation of polyphenols and anthocyanins from pomegranate (*Punica granatum*) juice was also done by spray drying with maltodextrin and soybean protein isolates under different process and formulation conditions and gave greater stability to these bioactives even when added to yogurt (Robert et al., 2010). A combined process of concentration, emulsification, and encapsulation by spray drying of polyphenols from carob (*Ceratonia siliqua*) was proposed by Wang et al. (2020) resulting in significant polyphenol encapsulation efficiencies ($87.0\% \pm 0.2\%$ and $77.4\% \pm 0.4\%$) and increased shelf life. Whey protein combined with maltodextrin at different ratios was used to encapsulate grape pomace polyphenols (Farrag et al., 2018) and showed a dependence of encapsulation efficiency, particle size, zeta potential, and polyphenol release under whey protein and maltodextrin ratio.

The spray chilling or cooling method can be also applied to encapsulate natural phenolic and polyphenols (Cutrim et al., 2019). Polyphenols from green tea extract were encapsulated by spray chilling in hydrogenated palm oil and far from hydrogenated/interesterified vegetable oil carriers and compared to beads obtained by ionic gelation. Both methods resulted in adequate microparticles obtained in both methods, with encapsulation efficiency of $83.5\% \pm 2.8\%$ and $72.6\% \pm 0.4\%$ and antioxidant activity (DPPH [2,2-diphenyl-1-phenylhydrazyl free

radical], IC 50 [inhibition concentration to quench 50% of free radical]) of $33,169.4 \pm 123.8$ and 2099.7 ± 35.31 g/ml for spray chilling and ionic gelation, respectively (Cutrim et al., 2019).

Recently, the techniques of electrospinning and electrospraying have gained attention due to being a one-step process at room temperature (Bhardwaj & Kundu, 2010; Chakraborty, Liao, Adler, & Leong, 2009) and also simple scale-up. In the electrospinning case, a solution is injected from an electric-charged nozzle into a collecting surface with opposite charge. The charge difference causes the liquid stream to accelerate and overcome surface tension and thus generating an elongated, fiber-like structure (Bhardwaj & Kundu, 2010). When the liquid stream breaks into spherical droplets due to surface tension, the technique is called electrospraying and results in micro- or nanoparticles (Chakraborty et al., 2009; Gómez-Estaca, Balaguer, Gavara, & Hernandez-Munoz, 2012; Dicastillo et al., 2019). Encapsulation of EGCG from green tea was performed by the electrospray technique and resulted in encapsulation efficiencies around 100% and full retention of antioxidant activity (Gaona-Sánchez et al., 2015).

8 | EFFECTS OF DRYING METHODS ON THE STABILITY OF POLYPHENOLS

8.1 | Lyophilization sustains the stability of polyphenols

The presence of water, light, oxygen, initial antioxidant concentration, availability of certain food components, and their chemical structure are the factors that affect the stability of phenolics (Ramírez, Giraldo, & Orrego, 2015). Among these factors, the presence of water can be considered as one of the most important ones because water is essential for the progression of many chemical reactions. It was reported that the stability of phenolic compounds such as alkaloids, terpenoids, and terpenes is mainly affected by water activity (Yıldırım, Duran, & Koç, 2018). High water activity increases the mobility of water and consequently causes an increase in oxygen transfer rate, which accelerates the degradation reactions of the phenolic compounds (Tonon, Brabet, & Hubinger, 2010). Therefore, the most convenient drying method to reduce the water activity and moisture content provides also the retention of the highest quantity of bioactive compounds in the final product (Samoticha, Wojdyło, & Lech, 2016). Freeze drying is one of the drying methods applied for long-term preservation of bioactive food compounds. Based on the phenomenon of sublimation of water under reduced pressure, it contributes to the retainment of most of the original raw material properties such as biological activity, appearance, shape, taste, dimensions, flavor, texture, and color (Ramírez et al., 2015).

Freeze drying is a unit operation where removal of moisture from the food products is carried out through sublimation. In the context of food drying, sublimation is a phase change process whereby the ice crystals (solid) within the frozen food are converted to water vapor (gas) during the moisture removal process under vacuum condition (Ratti, 2001) (Figure 8). This is usually achieved by lowering the

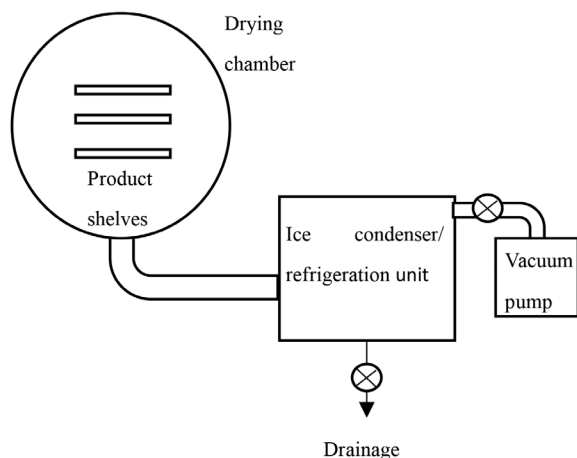


FIGURE 8 Schematic sketch of a freeze dryer

pressure of the drying system below the triple point of water (0.01°C or 273.16 K). Historically, freeze drying was first invented by Jacques-Arsene d'Arsonval from France in 1906 but it was not until in the 1950s that it was used in industrial-scale food production. Figure 8 shows a typical schematic sketch of a freeze dryer that is normally used in food drying.

A typical setup of a freeze dryer consists of a drying chamber, a refrigeration unit, and a vacuum pump. Selection of the operating pressure depends on the products and it can be categorized as rough vacuum ($1013\text{--}1\text{ mbar}$), medium vacuum ($1\text{--}10^{-3}\text{ mbar}$), and high vacuum ($10^{-3}\text{--}10^{-7}\text{ mbar}$). The operating temperatures with incorporation of a heated shelf are between -40 and -80°C during drying. Freezing is necessary for all food products that are subject to freeze drying. A complete freeze drying cycle includes freezing, sublimation, and final drying. The advantages of freeze drying include little thermal damage, good retention of flavors and nutrients, rapid product hydration, low final moisture content, no product shrinkage, and good retention of products' bioactivity. The disadvantages of freeze drying are also considerable, such as high fixed and operating costs, low sublimation process, application of refrigerants, requirement of prefreezing, and expensive packaging for dried product.

Freeze drying has been investigated by many researchers and applied in commercial production of food products. Many applications of freeze drying to sustain the stability of polyphenols are given in Table 2. Positive results have been reported especially in the preservation of nutrients and bioactive compounds that also include polyphenols. The low/mild-temperature and vacuum conditions are conducive in preserving bioactive compounds such as polyphenols. The anaerobic condition prevents the oxidation process and the mild temperature conditions prevent possible thermal changes of the compounds.

