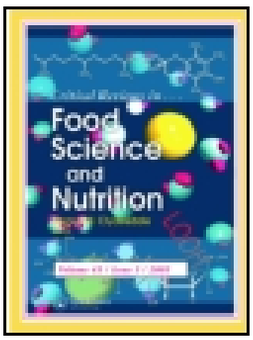

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REVIEW

Recent advances in food products fortification with anthocyanins

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ABSTRACT

Anthocyanins are polyphenolic compounds belonging to the group of flavonoids in charge of providing red, purple, and blue colourations to different parts of trees and plants, such as leaves, flowers, fruits, roots, and stems. These substances have potential health benefits due to characteristics such as antioxidant and anti-inflammatory properties, which could be leveraged in the food industry. However, the use and handling of anthocyanins are conditioned due to the low stability of these molecules. For this reason, the application of adequate extraction, purification and stabilization techniques is required for its subsequent management. In this regards, green extraction methods and novel stabilization techniques are of particular interest in the utilization of these biocompounds. This review provides in-depth information about the extraction, purification, and stabilization of anthocyanins from different plant sources. Additionally, this work highlights the potential use of anthocyanins in the food industry for the formulation of different fortified foods and beverages, which could have beneficial health effects. Green technologies, are a promising tool to recover extracts rich in anthocyanins from different vegetable matrices, including by-products. The extracts obtained can be easily used in the fortification of baked foods, dairy products, and different beverages.

KEYWORDS

Anthocyanins; green extraction methods; purification; stabilization; fortified; and functional foods

Introduction

Anthocyanins are the most essential and abundant water-soluble and vacuolar pigment found in the plant kingdom (Delgado-Vargas and Paredes-López, 2003). They are situated in leaves, flowers and fruits and other localizations such as roots, tubers, stems and storage organs of plants (Delgado-Vargas & Paredes-López, 2003; Dey & Harbone, 1993; Mazza & Miniati, 1993). Specifically, these substances are displayed in vegetables like grapes, berries, apples, red cabbage, radishes, tulips, roses and orchids, amongst others (Dey and Harbone, 1993). The primary function of anthocyanins is to impart color because these substances are responsible for cyanic hues comprising from salmon pink through red and from violet to dark blue (Cavalcanti et al., 2011). For this reason, these biochemical compounds play a vital function in the attraction of animals for pollination and seed dispersal. Furthermore, anthocyanins in plants act as an ultraviolet light protector, antioxidant, phytoalexins, antibacterial and protect against damage from certain insects (Harborne and Baxter, 1993; Kong et al., 2003).

Also, anthocyanins have been shown to possess protective and promoting effects on human health (Tarone et al., 2020). For instance, nutraceutical properties have been revealed due to its exceptional antioxidant capacity, anti-inflammatory effect and anti-biofilming activity (Fernández, García, Monte, Villar, & Lombó, 2018; Zhang et al., 2020). Simultaneously, the anthocyanin intake exerts a protective effect against several disorders such as diabetes (Wedick et al., 2012), cardiovascular diseases (Álvarez-Suarez et al., 2014), inflammation and cancer (Fernández et al., 2018). Additionally, these compounds supply colors that change/switches among orange, red, violet and blue displaying great potential as natural colorant owing to their low toxicity (Tonon et al., 2010). For this reason, certain vegetables and fruits (with their by-products inclusive) can be utilized in food, cosmetic and pharmaceutical products. Concretely, in the food industry, anthocyanins have novel applications as natural colorants and/or bioactive compounds (Santos-Buelga and González-Paramás, 2019). The main justification for this scenario is the market of functional foods ingredients. In 2017, this market was valued in more than 64 billion dollars in 2017 and has a projection of 6.6% increase for 2023 when the market value will surpass the 64 billion dollars (MarketsandMarkets, 2018).

However, the use of anthocyanins as a food additive is conditioned by the prior extraction, purification and

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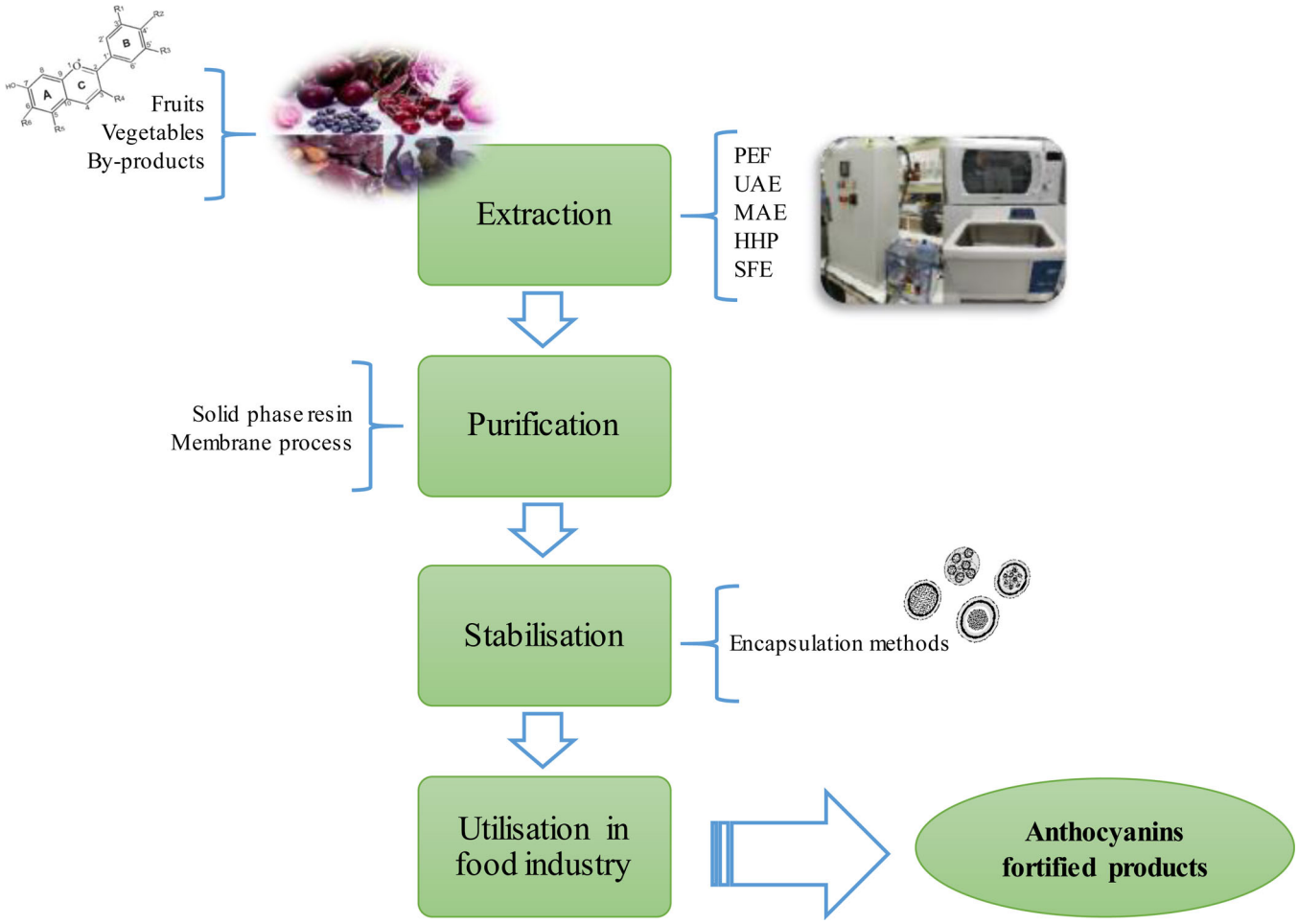

Figure 1. Operations carried out to obtain extracts rich in anthocyanins for their subsequent addition to fortified foods.

stabilization of these molecules (Figure 1) (Ghafoor et al., 2009). In light of this matter, we collate recent scientific literature and present a critical account on different anthocyanins extraction, purification and stabilization in order to improve the handling of these compounds in the food industry. Additionally, this work also explores the potential of these biomolecules as natural ingredients in the formulation of fortified foods and beverages.

Chemical structure

Anthocyanins are phenolic substances of the flavonoid family (Yousuf et al., 2016). These compounds are polyhydroxylated or polymethoxylated glycosides or acyl glycosides of their respective aglycone anthocyanin form (anthocyanidin), which are oxygenated derivatives of 2-phenyl benzo pyrylium or flavylium salts (Brouillard, 1982; Mazza & Miniati, 1993). Their chemical structure is characterized by a C15 skeleton based on C6–C3–C6 core structure which possesses eight conjugated double bonds and includes two benzoyl rings (A and B) joined by three carbons in an oxygenated heterocycle ring (C) (Figure 2) (Horbowicz et al., 2008; Shipp and Abdel-Aal, 2010; Yi et al., 2010). In nature, 23 different anthocyanidins can be found through distinct substitution patterns (Andersen and Jordheim 2006; Chemical Studies of Anthocyanins: A Review, 2009). Nevertheless,

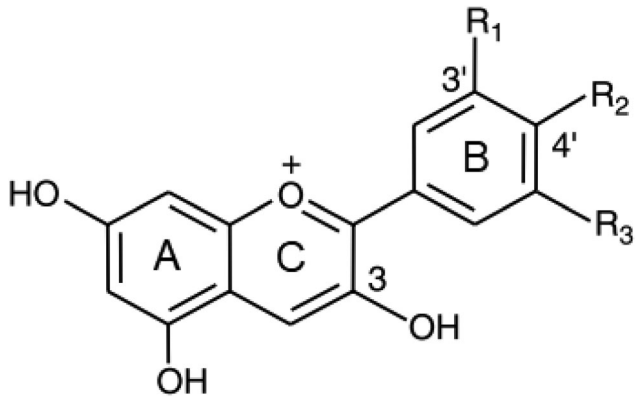

Figure 2. General chemical structure of aglycone anthocyanin form (anthocyanidin).

only six anthocyanidins are frequently displayed in plants as a consequence at differences discovered in 3′, and 5'locations of the B-ring. Concretely, these six molecules are cyanidin (Cy), delphinidin (Dp), malvidin (Mv), pelargonidin (Pg), peonidin (Pn), and petunidin (Pt). Thereby, only these six anthocyanidins are usually found forming part of the anthocyanins molecules (Ghosh and Konishi, 2007; Giuseppe Mazza, 2007; Valencia-Arredondo et al., 2020).

On the other hand, most natural sugars such as arabinose, galactose, glucose, rhamnose and xylose with their mono-, di- or trisaccharide forms are attached to anthocyanidins to form

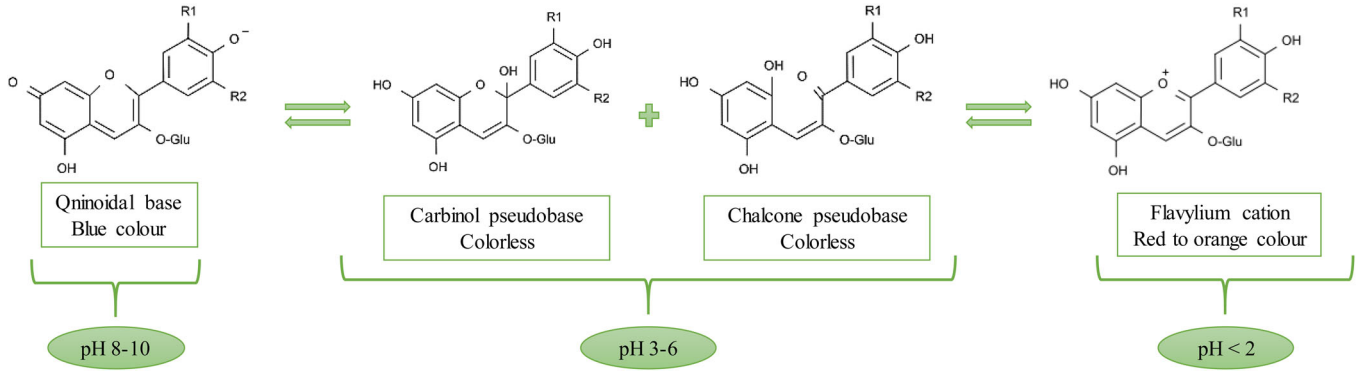

Figure 3. Effect of pH conditions on color stability of anthocyanins.