Although Aydin and Gocmen (2015) reduced the water activity of pumpkin flour by freeze drying and hot air oven drying, they stated that the deep orange color and additionally some technofunctional properties such as water holding capacity, oil binding capacity, emulsion capacity, and emulsion stability were strengthened by freeze drying. However, compared to hot air oven dried pumpkin flour, freeze-dried flours provided lower antioxidant capacities and phenolic acid

concentrations and less bioaccessible phenolic compounds. It could be related to the inactivation by hot air oven drying of PPO enzyme that catalyzes the oxidation of antioxidant phenolics, whereas the freeze-drying conditions would let the degradation reactions continue. When Sinrod et al. (2019) dried pitted olive pomace by using different drying methods, compared to the antioxidant capacity of fresh samples, freeze-dried ones had the highest decrease in antioxidant capacity, followed by hot air and drum-dried samples. The retention of individual phenolics was different among the drying methods, for example, by freeze drying, only 12% and 10% of vanillic acid and luteolin, respectively, were lost compared to fresh sample, whereas rutin was protected best by drum drying and hot air drying. The amount of hydroxytyrosol and tyrosol in fresh and freeze-dried samples was determined as lower than those of drum- and hot air-dried samples. The difference could be related both to the possible thermal inactivation of enzyme in the olive pomace and higher drying temperature, which would increase the extractability of phenolic compounds by the disruption of cell membranes. Similarly, Ucar and Karadag (2019) found that compared to freeze drying, vacuum drying was a better alternative in terms of extractability of phenolics from the oyster mushroom powders by simulated digestive fluids. In another study, freeze drying was used to ensure the stability of chokeberries polyphenols. The authors reported that the TPC of fresh chokeberries was $8008\text{ mg}/100\text{ g dm gallic acid equivalents}$, whereas the freeze-dried berries contained $7265\text{ mg}/100\text{ g dm gallic acid equivalents}$. The freeze-drying method causes only a loss of 9% of fresh chokeberries polyphenolic content, but the polyphenol loss increased up to 38% by using vacuum-microwave drying, vacuum drying, convection drying, and convection vacuum-microwave drying methods (Samoticha, Wojdyło, & Lech, 2016). In the study of Wojdyło, Figiel, and Oszmiansk (2009), proanthocyanidins' content of freeze-dried strawberry samples was found to be similar to fresh strawberries. Moreover, the catechin levels of freeze-dried strawberries were found to higher than fresh ones because of depolymerization of proanthocyanidins and conversion into elementary units. Cheng et al. (2017) compared the stability of freeze-dried and spray-dried bayberry powder; the value of the total polyphenols remaining in the freeze-dried powder was slightly higher than that of the spray-dried powder because application temperature of -50°C during freeze drying was effective in ensuring the stability of polyphenols in the powder. After 50 days storage at 25°C , TPCs of freeze-dried and spray-dried bayberry powder were found as $14.187\text{--}11.032$ and $7.38\text{--}5.69\text{ mg/g dW}$, respectively (Cheng et al., 2017). Four different fruits, blueberry, cherry, cranberry, and strawberry, known for their high antioxidant capacity, were dried using a variety of drying methods, such as freeze drying, hot air drying, and refractance window drying, and freeze-dried fruits had higher value of antioxidant capacities and polyphenol content (Nemzer, Vargas, Xia, Sintara, & Feng, 2018). In spite of the advantages offered by freeze-drying method such as retention of bioactive compounds and higher quality attributes of final products, the duration of the process was long and required high energy and operational costs (Schulze, Hubbermann, & Schwarz, 2014). New approaches combining key benefits of different drying methods have been described. Teixeira et al. (2017) dried *Baccharis dracunculifolia*

TABLE 2 Some applications of freeze drying to sustain the stability of polyphenols

Raw material	Operation conditions	Results	Reference
Pitted olive pomace	<ul style="list-style-type: none"> Duration: 69 h (13 h at -10°C, 10 h at -5°C, 12 h at 5°C, 10 h at 10°C, and 24 h at 20°C) 3 mTorr vacuum strength 	Antioxidant capacity: Drum-dried: 13% decrease from a fresh sample Hot air-dried: 27% decrease from a fresh sample Freeze-dried: 35% decrease from a fresh sample Individual phenolics: The freeze drying lost 12% of vanillic acid content. The freeze drying lost 10% of luteolin content	Sinrod et al., 2019
Chokeberries	<ul style="list-style-type: none"> Pressure: 0.960 kPa Temperature: -60°C Duration: 24 h 	Total phenolic content: gallic acid equivalents (mg/100 g dm) Fresh sample: 8008 ± 30 Freeze-dried sample: 7265 ± 81 Anthocyanins (mg/100 g dm) Fresh sample: 3917 ± 24 Freeze-dried sample: 2227 ± 37	Samoticha et al., 2016
Strawberry Fruits	<ul style="list-style-type: none"> Temperature: -60°C Pressure: 65 Pa 	Procyanidin (mg/100 g of dw): Fresh: 63.2 Freeze-dried: 61.5 Catechin (mg/100 g of dw): Fresh: 42.3 Freeze-dried: 49	Wojdylo et al., 2009
Bayberry	<ul style="list-style-type: none"> Temperature: -50°C Duration: 48 h 	Storage stability (at 25°C) of freeze-dried fruit: Polyphenols Day 0: 14.19 mg/g; Day 50: 11.03 mg/g Anthocyanins Day 0: 1.21 mg/g; Day 50: 0.9 mg/g	Cheng et al., 2017
Blueberry Cherry Cranberry Strawberry	<ul style="list-style-type: none"> Pressure of 0.5 mmHg Temperature: -64°C Duration: 24 h 	The total concentrations (%): Phenols: 1.64–3.84 Anthocyanin: 0.15–1.16 Flavonoids: 0.8–2.31 Antioxidant capacity ORAC ($\mu\text{mol Trolox equivalent/g}\cdot\text{dm}$) values: 273.75–444.50	Nemzer et al., 2018
Apple slices	<ul style="list-style-type: none"> Duration: 72 h 	Percentage of total quercetin: Freeze-dried: 5.9% Microwave vacuum dried: 4.7%	Schulze et al., 2014
<i>Baccharis dracunculifolia</i> extract	Spray-freeze drying <ul style="list-style-type: none"> Cold airflow: $0.9\text{ m}^3/\text{min}$ Air entrance temperature: -18°C Air exit temperature: -5°C Spray nozzle orifice: 1 mm 35 L/min of air at 5 bar and a liquid flow 6 mL/min 	The shelf life of up to 10 years Prolonged antioxidant activity at 4°C in the absence of light	Cardoso et al., 2017

extract by using spray-freeze-drying method and the product obtained was characterized by a shelf life of up to 10 years and prolonged antioxidant activity at 4°C in absence of light.

8.2 | Other drying methods

Effects of drying methods on the stability of polyphenols have been investigated by many researchers (selected examples as shown in Table 3). A recommended drying strategy to preserve polyphenols (besides freeze/vacuum drying options) is to shorten the drying time by

increasing the drying rate in order to reduce the activity of the enzymes (e.g., using microwave-assisted drying). However, convective air drying could have either positive or negative impact on the phenolic compounds.