anthocyanins. They are principally bounded at the 3′-location or 5′, 7′-position on the C- and A-ring, respectively (Mazza & Miniati, 1993; Prior & Wu, 2006). Moreover, common acylating agents of the sugar residues are cinnamic (*p*-coumaric, caffeic, ferulic, sinapic, gallic and *p*-hydroxybenzoic) and/or aliphatic (acetic, malonic, malic, succinic and oxalic) acids (Giusti & Wrolstad, 2003; Mazza & Miniati, 1993). To such a degree in nature, more than 600 different anthocyanins have been described (Santos-Buelga and González-Paramás, 2019; Sigurdson et al., 2017) according to the number and position of hydroxyl and methoxyl groups on the basic skeleton; the constitution, number and localization of sugars bound to the molecule; the degree of acylation of the sugar and the identity of the acylating agent (Brouillard, 1982; Mazza & Miniati, 1993; Shipp & Abdel-Aal, 2010; Yousuf et al., 2016).

Stability and color of anthocyanins

The chemical structures of anthocyanins are closely related to their stability and color. Primarily, the conjugated double bonds present in anthocyanins allow these compounds to absorb light at a wavelength of approximately 500 nm. Therefore, these natural pigments generate red, purple, blue and intermediate hues (Karakaya et al., 2016; Mazza, Cacace, & Kay, 2004). Nonetheless, anthocyanins are easily susceptible to degradation because they are highly unstable and strongly affected by glycosylation and acylation degree, pH, temperature, relative humidity, light (UV-visible), oxygen, enzymes, ascorbic acid and other substances such as metal ions and co-pigments (Chemical Studies of Anthocyanins: A Review, 2009). In general, glycosylation and acylation improve their structural stability. For instance, di-, tri-, or polyacylated anthocyanins have improved stability compared to monoacylated and simple forms (Mazza et al., 2004). Furthermore, the degree of hydroxylation and methoxylation in the molecule affects the stability and color of these pigments. In this fashion, the major methoxyl groups present cause an increase in stability and redness, while the available hydroxyl groups result in unstable molecules and a more bluish shade (Delgado-Vargas and Paredes-López, 2003; Heredia et al., 1998). Concurrently, the pH value directly affects anthocyanin stability and color to a large extent (Figure 3). Specifically, anthocyanins are more stable at low pH values (acidic) (Giusti & Wrolstad, 2003). Moreover, at acidic conditions (pH 1-3), the flavylium cation is red or orange colored, whereas, at alkaline conditions (pH 7-8), the blue-purple quinoidal bases are originated. Nevertheless, at pH 5, the carbinol pseudobase is colorless (Ghosh and Konishi, 2007). Moreover, there is a rise in temperature and the presence of light, oxygen, and certain enzymes such as glycosidases, peroxidases and phenolases, reduce the stability of anthocyanins during food processing and/or storage (Cavalcanti et al., 2011; Markakis, 1982; Mercadante and Bobbio, 2008). Also, the presence of ascorbic acid and its breakdowns products, increase anthocyanins degradation rate (Pacheco-Palencia and Talcott, 2010).

Besides the factors mentioned above, anthocyanins association reactions also affect the stability and coloration from anthocyanins via protection of the colored flavylium cation from hydration. These reactions fall into three different categories: (1) self-association between anthocyanins through the hydrophobic interactions that take place between their aromatic nuclei; (2) associations with other compounds (copigmentation); and 3) associations of the *o*-hydroxy groups of anthocyanins with metals such as magnesium and aluminum (Cavalcanti et al., 2011). Regarding copigmentation, there are two types of copigmentation, namely inter- and intramolecular, which affect the final coloration differently (Delgado-Vargas and Paredes-López, 2003; Heredia et al., 1998). The intermolecular copigmentation is observed when anthocyanins react with some colorless substances such as aurones, flavones and flavanols, resulting in significant changes in color (Gonnet, 1999). Whereas, intramolecular copigmentation is caused by the interaction of the aromatic groups of the acyl moiety of anthocyanin with their basic structure and is more effective than intermolecular copigmentation (Figueiredo et al., 1996).

Extraction of anthocyanins

The recovery of different compounds available in vegetables, fruits and their by-products is a crucial step in bioactive substances production and their subsequent use as a food supplement, nutraceutical and/or food additive (Ghafoor et al., 2009). With anthocyanins as no exception, their extraction processes/operations acquire a particular interest due to high instability and easy degradation ability of these molecules (da Silva Carvalho et al., 2016; Giusti and

Wrolstad, 2003). For example, on account of their sensitivity to pH and thermal susceptibility, high pH values and temperatures (above 45 °C) can decrease anthocyanin concentrations (Cacace and Mazza, 2003; Condurache et al., 2020).

Before anthocyanin extraction, the origin matrices are usually prepared with various pretreatments which favors the anthocyanins release from the cell organelles, improving their accessibility. This pretreatment process includes particle size reduction. It increases the contact area and promotes solid particles diffusion into the solvent (Dutta, 2007; Lorenzo et al., 2018). Furthermore, other pretreatment processes, such as lyophilization and drying, are used. These processes help to concentrate anthocyanin content of the matrix (Chemical Studies of Anthocyanins: A Review, 2009). The extraction process can be carried out after the prior sample conditioning/preparation of the sample has been accomplished. On this matter, the classical standpoint employed to recover anthocyanins from plant tissues was based on solvent extraction (Chemical Studies of Anthocyanins: A Review, 2009). These traditional methods are customarily performed with polar substances since these solvents facilitate the dissolution of anthocyanins (Kerio et al., 2012; Silva et al., 2017). In this way, combinations of ethanol, methanol or acetone and water are frequently used to optimize process efficiency (Abdel-Aal et al., 2018; Vayupharp and Laksanalamai, 2015). In the case of pure water, although it is a polar and an innocuous solvent, the dissolution of anthocyanins in this solvent is hindered mainly due to the interactions with ionic carbohydrates present in the source matrices. Therefore, it is recommended to combine it with other compounds or solvents (Fernandes et al., 2014; Takahama et al., 2013). Moreover, due to the low stability of anthocyanins in non-acidified solutions, extraction solvents are usually acidified with different acids such as acetic, formic, phosphoric and hydrochloric acid (Hong et al., 2020; Kong et al., 2003; Yousuf et al., 2016). Additionally, high temperatures are favorable to aid the release of anthocyanins in traditional solid-liquid extraction. These high temperatures help to degrade the cellular structures where various anthocyanins are located (Bursać Kovačević et al., 2015).

Nevertheless, the use of these conservative methods has several disadvantages in anthocyanins recovery (Ghafoor et al., 2009). Some of these drawbacks include highly toxic nature of solvents such as methanol acidified with hydrochloric acid, propanone and n-hexane to human; low target compound recovery rate; and high energy consumption due to high-temperature usage. This could degrade the anthocyanins (Barba et al., 2016; Cavalcanti et al., 2011; Lorenzo et al., 2018). Currently, there is no single standardized solvent extraction method. The choice of method depends on factors such as its cost, accessibility, simplicity, efficiency and its range of application (Ignat et al., 2011). Due to these demerits as mentioned above, the use of the new green technologies such as pulsed electric field, ultrasounds, microwave, high hydrostatic pressurized and supercritical fluids extraction favor the extraction of high-quality and minimally processed anthocyanins extracts (Table 1). Simultaneously, these techniques favor the reduction of treatment duration and temperatures. They also increase mass transfer processes and the extraction yields, while minimizing the utilization of organic solvents and the energy consumption (Barba et al., 2016).

Pulsed electric field treatment

Pulsed electric field (PEF) treatment involves the formation of temporary (reversible) or permanent (irreversible) pores in the cell membranes. This phenomenon, known as electroporation, facilitates the mass transfer process and consequently, anthocyanins release into the medium (Corrales et al., 2008; Gachovska et al., 2010; Galanakis, 2012; Knorr & Angersbach, 1998; Koubaa et al., 2015). The working conditions commonly used in the PEF technique include exposures of the sample to moderate electric field with voltages ranged between 1-10 kV/cm, specific energies (approximately 10 kJ/kg) and the number of pulses (between 5 and 50) at ambient temperature (Barba et al., 2016; Silva et al., 2017). Corrales et al. (2008) observed that the PEF treatment of grape (Dornfelder *Vitis vinifera* ssp.) skin increased anthocyanin extraction up to 17% compared to conventional and ultrasonic extraction (14.05 *vs* 7.93 and 7.96 mg Cy-3-glu eq/g DM, respectively), and by 10% when compared to high hydrostatic pressure (HHP) assisted extraction (14.05 mg Cy-3-glu eq/g DM for PEF extraction *vs* 11.21 mg Cy-3-glu eq/g DM for HHP-treatment). Furthermore, they observed that PEF improved the anthocyanin monoglycosides recovery (Corrales et al., 2008). These results were in agreement with work by (Puértolas et al., 2013). The authors observed that after the treatment of purple-fleshed potatoes (*Solanum tuberosum* var. Vitelotte) with PEF method, the extraction yields were always higher, regardless of the extraction temperature and the solvent The concentration of anthocyanins were between 8.1 and 63.9 mg/100 g FW in the untreated purple-fleshed potatoes and amounts among 14.1 and 67.9 mg/100 g FW in the PEF treated purple-fleshed potatoes. (Gachovska et al., 2010) also observed that PEF technique improved the total extraction of anthocyanins in red cabbage (3.46 *vs* 1.65 µg/mL for PEF and control treatment, respectively). Thus, the PEF extraction was 2.15 times greater than the extraction without this green method. Furthermore, these authors determined a higher proportion of non-acylated anthocyanins in PEF extracts.

Ultrasound-assisted extraction

This extraction process is characterized by the generation of cavitation bubbles, whose vibration creates fluid currents and disruptive forces in nearby cells and particles (Golmohamadi et al., 2013). The ultrasonic favors the hydration of the vegetal tissue and cause enlargement of the pores, and sometimes the cell wall rupture via the sonication effect (Espada-Bellido et al., 2017). This enlargement and breakage facilitate the transfer of matter, permitting cellular contents washing and anthocyanins recovery (Huie, 2002; Ravanfar et al., 2015). The operating conditions of power

Table 1. Anthocyanin concentration and major findings in anthocyanin recovery according to the vegetable source and the green extraction method used.