The degradation of polyphenols during drying can be caused by enzymatic or nonenzymatic browning reactions (McSweeney & Seetharaman, 2015). Enzymatic browning involves enzymes (e.g., PPO) that are responsible for the production of brown pigments (melanins) at the end of drying. Nonenzymatic reactions are represented mainly by the Maillard reactions that involve reducing sugars and free amino groups of amino acids, peptides, and proteins (Yamada, Ando,

TABLE 3 Effects of drying on stability of polyphenols

Food materials	Drying conditions	Findings	Reference
Raspberries	Freeze drying, hot air drying (76.6°C for 4.5 h), microwave-vacuum drying (65.5°C for 90 min at 3000 W and 2.6 KPa), and hot air (98.8°C for 45 min) and microwave vacuum (1.1°C for 60 min at 3000 W and 2.6 KPa) combination drying	Freeze drying and combination drying showed higher retention in total polyphenols and total anthocyanins. Factors such as use of vacuum, low temperature (freeze drying), and reduced time and temperature (combination drying) helped in retaining the polyphenols in samples. Hot air drying showed the highest negative effect.	Mejia-Meza et al., 2010
Dates	Commercial sun drying (30–50°C for 7–10 days)	No anthocyanins were detected in sun-dried dates possibly due to thermal destruction/browning reactions. Total phenolic contents were significantly higher than those of fresh samples (22.5%–153%) due to the release of phenolic compounds.	Al-Farsi, Alasalvar, Morris, Baron, & Shahidi, 2005
Broccoli	Hot air (60°C with an air velocity of 1 m/s) and microwave drying (power intensity at 18, 36, and 54 W/g)	Total phenolic content (TPC) reduced after drying. Samples exposed to 54 W/g microwave power intensity had comparable TPC results (759.8 mgGAE/100 g) to fresh samples (892.4 mgGAE/100 g). The fast drying rate and short drying time improved retention of phenolic compounds.	Yilmaz, Şakiyan, Barutcu Mazi, & Mazi, 2019
Boletus mushrooms	Steaming (8 min), pressure cooking (5 min), microwave (900 W for 1.5 min), frying (160°C for 3 min), and boiling (10 min).	All cooking methods reduced the TPC. Boiling retained the least amount (33%–46%), whereas microwaving retained the highest amount (71%–98%). Cooking with heating could have negative impact/destroy the structures of phenolic and decrease their contents.	Sun, Bai, & Zhuang, 2014
Chinese cabbage and nightshade	Sun drying, shade drying, solar cabinet drying, hot air drying, and freeze drying	Freeze drying showed the best preservation, but solar drying was recommended due to moderate retention of antioxidant activity due to cost reason. Sun drying was not recommended.	Managa, Sultanbawa, & Sivakumar, 2020
Yam peels	Freeze drying and hot air drying Final moisture content 6.0%–6.5%. Pretreatment with blanching at 85°C water bath for 30 s.	Blanching caused significant reduction in TPC due to high solubility of the compounds in in water at 85°C. Unblanched samples showed the highest TPC (Freeze-dried samples = 6.09–11.14 mgGAE/g and hot air-dried samples = 6.42–8.76 mgGAE/g)	Chung et al., 2008
Plums	Air flow cabinet dryer (60 and 85°C)	Total polyphenols markedly decreased especially in samples dried at 60°C. Slightly higher amount of polyphenols was retained at 85°C. Antioxidant activity was higher in later case as polyphenols in an intermediate stage of oxidation have greater antioxidant power.	Piga, Del Caro, & Corda, 2003
Salak	Intermittent heat pump (26–37°C)–hot air (40–50°C) drying.	Degradation of polyphenols was higher in intermittent drying with longer hot air drying duration. Heat pump drying preserved the higher TPC (44.1 mgGAE/g) as compared to hot air drying (39.6 mgGAE/g). The mild and dehumidified air conditions are conducive in preserving polyphenols.	Ong, Law, & Hii, 2012

(Continues)

TABLE 3 (Continued)

Food materials	Drying conditions	Findings	Reference
Kedondong	Freeze drying and convective air drying (60–80°C).	Freeze drying retained the highest TPC (18 mgGAE/g) as compared to convective air drying. Convective air drying recorded the lowest (11.95 mgGAE/g) due to the longer exposure of the fruit sample to aerated drying condition.	Ee et al., 2019
Papaya leaf	Freeze drying, hot air drying (60–80°C), and shade drying	Freeze drying preserved the most polyphenols (2158 mgGAE/100 g) followed by hot air (1940–2093 mgGAE/100 g) and shade drying (1492 mgGAE/100 g). Shade drying prolonged drying duration and enzymatic browning.	Yap et al., 2020
Olive leaf	Solar drying and oven drying (40°C) with blanching pretreatment (90–95°C)	Blanching time more than 20 s inactivated the activity of the enzyme (PPO) and increased the phenolic content by 61.7%. Oven-dried leaves showed the lowest phenolic content.	Zeitoun, Mansour, Ezzat, & El Sohaimy, 2017
Persimmon	Air drying (30°C and 30%–45% RH) for 13 days	Drastic drop in polyphenols from ~12 to <1 mg/g for the first 7 days of drying. No significant astringency taste was observed at this stage. The drastic drop of the polyphenols could be due to insolubilization by acetaldehyde accumulation. Nonenzymatic browning might play only a minor role.	Yamada et al., 2009
Black tea leaf	Superheated steam drying (120–200°C)	TPC decreased with increased drying time and temperature. The phenolic compounds are heat labile and high-temperature/long drying periods could cause further deterioration.	Rumaisa, Hanim, & Hii, 2018

Tsutani, Amano, & Yamamoto, 2009). In most reported studies, enzymatic browning is more severe especially when the drying temperature is mild (35–60°C) at atmospheric conditions and this results in prolonged activity of the PPO that contributes to a more intense browning. Kyi et al. (2005) have reported reaction rate constants of cocoa polyphenols degradation due to PPO that are greatly affected by temperature and relative humidity (RH) of the drying air. The higher the temperature (40–60°C) and RH (50%–80%) of the drying air, the faster the chemical reactions. It was reported that the activation energies to initiate the browning reactions in cocoa beans were determined at 27.8–30.3 kJ/mol·K.

In some cases, the amounts of polyphenols are reported to be higher than the fresh samples after drying. For instance, Serratos, Lopez-Toledano, Merida, and Medina (2008) had reported increased phenolic content in raisins after drying due to the hydrolysis of tannins, lignins, and oligomers. Chang, Lin, Chang, and Liu (2006) also reported similar observation in tomatoes, which could be due to liberation of the phenolic compounds from the matrix under high drying temperatures. On the other hand, pretreatment before drying could also preserve polyphenols due to the inactivation of enzymes, for instance, through blanching with hot water and sulfur dioxide (Williamson & Carughi, 2010). There-

fore, correct combinations of drying methods and operating conditions must be selected very specifically for the type of food product in order to preserve its polyphenols after drying.

9 | EFFECTS OF POLYPHENOL-PROTEIN INTERACTION ON THE STABILITY

Due to the large number of available functional groups, proteins are ideal candidates to form covalent and noncovalent interactions with phenolic compounds. Many publications presented and reviewed the possibility of noncovalent interactions between polyphenols and proteins (Xiao & Kai, 2012). Although the focus of those studies is often on mechanistic aspects, a possible impact can also be expected on bioaccessibility/bioavailability and antioxidant capacity, as well as on the structure and physical properties of the molecules. The nature of noncovalent interactions is suggested to be based on hydrogen and/or hydrophobic interactions (Xiao & Kai, 2012). The type and strength of these interactions depend on specific protein and phenolic compounds, because hydroxylation, methylation, and steric hindrance have been reported as governing factors (Li et al., 2020).

TABLE 4 Effect of noncovalent polyphenol–protein interactions on the stability of polyphenols

Protein systems	Polyphenol	Testing conditions	Stability effect	Reference
B-Lactoglobulin	EGCG	pH 6.85, 25°C oxidation by H ₂ O ₂ ; illumination	Protection	Shpigelman et al., 2010
Bovine whey protein	Anthocyanins extract from grape skin	pH 6.3, 80°C	Protection	He, Xu, Zeng, Qin, & Chen, 2016a
Human plasma and DMEM	Flavonols	37°C, 5% CO ₂	Protection	Xiao & Högger, 2015
Human serum albumin	Stilbenoids	37°C, 5% CO ₂	Protection	Cao, Jia, Shi, Xiao, & Chen, 2016
Model dairy beverages	Tea flavan-3-ols	pH 6.30, 62°C	Stabilization against thermal treatment.	Song et al., 2015
Whey protein	Anthocyanins from purple carrot	pH 3, 40°C	Protection	Chung et al., 2015
α- and β-casein	Malvidin-3-O-glucoside	pH 6.3, 80°C; oxidation by H ₂ O ₂ ; illumination	Protection	He, Xu, Zeng, Qin, & Chen, 2016b
Preheated milk proteins	Anthocyanins extract from grape skin	pH 6.3, 80°C; oxidation by H ₂ O ₂ ; illumination	Protection	He et al., 2016a
Preheated soy protein	Cyanidin 3-O-glucoside	pH 7.4, 80°C; oxidation by H ₂ O ₂ ;	Protection	Attaribo et al., 2020
Whey protein, soy protein	Anthocyanins from purple-fleshed sweet potatoes	pH 3, flushed with N ₂ ; 100°C, 25°C	Improve the thermal stability, no effect on stability during storage	Quan et al., 2020
Casein, whey protein, soy bean protein	Fisetin, quercetin	pH 6, 6.8, 7.5, 37°C	Decrease the degradation rate.	Wang & Zhao, 2016
Preheated silkworm pupae protein	Cyanidin-3-O-glucoside	pH 6.5, 80°C; H ₂ O ₂ oxidation 25°C	Protection	Attaribo et al., 2020
Black soybean protein isolate	Cyanidin 3-O-glucoside	pH 7.0, 85°C, 100°C	Protection	Wang & Xie, 2019
Soy protein isolate	Grape seed procyanidins	50°C	Protection	Zou et al., 2019
Ferritin (recombinant)	Cyanidin-3-O-glucoside	pH 7.5, 50°C	Improve thermal stability and photostability	Chen & Hagerman, 2005

9.1 | Noncovalent polyphenol–protein interactions

It is also well known that the noncovalent polyphenol–protein affinity is dependent on the polyphenol structure (Xiao et al., 2011). Many of the studies focused on the effect of polyphenols–proteins interactions on polyphenol stability and bioactivity examined polyphenols encapsulation by specific purified proteins (Hu, Liu, Zhang, & Zeng, 2017; Shpigelman, Israeli, & Livney, 2010) or interactions with specific bioactive proteins (Sugiyama et al., 2007). Some studies also monitored the stability of polyphenols in whole protein-rich foods, for example, milk and milk-based products (Song, Manganais, & Ferruzzi, 2015). The effect of the presence or interaction with proteins on the stability of polyphenolic compounds could play a major role in the engineering of processing (Song et al., 2015), shelf life (Shpigelman et al., 2010), and the digestive fate (Nagar, Okun, & Shpigelman, 2020) of polyphenol-rich products. In addition, when discussing bioactivity studies, from an analytical point of view, often the culture media contain proteins that can affect the outcome of the bioactivity study (Tang et al., 2017).

Table 4 summarizes the reported effects of protein–polyphenol interaction on polyphenols stability. It can be observed that most studies focused on anthocyanins, likely due to their sensory importance (color), chemical instability, relative solubility, and the possibility to determine their concentration by monitoring the visible spectra. It can be observed that practically in all reported cases, the chemical stability during exposure to degrading external conditions (time–temperature combination, H₂O₂, light, etc.) of tested compounds increased in the presence of proteins. The increased stability is often reported to be related to the affinity, even though further work is still needed to verify the protective mechanism. Several studies tested also the effect of preheating for the formation of the complexes (Attaribo et al., 2020; Chen et al., 2019; Chung, Rojanasathira, Mutilangi, & McClements, 2015; He et al., 2016; Shpigelman et al., 2010), in some cases showing that preheating enhances interactions (Shpigelman et al., 2010) and can improve stability (beyond the effect of the nonpreheated protein), yet an optimal preheating temperature was also reported to exist (Attaribo et al., 2020). Although noncovalent polyphenol–protein interactions

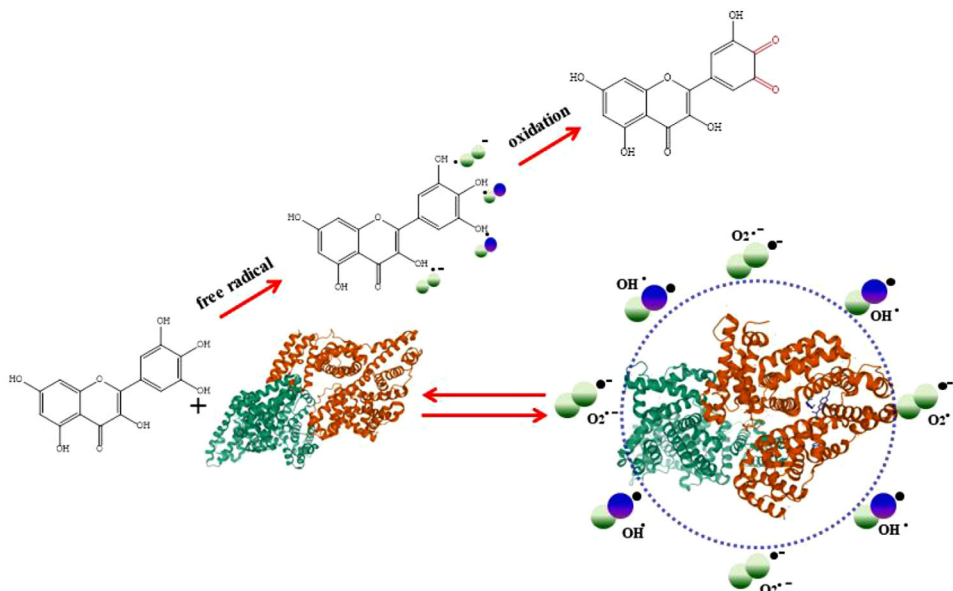


FIGURE 9 Suggested protective effect of polyphenol–protein interaction against the oxidation of polyphenols by free radical

are well known to be polyphenol structure dependent, less is known on how the protein structure and amino acid sequence affect the stability (except for the improved interaction of preheated proteins), yet a difference between the impact of different proteins was reported (Wang & Zhao, 2016). It was suggested that noncovalent polyphenol–protein interaction protects polyphenols against the oxidation caused by free radical (Figure 9).

He et al. (2016) evaluated the interactions between α - and β -casein with malvidin 3-O-glucoside (10) using a number of methodologies. Casein–malvidin mixtures were exposed to thermal (80°C/2h), oxidation (0.005% H_2O_2 /1 h), and photostability (5000 lux/5 days) analyses, which showed significant improvements due to protein–phenolic interactions. For example, thermal degradation rate decreased by 37%, chemical oxidation by 18%, and photodegradation by 29%. Authors proposed that their findings may be due to the formation of casein–malvidin complexes, which likely occurred by a combination of hydrogen bonds and hydrophobic interactions. Thus, casein-containing food products may be enriched with anthocyanins, which can protect them against thermal, chemical, and photodegradation.