Extraction	source	Solvent	Solid: liquid ratio	Extraction conditions	Anthocyanin concentration	Major findings	References
PEF	Grape skin of Dornfelder *Vitis vinifera* ssp.	50% ethanol	1:4.5	3 kV/cm, 30 pulses, 15 s, 10 kJ/kg, 70 °C, 1h	14.05 mg Cy-3-glu eq/g DM	Extraction yield 17% greater than control Extraction yield 10% greater than HHP technique Good recovery of monoglycoside anthocyanins	(Corrales et al., 2008)
	Solanum tuberosum var. Vitelotte	Water, 48, 96% ethanol	1:10	3.4 kV/cm, 35 pulses, 105 μs, 8.92 kJ/kg, 10-40 °C, 1-8 h	14.1-67.9 mg/100 g FW	Improvement of anthocyanin extraction yield (independent of solvent or temperature)	(Puértolas et al., 2013)
	Redd cabbage	Water	1:2.5	2.5 kV/cm; 50 pulses, 750 μs, 15.63 kJ/kg, 22 °C, 4 h	3.46 μg/mL	Improvement in Extraction efficiency (2.12 times) Higher efficiencies for nonacylated anthocyanins Stable anthocyanins degradation	(Gachovska et al., 2010)
Ultrasound	Grape skin of Dornfelder *Vitis vinifera* ssp.	50% ethanol	1:4.5	35 kHz, 70 °C, 1h	7.76 mg Cy-3-glu eq/g DM	No differences with respect to control samples Lower extraction/recovery compared to PEF and HHP techniques	(Corrales et al., 2008)
	Brassica oleracea L. var. Capitata f. Rubra	Water	1:50	30 kHz, 100 W, 15 °C, 30 min	18.6 mg/L	Enhancement the yield about 2 times compared to water bath Improvement in the extraction efficiency compared to water bath	(Ravanfar et al., 2015)
	Marc *Vaccinium uliginosum* L.	70% ethanol (0.02% carnosinic acid)	1:16	80 kHz, 200 W, 55 °C, 40 min	13.95 mg/g	Improvement in extraction efficiency Reduced loss of effective ingredients	(Jin et al., 2019)
	Rubia sylvatica fruits	30% ethanol	1:20	40 kHz, 400 W, 55 °C, 20 min	22.35 mg Cy-3-glu/g	Higher yield compared to water bath Shorter extraction time compared to water bath	(Chen et al., 2020)
MAE	Lyophilizer *Morus atropurpurea* Roxb	59.6% methanol (1% TFA)	1:25	2450 MHz, 425 W, room temperature, 132 s	54.72 mg Cy-3-glu/g	Enhancement in yield compared to traditional techniques	(Zou et al., 2012)
	Red stigmas of *Crocus sativus* L.	25-50% ethanol (pH = 2 HCl)	1:77.5	360 W, 48 °C, 558 s	101 mg Cy-3-glu eq/g DM	Rapid and efficient technique Disruption of cell walls	(Jafari et al., 2019).

(*continued*)

Table 1. Continued.

Extraction	source	Solvent	Solid: liquid ratio	Extraction conditions	Anthocyanin concentration	Major findings	References
	Prunus cerasus var. Marasca	80% methanol (0.1% HCl)	1:8	2450 MHz, 400 W, 60 °C, 6-9 min	1.73 mg TA/g	Higher efficiency than conventional method	(Elez Garofulić et al., 2013)
HHP	Grape skin of Dornfelder *Vitis vinifera* ssp.	50% ethanol	1:4.5	600 MPa, 70 °C, 1 h	11.21 mg Cy-3-glu eq/g DM	Enhanced yield compared to the control 10% lower yield than in PEF technique Considerably greater extraction of acylated anthocyanins	Corrales et al., 2008)
	Grape skin of Dornfelder *Vitis vinifera* ssp.	50% ethanol	1:4.5	200-600 MPa, 30 min	8.15- 12.22 mg Cy-3-glu eq/g DM	Improved extraction performance/efficiency An optimum is achieved with 200 MPa	(Margarita Corrales et al., 2009)
	Blueberry (*Vaccinium ashei*) pomace	63% ethanol	1:41	443 MPa, 95 s	107.9 mg/100 g	Improved yield compared to a control	(Zhang and Ma, 2017)
SFE	Pressing residues of cranberry (*Vaccinium macrocarpon* Ait.)	SC-CO_2 and different mixtures of CO_2 + ethanol + water	–	40 MPa, 1.827 g/min flow rate, 50 °C	2.4-96% (compared to total solid-liquid extraction)	Similar results to solid-liquid extraction using 70% ethanol Reduce up to 88% the use of ethanol Presence of water in the solvent was essential to recovery	(Kühn and Temelli, 2017)
	Vaccinium myrtillus L waste (peel, seeds, and pulp)	90% SC-CO_2 + 5% H_2O + 5% ethanol	–	20 MPa, $1.4 \cdot 10^{-4}$ kg/s flow rate, 40 °C	1071 mg/100g	Anthocyanins recovery was satisfactory 16 different anthocyanins were identified	(Paes et al., 2014)
	Solanum melongena L. peel	SC-CO_2 + citric acid	–	10-15 MPa, 2 L/min flow rate, 40-60 °C	295-1704 Dp-3-glu eq mg/100g	Optimum efficiency achieved with 10 MPa, 60 °C Lower recovery compared to acidified GRAS solvent extraction method	Chatterjee, Jadhav and Bhattacharjee, 2013)

PEF: pulsed electric field; MAE: microwave assisted extraction; HHP: high hydrostatic pressure; SFE: supercritical fluid extraction. SC-CO_2: super critical CO_2. TFA: trifluoroacetic acid. DM: dry material, FW: fresh weight. TA: total anthocyanins; Cy: cyanidin; Dp: delphinidin; eq: equivalents. GRAS: generally recognized as safe.

ultrasound frequencies vary from 20 kHz to 1 MHz, although frequencies range of 20–100 kHz are usually employed in food processing because it favors the formation of cavitation bubbles (Golmohamadi et al., 2013; Silva et al., 2017). According to various studies, this method has shown an increase in anthocyanins extraction yield. For instance, (Ravanfar et al., 2015) found that the ultrasound applied to red cabbage (*Brassica oleracea* L. var. Capitata f. Rubra) improved the performance of the anthocyanins extracted compared to the traditional extraction in a water bath, almost doubling it. Similar results were observed for bilberry (*Marc Vaccinium uliginosum* L.) by Jin et al. (2019). The authors observed a reduction in the loss of active ingredients and an improvement in extraction efficiency. In addition, Chen et al. (2020) demonstrated that the ultrasound treatment of *Rubia sylvatica* fruit resulted in higher yields within shorter extraction period when compared to extraction using a conventional water bath. However, Corrales et al. (2008) did not find an improvement in anthocyanin extraction on grape (Dornfelder *Vitis vinifera* ssp.) skin. The investigators found that the ultrasound treatment had similar yields to conventional extraction (7.76 and 7.96 mg Cy-3-glu eq/g DM for the ultrasound and conventional extraction, respectively) and lower when compared to other new technologies such as PEF (14.05 mg Cy-3-glu eq/g DM) and HHP (11.21 mg Cy-3-glu eq/g DM).