For some polyphenols, several studies also dealt with the effect of pH. Interestingly, oxidation of pentagalloyl glucose by NaO_4 shifted the formation of quinone to a lower pH (Chen & Hagerman, 2005). A combination with additional biopolymers is also currently studied presenting further protective effect (Cuevas-Bernardino et al., 2018). It is important also to note that after oxidation, covalent interactions can form between the polyphenol degradation products and the protein, changing the protein's functional properties (Prigent, Voragen, Visser, van Koningsveld, & Gruppen, 2007). Furthermore, studies at relatively high pH did not confirm the lack of covalent bonds' formation that is known to occur at alkaline conditions (Liu et al., 2019) and should be further verified. Although the affinity of polyphenol–protein interaction as a function of polyphenol structure is relatively

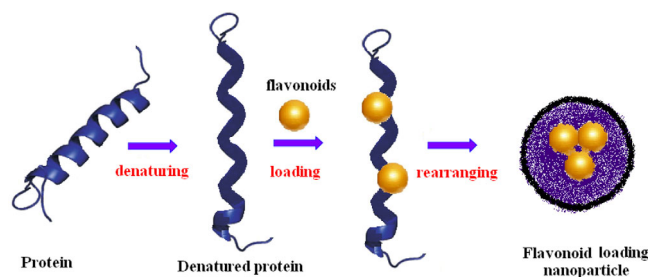


FIGURE 10 Polyphenol loading nanoparticles with denatured protein via rearrangement

well described, still a significantly larger gap exists regarding the impact of such interactions on polyphenols stability, especially for nonanthocyanin polyphenols.

9.2 | Covalent polyphenol–protein interactions

The polyphenol loading nanoparticles can form between denatured protein and polyphenol after rearrangement (Figure 10). Numerous parameters are modified when β -lactoglobulin and caffeic acid are coupled together, in contrast to individual molecules. For example, Abd El-Maksoud et al. (2018) showed that thermal stability, radical scavenging (DPPH), and inhibition of lipid oxidation in emulsions increased when these compounds were covalently linked. Based on their data, authors suggest that the phenolic–protein structure improves the functionality of the protein, which could be used as an ingredient in food products, with particular emphasis on its increased antioxidant activity.

Liu et al. (2018) prepared a nanoparticle core–shell system (zein–EGCG as hydrophobic core and rhamnolipid as shell) and used it to encapsulate curcumin and resveratrol. The system's stability was

sensitive to acid conditions, but was stable over a wide pH range. The stability of curcumin was significantly improved, because 50% of the molecule degraded over the first 40 min and 65% after 120 min; in contrast, only 20% was lost when encapsulated. Authors propose that the enhanced stability was due to inhibition of deprotonation and direct contact with the exterior aqueous phase. Regarding resveratrol, its stability was increased when exposed to UV radiation for 1 h, according to a 90% compound retention when encapsulated, as compared to 65% when free. Additional experiments also showed an adequate performance under digestion and enhanced antioxidant capacity, suggesting that the protein–lipid system exerted significant benefits to the stability and functionality of compounds tested. Correia, Grace, Esposito, and Lila (2017) used wild blueberry pomace as source of phenolic compounds, which were then encapsulated (spray drying) using either wheat flour, chickpea flour, coconut flour, or soy protein isolate (SPI). Authors determined that SPI was the most effective material among those tested, because it had the highest phenolic incorporation, while also effectively protecting these compounds against degradation during a 16-week storage period at 4 and 20°C. According to these results, protein–phenolic interactions can exert significant improvements on phenolic compounds; however, it is apparent that choosing the correct type and/or source of protein is crucial, because each protein tested behaved significantly different. Pham, Wang, Zisu, and Adhikari (2019a) evaluated covalent modification of flaxseed proteins with flaxseed phenolics, ferulic acid, and hydroxytyrosol. When covalently conjugated, the thermal stability and antioxidant capacity were increased. Authors therefore suggested that their system can be used to stabilize bioactive ingredients, as well as other food-related applications. In a related paper (Pham, Wang, Zisu, & Adhikari, 2019b), they add that phenolic compounds can be used to stabilize flaxseed protein emulsions, while also increasing their antioxidant capacity. Most of these results are related to changes in protein structure, such as folding/unfolding, as well as molecular characteristics of the particular phenolics used. Zhou, Lin, Xu, Meng, and Dong (2020) evaluated phenolic–protein interactions between EGCG and SPI. The covalent and noncovalent interactions allowed formation of an SPI–EGCG complex, both of which were stable, although covalent ones performed better. EGCG was found to have a higher tolerance to oxidation and thermal degradation, which the authors propose was indicative of a protective effect on the compound. Further experiments showed that protection extended to the digestive process, where stability was also increased. Thus, their evidence showed that SPI–phenolic complexes may be used as functional food ingredients.

10 | ENCAPSULATION OF PHENOLIC COMPOUNDS WITHIN EMULSION-BASED NANOCARRIERS

Phenolic compounds have received a great attention in both pharmaceutical and nutraceutical industries due to their diverse health-promoting benefits (Rahaiee, Assadpour, Esfanjani, Silva, & Jafari, 2020). Alongside their desirable properties, there are some drawbacks,

such as adverse effects on the organoleptic characteristics of the final products including color, odor, and a bitter flavor (Faridi Esfanjani & Jafari, 2016). On the other hand, they have a low water solubility and stability, which diminishes their biological functions as they cannot reach the target organs safely and efficiently (Assadpour, Jafari, & Esfanjani, 2017). A further problem is the sensitivity of the phenolic compounds against food processing/storage conditions or the gastrointestinal environment leading to their degradation and a low bioavailability (Faridi Esfanjani, Assadpour, & Jafari, 2018). Therefore, development of appropriate carriers and delivery systems has been one of the best strategies to tackle these issues. Among different proposed encapsulation techniques, nanocarriers based on emulsions have been very popular within the scientific community (Akhavan, Assadpour, Katouzian, & Jafari, 2018; Yousefi, Ehsani, & Jafari, 2019).

Fortunately, emulsion-based nanodelivery systems have a high versatility and different options can be proposed for the nanoencapsulation of phenolic compounds by these cargos including (i) simple nanoemulsions with a dispersed phase droplet size of 100–500 nm and in the two forms of water-in-oil (W/O) or oil-in-water (O/W) nanoemulsions (Assadpour & Mahdi Jafari, 2019)—for hydrophobic phenolics such as curcumin (Rafiee, Nejatian, Daeihamed, & Jafari, 2019) and hydrophilic ones such as anthocyanins (Sharif, Khoshnoudi-Nia, & Jafari, 2020), O/W and W/O nanoemulsions can be used, respectively; (ii) double nanoemulsions that can be simply defined as the three-phase systems made of alternative oil and water phases (Mehrnia, Jafari, Makhmal-Zadeh, & Maghsoudlou, 2017); the two most important double nanoemulsions are $W_1/O/W_2$ and $O_1/W/O_2$ systems that are designed for the hydrophilic and hydrophobic phenolics entrapped within W_1 and O_1 phases, respectively (Gharehbeglou, Jafari, Homayouni, Hamishekar, & Mirzaei, 2019). An advantage of these nanocarriers is the possibility of the co-encapsulation of another bioactive compound or antioxidants in the middle phase/layer, which can result in a higher efficiency for the main encapsulated phenolic compound and its protection; (iii) microemulsions (droplet size <100 nm) have also been used to encapsulate phenolics and they have a much better stability than nanoemulsions, although their main drawback is the high usage of chemical surfactants (Mehrnia, Jafari, Makhmal-Zadeh, & Maghsoudlou, 2016); similarly, they can be in the form of both O/W and W/O emulsions appropriate for hydrophobic and hydrophilic phenolics, respectively; (iv) multilayer-coated nanoemulsions or double nanoemulsions that have an extra layer of a biopolymer or complexed/conjugated biopolymers at their oil–water interface rather than simply a single layer of emulsifier/surfactant (Shamsara, Jafari, & Muhidinov, 2017). Different proteins (whey proteins, caseinate, and soy proteins) and polysaccharides (pectin, chitosan, and gums) have been utilized as the coating layer of dispersed phase droplets in these nanodelivery systems for the phenolic compounds such as olive leaf phenolic extract (Mohammadi, Jafari, Assadpour, & Esfanjani, 2016); and (v) Pickering nanoemulsions that are stabilized by colloidal organic/inorganic nanoparticles such as silica, chitosan, caseinate, and many other nanoparticles (Jafari, Doost, Nasrabadi, Boostani, & Van der Meer, 2020). In fact, these systems do not have a conventional emulsifier/surfactant at their oil–water interface and their formation

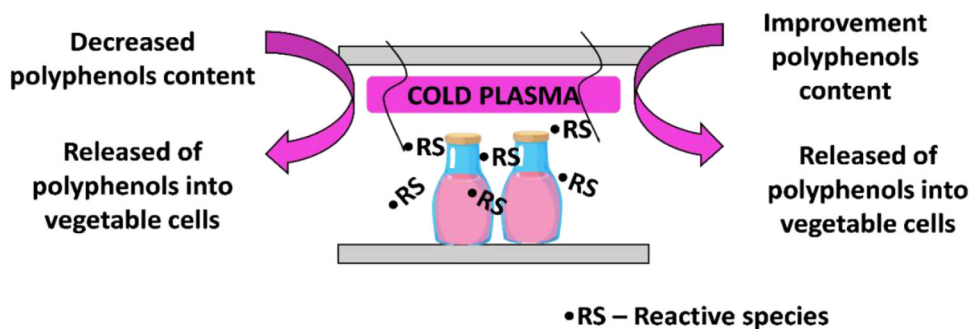


FIGURE 11 Mechanisms of action of cold plasma in polyphenols

and stability is due to coverage of dispersed phase oil or water droplets by colloidal solid nanoparticles. These nanodelivery systems have also been applied for the encapsulation of phenolic compounds (Rezaei, Fathi, & Jafari, 2019).

There are some other lipid-based nanocarriers that can be considered somehow as an emulsion system because they are also composed of dispersed nanodroplets/nanoparticles stabilized by emulsifiers including solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), nanoliposomes, niosomes, hexosomes, and cubosomes (Dima, Assadpour, Dima, & Jafari, 2020). In SLNs, a solid lipid nanoparticle loaded with phenolic compounds is dispersed in an aqueous phase and stabilized/coated by emulsifiers, similar to NLCs, whereas the latter is composed of a combination of liquid oil and solid fat within the nanoparticles (Katouzian, Esfanjani, Jafari, & Akhavan, 2017). Nanoliposomes are vesicular nanoparticles made with phospholipids such as soy lecithin that have a polar core (suitable for hydrophilic phenolics) and a nonpolar bilayer surrounding this core (for the entrapment of hydrophobic phenolics) (Maqsoudlou, Assadpour, Mohebodini, & Jafari, 2020). There is a special nanoliposome designed for phenolic compounds called nanophytosomes that are approximately like nanoliposomes, but the interaction/entrapment of phenolics happens at the head polar groups of bilayer rather than encapsulation within the central core (Babazadeh, Jafari, & Shi, 2019). Niosomes are structurally similar to nanoliposomes made with small surfactants including Tween or Span, instead of using phospholipids (Koshani & Jafari, 2019). Finally, cubosomes/hexosomes are liquid crystalline phase surfactants with characteristic morphologies and are applied for the nanoencapsulation of different bioactive compounds including phenolics (Faridi Esfanjani et al., 2018).

In order to prepare emulsion-based nanodelivery systems for the encapsulation of phenolic compounds, generally there are two available techniques called high-energy and low-energy methods (Jamali, Assadpour, & Jafari, 2020). In the first group, emulsion nanocarriers are fabricated with special equipment of a high energy density including ultrasonication devices, high-pressure homogenizers, and microfluidizers. In low-energy methods, the fabrication of emulsion-based nanocarriers is based on the art of formulation and ingredients so that some routine lab equipment is enough such as stirrers and rotor–stator mixers/homogenizers (Jafari, Paximada, Mandala, Assadpour, & Mehrnia, 2017).

11 | COLD PLASMA TREATMENT

To date, nonthermal technologies have been considered significantly interesting as efficient alternative to conventional thermal methods (such as pasteurization), because they allow the preservation of the original nutritional and sensory characteristics of fresh foods (Bursac Kovačević et al., 2016), which can be compromised with thermal processing of foods. Cold plasma is an emerging process technology that is widely applied for surface and materials modifications and is under investigation for applications in the biomedical arena. Both of these sectors rely heavily on noble gases in plasma processes, which is unsustainable for applications in the food and ingredients processing sector; thus, research and development is focused on systems and processes employing air as the inducer gas. Cold plasma has gained significant interest as a nonthermal technology for food processing (Pankaj, Wan, & Keener, 2018), mainly because it can be integrated with standard unit processes within fresh food preparation such as washing (Schnabel et al., 2020) or packaging stages (Ziuzina et al., 2020). The advantages are based on the relatively stable composition of food during and after processing, the low energy cost, and short processing time (Alves Filho, de Brito, & Rodrigues, 2020).

Phenolic compounds are usually affected by oxidation, reduction, or photochemical reactions occurring at the plasma–liquid interface, which can result on their degradation due to the presence of nitrogen, oxygen, as well as other gases (Figure 11) (Castro et al., 2020a; Fernandes, Santos, & Rodrigues, 2019). However, plasma processing has potential to increase the bioavailability of these compounds, depending on the tunability of the plasma processing parameters such as treatment time, discharge type, inducer gas and flow rate, and mode of exposure. Free radicals from cold plasma can interact with the membrane of plant cells, causing damage to their structure and releasing intracellular polyphenols (Porto et al., 2020; Sousa et al., 2011). Studies to date have focused both on extraction, release, and concentration of bioactive or polyphenol compounds and on the development of a minimal process to maintain polyphenols or interfere with the action of PPO. Ravindran et al. (2019) demonstrated significant delignification in the plant material feedstock of dried brewers' spent grain. Castro et al. (2020b) observed that different frequencies of plasma excitation can influence the concentration of polyphenols in camu-camu juices, resulting in the increase of the TPC when the excitation frequency was

increased. Increased oxygen concentration in the cold plasma process increased the phenolic compounds' concentration in blueberry juices (Hou et al., 2019). Time and synthetic air flow combination favored the release of polyphenols from plant cells and camu-camu juices (Castro et al., 2020a), as well as cashew (Rodríguez, Gomes, Rodrigues, & Fernandes, 2017) exposed to cold plasma technique. TPC of cloudy apple juice was increased by 69% and 64% after 4 and 5 min of cold plasma treatment, respectively, using spark discharge mode to treat the juice (Illera et al., 2019). The same study reported useful inactivation of PPO within 5 min of treatment, which was attributed to the action of the plasma treatment as the heat controls to 41°C did not result in PPO inactivation in cloudy apple juice. During storage for up to 28 days, all plasma-treated cloudy apple juice samples had a decrease in the TPC content, but after 28 days, these values were still higher than in the untreated juice (Illera et al., 2019).