Microwave assisted extraction

Microwave energy is a non-ionizing electromagnetic wave of frequency range 300–3,00,000 MHz (Mandal et al., 2007). In this process, molecular motion is generated by ion migration and rotation of dipoles. This motion generates a thermal disorder which stops once the electric field decreases, emitting thermal energy (Camel, 2000). In the presence of water, the internal overheating in plant matrices causes cell interruption and facilitates the extraction process (Jafari et al., 2019). Due to this, microwaved assisted extraction (MAE) has been hailed as a quick and efficient technique for the extraction of bioactive substances from vegetables (Teo et al., 2009). Nevertheless, the rise in temperature caused by this technique generates thermal degradation during anthocyanins recovery (Mandal et al., 2007). In spite of that, some works had reported improvements in anthocyanin extraction yields using MAE. According to Zou et al. (2012), MAE significantly improved the recovery of anthocyanins in a lyophilized mulberry (*Morus atropurpurea* Roxb) matrix compared to conventional extraction (54.72 *vs* 44.83 mg Cy-3-glu mg Cy-3-glu/g). In the same vein, it was observed that in saffron tepal's (*Crocus sativus* L.) the application of MAE was a rapid and efficient technique for the recovery of anthocyanins owing to disruption of cell walls generated by this treatment (Jafari et al., 2019). Additionally, Elez Garofulić, Dragović-Uzelac, Režek Jambrak, & Jukić (2013) demonstrated that MAE was more efficient than the conventional extraction for the recovery of anthocyanins in sour cherry (*Prunus cerasus* var. Marasca).

High hydrostatic pressure assisted extraction

High hydrostatic pressure assisted (HHP) extraction is a technique employed for the recovery of active ingredients through the application of a cold isostatic superhigh hydraulic pressure, which can vary between 100 to 800 MPa or more (Paul and Morita, 1971; Shouqin et al., 2005). These high-pressure conditions favor the penetration of liquid into air gaps present in plant tissues. When pressure is reduced, the air occluded in the pores leaves, causing damage to the cell membrane (Garcia et al., 2001). Simultaneously, high-pressure cause deprotonation of charged groups and disruption of hydrophobic bonds and salt bridges, consequently leading to protein denaturation (Barbosa-Cánovas et al., 1998). Additionally, during extraction, the HHP technique can reduce the medium pH during the extraction owing to deprotonation of molecules present in the extracts (Corrales et al., 2008). Therefore, HHP increases mass transfer and stability of anthocyanins, proving to be a great potential extraction technique in the food industry (Jun, 2013; Rastogi et al., 2003). Several studies demonstrated improvement in the extraction of anthocyanins through HHP technique. For example, Corrales et al. (2008) found a greater recovery of anthocyanins when HHP extraction was applied to grape (Dornfelder *Vitis vinifera* ssp.) skin compared to conventional and ultrasound extractions (11.21 *vs* 7.93 and 7.76 mg Cy-3-glu eq/g DM, respectively). Specially, these authors obtained good recoveries for acylated anthocyanins (5.15 mg Cy-3-glu eq/g DM). Nevertheless, the extraction yield was 10% less than in the case of the PEF technique for this same matrix, where 14.05 mg Cy-3-glu eq/g DM was obtained. In a later study and using the same plant matrix, it was also determined that the application of HHP favored the extraction of anthocyanins compared to its control (Margarita Corrales et al., 2009). Similar outcomes were showed by Zhang & Ma (2017) who observed improved extraction performance (107.9 *vs* 67.63 mg of anthocyanins/100g, respectively) in a blueberry (*Vaccinium ashei*) pomace matrix using assisted/tandem extraction (traditional hot water bath and HHP technique).

Supercritical fluid extraction

Supercritical fluid extraction (SFE) is a technique involving the use of a gas (mainly CO_2) above its critical temperature and pressure, so that the carrier gas displays transitional physicochemical properties between liquid and gas (Galanakis, 2012). The use of this technology allows the extractions of biocomponents in the absence of light and air, which minimize degradation reactions. Concurrently, carbonic acid is generated from the water in the extraction matrix when it contacts CO_2. Hence, SFE eliminates the use of acids in the extraction process (Paes et al., 2014). However, due to the non-polar character of CO_2, this technique sometimes employs other co-solvents such as ethanol and methanol but in lower concentrations (Barba et al., 2016). Kuhn and colleague (Kühn and Temelli, 2017) observed that anthocyanins recovery/yield using supercritical

ternary mixtures (CO_2, ethanol and water) and conventional solid-liquid extraction with 70% acidified ethanol for cranberry (*Vaccinium macrocarpon* Ait.) pomace were similar (Kühn & Temelli, 2017).In the former approach, ethanol concentration was reduced by 88%.

Also, Paes et al. (2014) found satisfactory recoveries with the SFE method for anthocyanins extraction from blueberry (*Vaccinium myrtillus* L.) wastes (peel, seeds, and pulp). The authors recovered 1071 mg of anthocyanins/100 g when water and ethanol were used as co-solvents. On the contrary, Chatterjee, Jadhav, & Bhattacharjee (2013) carried out a supercritical CO_2 extraction in citrus acidified aubergine (*Solanum melongena* L.) skin extracts, obtaining 1704 mg of anthocyanin/100 g DW under optimal conditions. The yield was lower than those displayed by extraction with acidified GRAS solvents (1975 mg of anthocyanin/100 DW).

In addition to the green technologies mentioned above, other methods can also be used to obtain anthocyanins. For instance, enzyme-assisted extraction is used to improve anthocyanin recovery from vegetables with thick cell walls and rich in pectin (Buchert et al., 2005).

Ohmic assisted extraction has also been used to increase membrane permeability by transforming electrical energy into thermal heating (Loypimai et al., 2015). However, these techniques have some disadvantages. For example, enzyme-assisted extraction may hydrolyze the anthocyanins and their aglycone counterpart (Buchert et al., 2005; Landbo and Meyer, 2001) and ohmic assisted extraction could degrade anthocyanins due to heating caused by this technology (Silva et al., 2017).