The diversity of plasma system configurations requires that the system, process, and target parameters are clearly defined when seeking to enhance or retain polyphenol content or activity in plasma processes for foods and bioactives. Nonetheless, there is opportunity for extraction of functional polyphenols from co-products in food processing to support a circular bioeconomy, in addition to enhanced polyphenol retention and availability in plasma processed foods. The combination of delivery and stability systems with cold plasma processing for polyphenols bioactivity retention or improvement is under-explored to date.

12 | EFFECT OF COOKING ON THE STABILITY OF POLYPHENOLS

Cooking is the process of producing edible and safe foods by preparing and combining ingredients, and (in most cases) applying heat. Without cooking, many foods would be unsuitable for human consumption. By the effect of cooking, food is preserved, food safety is ensured, and some properties of foods including their nutritional value, quality, and structural properties may be enhanced. The effects of cooking on food constituents depend on the cooking technique and the characteristics of the food material (Hidalgo & Zamora, 2017), and could be positive, negative, or neutral depending on these parameters. For example, thermal treatments may cause the formation of new bioactive molecules (Maillard reaction products) but also impair the stability of some important compounds such as vitamins and polyphenols (Kucner, Papiewska, Klewicki, Sójka, & Klewicka, 2014; Tamanna & Mahmood, 2015).

Polyphenols are commonly known for their low thermal stability. Even so, it is possible to maintain some of their stability with an optimal combination of time and temperature of the treatment applied. In thermal treatments, as reported in many food matrices, high-temperature, short-time (HTST) treatment can be less destructive on food components compared to that of low-temperature, long time treatments. This is also the case for polyphenols as reported in many studies. For example, Cheng, Xiang, Liu, and Zhu (2019) investigated the stability of polyphenols and their antioxidant capacity in mulberry juice-enriched dried minced pork slices concerning different drying and baking times

and temperatures. In the study, four different treatments were performed, including two different temperatures and time conditions for drying (40°C/10 h or 55°C/6 h) followed by baking (120°C/5 min or 150°C/3 min). They have reported that the TPC, TFC, and total antioxidant capacity decreased in all treatments. Combined treatment of 40°C/10 h (drying)-150°C/3 min (baking) exhibited the lowest decrease (27.87%) in the total antioxidant capacity. This treatment also showed a loss of 32.42% in TPC and 52.01% in TFC. Another treatment (40°C/10 h [drying]-120°C/5 min [baking]) showed a lower level of loss in TFC (51.38%) but the loss in TPC (33.68%) and total antioxidant capacity (33.43%) was higher.

The positive effects of high-temperature treatments observed in some studies could be related with the fact that some new compounds with antioxidant activity may be formed during thermal treatments while some others are lost (Jeong et al., 2004). Therefore, to better understand the effect of thermal treatments on polyphenol stability, investigations on specific compounds are necessary instead of measuring the total polyphenol content or antioxidant activity. In a study performed by Hirth, Preiß, Mayer-Miebach, and Schuchmann (2015), the effect of HTST extrusion cooking on the stability of TPC as well as the main polyphenols (hydroxycinnamic acids, cyanidin glycosides, and cyanidin aglycone) in chokeberry was investigated. The authors reported a decrease of total polyphenols (up to 19%) and anthocyanins (42-90%) in all extrusion trials, varying screw speed (300 and 500 1/min), barrel temperature (100 and 140°C), and water content (15 and 22 g/100 g, wet basis), but changes in these parameters had no significant ($p < 0.05$) effect on the retention of procyanidins and hydroxycinnamic acids. The highest retention of cyanidin glycosides (about 60%) was obtained at the highest water content (22 g/100 g, wet basis), irrespective of the barrel temperature and the screw speed. This study indicated that although heat treatments may reduce the amount of some phenolics, the degree of influence for each specific compound may vary.

Although the effect of thermal treatments on polyphenols has been widely investigated in different studies, these effects may differ when examined in terms of cooking methods considering the differences in the cooking parameters and environment. For example, phenolic compounds may get lost in some cooking processes such as boiling owing to the drain and loss in water. On the other hand, phenolic compounds mostly increase in processes at high temperatures with the addition of oil such as frying. Therefore, cooking method and conditions are also important factors in the stability of polyphenols. Ramírez-Anaya, Samaniego-Sánchez, Castañeda-Saucedo, Villalón-Mir, and De La Serana (2015) evaluated different domestic cooking methods (deep-frying, sautéing, boiling in water, and water/oil) on polyphenols of Mediterranean vegetables including pumpkin, tomatoes, eggplant, and potatoes. They found that the behavior of different phenolic compounds showed different trends according to the cooking method, and there was no correlation between the method and the level of either an increase or decrease of the investigated compounds. TPC increased with deep-frying cooking in all samples and the highest increase was observed in tomatoes (from 0.21 to 5.38 mg GAE/g fresh weight). Boiling in water decreased the TPC in potatoes and pumpkin, but

an increase was observed in eggplant. Sautéing increased the TPC of pumpkin but did not show a significant difference for other samples. In another study, Lima et al. (2017) investigated the effect of three different cooking methods including boiling, steaming, and microwave on cassava. TPCs of cassava samples increased with all treatments, boiling having the lowest increase (from 10.93 to 16.59 mg GAE/100 g), which is probably as a result of the pass of phenolics from the food matrix to water and this causes a loss in some phenolics. The effect of draining was investigated in another study and the authors reported that keeping water increases the TPC (Teixeira-Guedes, Oppolzer, Barros, & Pereira-Wilson, 2019).

Zhao et al. (2017) studied the influence of different cooking methods including boiling, baking, and microwave on polyphenols in blueberry. Caffeoylquinic acid, catechin, and quercetin glycosides were found to be the most stable compounds. Authors also mentioned that when they compared the stability of caffeoylquinic acid with the other studies in the literature, they observed differences in different vegetables and fruits in addition to the variation caused by the cooking method. The effect of baking for 5 min and boiling for different times (1, 3, and 10 min) did not result in a significant difference when compared with the fresh samples. Microwave cooking was indicated as the least effective method due to the highest loss in caffeoylquinic acid content (23.4%–29.0%) as a result of this process. Nevertheless, there are some studies in which different results using microwave cooking were observed. For example, Yilmaz (2019) reported that the microwave cooking showed the most positive effect on emmer bulgur instead of traditional and autoclave applications. However, Zhang and Hamazu (2004) observed no difference between the conventional and microwave-cooked broccoli.

In summary, it is known that thermal treatments destabilize polyphenols but also result in the formation of new compounds and may cause better extractability. On the other hand, when the cooking methods are compared it can be observed that there are many studies that reported that TPC increases (Jesús Ornelas-Paz et al., 2010; Teixeira-Guedes et al., 2019) or decreases (Melini, Panfili, Fratianni, & Acquistucci, 2019; Ti, Zhang, Li, Wei, & Zhang, 2015; Xu & Chang, 2008) after cooking depending on various factors. Therefore, the optimum cooking method should be determined for each food material and cooking method individually.

13 | EFFECT OF STORAGE ON THE STABILITY OF POLYPHENOLS

Various changes may occur in foods during storage depending on the storage conditions such as temperature, moisture, atmosphere, and time. Several compounds in foods degrade by the time and thus their nutritional value and quality decrease. As a result, it is important to find the optimum storage conditions to keep the stability of food components during storage. Polyphenols might easily degrade by the effect of some factors such as light, heat, oxygen, chemical modification, enzyme, and metal ions (Deng et al., 2018). Loss of phenolics during storage is inevitable. However, it can be prevented at least for a

while by selecting the optimum storage conditions for each specific product.

In order to improve the stability of food components, adjusting the temperature is the most common method during the storage of foods where the chemical reactions slow down at low temperatures and the degradation of bioactive compounds is delayed. In a study, Klimczak, Malecka, Szlachta, and Gliszczyńska-Świągło (2007) investigated the polyphenol stability and antioxidant activity of orange juice during storage at 18, 28, and 38°C for 2, 4, and 6 months. DPPH and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) tests were carried out to measure the antioxidant activity. TPC decreased as time progressed and temperature increased. At the end of the storage period, TPC and vitamin C contents of samples at various temperatures decreased approximately by 10%–22%. On the other hand, a slight increase (3%–8%) was observed in the antioxidant activity with only DPPH test, during first 2 months, which was associated with the Mailard reactions. After 6 months of storage, a decrease was observed in the antioxidant activity for samples at various temperatures by 18%–84% and 23%–57% according to the DPPH and ABTS tests, respectively. Besides, they reported that hydroxycinnamic acids and vitamin C were the most affected compounds, whereas narirutin (19), hesperidin (20), and didymin (21) were highly stable. In a similar study, Yang, Gadi, Paulino, and Thomson (2010) investigated the changes in phenolics and antioxidant activity of noni (*Morinda citrifolia* L.) juice and powder during storage at 4 and 24°C for 12 weeks. DPPH and ABTS methods were carried out for the assessment of antioxidant activity. They found similar results with the previous study indicating that the TPC and antioxidant activity decreased as time progressed and temperature was increased. TPC of noni juice was decreased by about 42% after 12 weeks of storage, whereas the percent reduction of TPC in noni powder was 2.9 times less than that of noni juice. They also investigated the effect of light by illuminating samples with fluorescent light. Samples that were illuminated with fluorescent light showed weaker polyphenol and antioxidant stability than the unilluminated ones. The results indicated that drying the sample and optimizing the storage conditions with respect to combining light and temperature parameters were significantly important for the stability of polyphenols and their antioxidant activity during storage.

Water activity is another important parameter that should be considered during storage of samples, and decreased water activities provide better stability results. Fang and Bhandari (2011) investigated the effect of water activity (changing between 0.11 and 0.44) and temperature (5, 25, and 40°C) on the storage stability of spray-dried bayberry polyphenols. They reported that loss of polyphenols increased when water activity and temperature were increased. For example, TPC of samples decreased by 6%–8% at 5°C and 7%–37% at 40°C of storage temperature. On the other hand, phenolic content also decreased approximately by 29% when the water activity of the sample was 0.44 compared to 0.11.

Lang et al. (2019) studied the effect of different drying temperatures (60, 80, and 100°C) and storage conditions including the normal atmosphere, nitrogen atmosphere, and vacuum atmosphere on black rice phenolics. TPC decreased up to 7% when the drying temperature

was increased. However, no decrease was observed in the content of free phenolics at all drying temperatures when stored under nitrogen atmosphere. As an inert gas, nitrogen may protect polyphenols from oxidation reactions, which may improve their stability. This was associated with an increase in enzymatic hydrolysis due to the increase in metabolic reactions in the conventional atmosphere. Bound flavonoids were converted into free flavonoids by the breakdown of the cell wall.

Instead of controlling storage conditions, some pretreatments may also increase the polyphenol stability. For example, Rękas, Ścibisz, Siger, and Wroniak (2017) applied microwave radiation (0, 2, 4, 6, 8, and 10 min, 800 W) to rapeseed and pressed the oil afterward. Then they investigated the effect of this treatment on phenolic compounds during storage for 12 months. Microwave pretreatment increased all detected major phenolic compounds (canonol, *trans*-sinapic acid, ferulic acid, *p*-coumaric acid, and sinapine) in the rapeseed oil. The highest increase was observed in canonol from 28.66 to 1807.63 $\mu\text{g/g}$ after microwave treatment for 10 min. In addition, microwave-treated samples were found to be more stable concerning phenolic compounds compared to nontreated samples, especially with a microwave pretreatment for 10 min. It should be noted that increasing the stability of polyphenols is generally based on slowing down the chemical reactions. Therefore, all these applied methods have shown favorable effects on improving the storage stability of polyphenols for almost all food products.

14 | CONCLUSION AND PERSPECTIVE

There is a great demand for high-added-value compounds such as polyphenols (flavonoids, stilbenoids, lignans, ellagic acids, and polymers of phenolic acids and flavonoids, namely, hydrolysable and condensed tannins). In this sense, the development of stability-friendly processing procedures, controlling the humidity, heating, pH, oxygen, light, interaction with metal ions and other food components, enzymes, high pressure, and *in vitro* digestive process contribute to the continued growth of this market. With the aid of mass spectrometry and NMR analysis, it can also identify the structure of degradation products and their degradation kinetics. Their stability can be improved with different operations: chemical modification, nanotechnologies, encapsulation, spray drying, emulsification lyophilization, and so on. The possibility of combining different operations in this stability purpose offers interesting new insights for redesigning flowsheets based on the scientific evidence of noncovalent and covalent polyphenol-protein interactions, cold plasma use versus thermal methods, and the effects of cooking (high-temperature, short-time treatment and low-temperature, long-time treatment) and storage conditions on their stability, bioaccessibility, and bioavailability. Innovative sustainable technologies offer a wide range of advantages for the extraction, purification, and preservation of phenolic compounds, and for the storage and cooking of enriched foodstuffs. Better control of the factors impacting their stability is of paramount importance, together with a high degree of selectivity, water savings, and low energy costs in the optimized operations and designed processes. It is a promising area for future investigations aimed at improving the stability of polyphenols

that play a protective role in preventing different pathologies such as cardiovascular disease, cancer, diabetes, and obesity.

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AUTHOR CONTRIBUTIONS

HC, JSG, and JX contributed as the senior authors and the principal investigators of this study, refined the study, and wrote the first draft of the manuscript. OS, AK, ZD, GZ, CACJ, GAGA, JO, WB, CMZ, LAPdF, AS, PHC, ECG, CLH, SMJ, YQ, PL, and MW critically revised the manuscript. All authors read, critically reviewed, and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Weibin Bai  <https://orcid.org/0000-0003-1729-2970>

Esra Capanoglu  <https://orcid.org/0000-0003-0335-9433>

Jesus Simal-Gandara  <https://orcid.org/0000-0001-9215-9737>

Jianbo Xiao  <https://orcid.org/0000-0003-3311-770X>

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