Purification and stabilization of anthocyanins

Due to the non-selectivity of the extraction techniques used, the recovery of anthocyanins from the plant matrices involves simultaneous removal of other undesirable compounds (Coutinho et al., 2004). For example, it is common to find extracts with large amounts of sugars and organic acids that can also interact with anthocyanins, degrading them and/or altering their functionality (Coutinho et al., 2004; He & Giusti, 2010). Therefore, it can sometimes be interesting to conduct purification operations once the extraction has been carried out (Silva et al., 2017). On this frontier, several techniques such as two-phase extraction and membrane processes have emerged (Valencia-Arredondo et al., 2020). Solid-phase extraction is a relatively cheap separation process widely employed. In this technique, dissolved anthocyanins are held in a resin packed column according to their physicochemical characteristics, and subsequently, they are separated from other compounds by increasing the polarity with distinct solvents (Chemical Studies of Anthocyanins: A Review, 2009; Liu et al., 2020; Meng et al., 2020; Silva et al., 2016; Liu et al., 2020; Xianzhe et al., 2015). Liu et al. (2020) purified an extract of *Lycium ruthenicum* Murr rich in anthocyanins with the help of an AK-8 macroporous resin column. The authors removed/eliminated impurities such as proteins, sugars, and polar compounds to enrich the original extract from 52.10 to 77.62% (Liu et al., 2020). Similarly, Jiang et al. (2018) employed an AB-8 macroporous resin to improve the purification of purple corn (*Zea mays* L.) extract rich in anthocyanins, obtaining 1.60 g of these compounds. Other non-polar copolymer styrene type macroporous resin, such as HPD-300, managed to enrich the anthocyanin content from a *Schisandra Chinensis* extract from 47.6 to 128.4 mg/g. Thus, effluent purity was increased 6 times (from 5.08% to 30.43%), and the anthocyanins recovery/yield was 96.5% (Yue et al., 2016). Additionally, other anthocyanin purification technique, which are pressure-driven membrane processes, such as ultra- and nanofiltration enable high recovery and concentration of extracts rich in these molecules (Cassano et al., 2018; Valencia-Arredondo et al., 2020). On this matter, Cissé, Vaillant, Pallet, & Dornier (2011) observed in an industrial trial that, ultrafiltration and nanofiltration of roselle (*Hibiscus sabdariffa* L.) extract served to produce anthocyanin extracts which were 6 times more concentrated than the initial roselle extract (control). Valencia-Arredondo et al. (2020) used a combination of micro- and ultrafiltration followed by adsorption to process red cabbage extract. The investigators were able to concentrate monomeric anthocyanins from 32.05 to 3221.45 mg eq cy-3-glu/L. Gilewicz-Łukasik, Koter, & Kurzawa (2007) also showed an effective purification of anthocyanins from aronia (black chokeberry) fruits through the use of nanofiltration in the presence of sodium sulfate (IV). The recovery of these pigments was 99%.

Due to the instability of anthocyanins, the extracts obtained after their recovery and purification are often subjected to stabilization processes in order to be used in the food industry (He et al., 2017; Sharif, Khoshnoudi-Nia, & Jafari, 2020). In this regard, various strategies allow increasing the stability of anthocyanins including the utilization of controlled atmospheres, encapsulation, the use of metal ions, and the addition of -SH group-containing compounds and polyphenolic compounds with stabilizing effect (Nieto, 2020). Among these techniques, the encapsulation method has been hailed and takes on special interest (Faridi and Jafari, 2016; Xiao et al., 2019). In this stabilization technique, sensitive ingredients, like anthocyanins, are covered with a coating or wall material, thereby obtaining particles with diameters of a few nm to a few µm with greater stability against oxidation, temperature and light (Jafari et al., 2016; Sharif et al., 2020). Various encapsulation strategies, namely spray drying, freeze-drying, gelation, lipid-based particles and electrohydrodynamic processes, have emerged with the most commonly used one been spray drying (Sharif et al., 2020). The same encapsulation technique can be modified, but there is a need to take into account diverse factors that affect anthocyanin's stability (Jin et al., 2020). Many types of research have proved the effectiveness of encapsulation to stabilize anthocyanins. For example, (He et al., 2017) studied the stability of a nano encapsulated blueberry-derived mixture rich in anthocyanins with chitosan in a model beverage. In this work, the authors observed that encapsulation improved stability during storage for the addition of free anthocyanins. They obtained stability of 84.5 *vs* 71.2% for

35 days at refrigeration temperatures (4 °C) and of 68.4 *vs* 49.7% after 12 days at room temperature (25 °C).

Furthermore, these authors demonstrated that the anthocyanin-loaded chitosan nanoparticles exhibited a slowed degradation in simulated gastrointestinal fluid. In the same loop, in vitro studies of microencapsulated blueberries derived anthocyanins using different combinations of carboxymethyl starch/xanthan gum showed that encapsulation protected anthocyanins in the stomach, improving their release in the intestine (Cai et al., 2019). The authors further showed that microencapsulation ameliorated the thermal stability of anthocyanins and provided more excellent antioxidant stability.

Also, Idham, Muhamad, & Sarmidi (2012) analyzed the effect of stability and encapsulation by spray drying of anthocyanins from roselle (*Hibiscus sabdariffa* L.) calyces of using different matrices (soluble starch maltodextrin, gum Arabic, a combination of maltodextrin and gum Arabic). They investigated the stability of the encapsulated pigments during 105 days of storage at three different temperatures (4, 25 and 37 °C). The investigators observed an increased half-life of anthocyanins for 4 different types of matrices at any storage temperature when compared with the non-encapsulated roselle extract. The encapsulation efficiencies were higher than 96% for the first three matrices and 99.87% in the case of the combined maltodextrin and Arabic gum matrix. Additionally, this combination showed the lowest level of degradation during storage at 4 °C and the least changes in the color parameters a* and b*.

In another study, Righi da Rosa et al. (2019) observed that microencapsulated anthocyanins extracts from blueberry (*Vaccinium* spp.) (Righi da Rosa et al., 2019) had a protective effect during storage when compared to the unencapsulated extract. Specifically, in this work, the effect of two encapsulating agents (maltodextrin DE20 and hi-maize) and three different inlet air temperatures (120, 140 and 160 °C) were studied. Highest microencapsulation efficiencies between 74 and 85% were observed for air inlet temperature conditions of 160 °C and 14% maltodextrin DE20 and 4% hi-maize coating. In addition, the authors demonstrated that the unencapsulated blueberry extracts had a lower compound delivery under dissimulated gastrointestinal conditions in all steps of the simulated gastric system when compared to microencapsulated extracts (Righi da Rosa et al., 2019).

Anthocyanins as natural ingredients in functional food products

Anthocyanins recovered from distinct vegetables, fruits and their wastes can be used for the reformulation of different foods (see Table 2) in order to increase the intake of polyphenols as well as to improve the acceptability of the elaborated product, its shelf life and avoid spoilage (McDougall, 2017). Moreover, this addition may transform the product into a functional food with beneficial properties (Konczak and Zhang, 2004). In a study Papillo et al. (2018) studied the influence of adding anthocyanin-rich extracts from Italian black rice (*Oryza sativa* L., var. Artemide) in baked biscuits. The authors used anthocyanin-rich hydroalcoholic extracts stabilized by spray-drying (with and without coating agents) and freeze-drying in biscuits baked at 180 °C for 25 min. Under these conditions, they obtained a biscuit with a higher anthocyanin content (between 194 and 230 µg/g cookie, according to the stabilization method of the extract) compared to the control, where anthocyanins were not detected. Simultaneously, the enriched cookies displayed higher antioxidant capacities than the control cookies (482–595 *vs* 283 µg TE/g biscuit). Cyanidin-3-*O*-glucoside was the most abundant anthocyanin in any of the fortified cookies (32–20 µg/g biscuit). However, it is important to comment that baking stage induced a reduction in the total anthocyanin content. According to the authors, the encapsulation of extracts produced a slightly protective effect on anthocyanins.

Maner, Sharma, & Banerjee (2017) obtained similar results after the application of wine grape pomace powder, containing anthocyanins, in the preparation of other cookies. The addition of 5, 10, 15 and 20% of an extract rich in anthocyanins also caused a higher content of anthocyanins in the finished product (2.03–3.51 mg/g of the samples with extract *vs* 0.16 mg/g of the sample control). The inclusion of this extract in the cookie dough increased the total phenolic compounds content, tannins, flavonoids and the antioxidant capacity of the final product. In a recent study, López et al. (2019) found that the incorporation of an extract rich in anthocyanins from *Arbutus unedo* L. var. San Luis simultaneously improved the antioxidant activity and provided a more attractive color in baked wafers.

Furthermore, Maner et al. (2017) observed that the anthocyanin-rich extract provided a higher coloration of the cookies, and in general had a positive impact on the organoleptic characteristics when compared to the control samples. Another study carried out by Pasqualone, Bianco, & Paradiso (2013) also revealed the suitability of grape pomace extract to enrich cookies with anthocyanins. Pasqualone and his teammates observed that the addition of a lyophilized extract increased the anthocyanin content while imparting a more intense reddish color than control. Similar results were obtained by Albuquerque, Pinela, Barros, Oliveira, & Ferreira (2020). These authors observed that the enrichment with jabuticaba (*Myrciaria jaboticaba* (Vell.) Berg.) epicarp extracts in macarons provided a more stable color than the commercial colorant E136, during 6-day shelf life.

Additionally, macarons produced with these extracts displayed a composition of macronutrients similar to the colored with E136 samples, although they sowed a higher fructose and glucose contents. Also, Sui (2017) demonstrated that biscuits enriched with anthocyanins from commercial anthocyanin-rich black rice extract powder (2 and 4%) were refractory to lipid oxidation, even to a greater extent than the synthetic additive butylated hydroxyanisole (BHA) (López et al., 2019). A(López et al., 2019)nother study carried out on gluten-free tortillas and cookies showed that the addition of a black bean seed coat extracts to nixtamalized maize flour favorably affected color but not physical

Table 2. Application of anthocyanins in different food products.

Food product	Matrix of extracts	Extract characteristics	Concentration	Major findings	References
Bakery biscuits	Italian black rice (*Oryza sativa* L., var. Artemide)	Different spray-drying and freeze drying	0.23%	Higher anthocyanin content protected Higher antioxidant capacities Cy-3-*O*-glu was the most abundant Protected from thermal damage during baking	(Papillo et al., 2018)
Bakery cookies	Wine grape pomace powder	Dried in oven, 45–50 °C, 72 h	5, 10, 15, 20%	Increased antioxidant properties and anthocyanin concentration Impart brown color to cookies Positive impact on the organoleptic characteristics	(Maner et al., 2017)
Bakery biscuits	Grape marc	Lyophilized	$103 \cdot 10^{-3}$ g/kg	Increased anthocyanin content Impart more intense reddish color Adequate acceptability	(Pasqualone et al., 2013)
Bakery biscuits	Commercial anthocyanin-rich black rice extract powder	–	2 and 4%	Protected biscuits from lipid oxidation (greater extent than BHA)	(Sui, 2017)
Macarons	*Myrciaria jabuticaba* (Vell.) Berg. epicarp	Heat-assisted extraction	–	No effect on macronutrients content Higher fructose and glucose contents than macarons with colorant E136 More stable color than macarons with colorant E136	(Albuquerque et al., 2020)
Wafers	*Arbutus unedo* L. fruits	Heat-assisted extraction	5.50 g	Improved antioxidant capacity Provided more attractive color	(López et al., 2019)
Tortillas and cookies	*Phaseolus vulgaris* L. var. San Luis	Solid-liquid extraction	3 and 7 g/kg	Affected the color No effect on the texture No effect on rheological parameters in the case of 3 g/kg Retained over 80% of bioactive compounds for tortillas Retained over 60% of bioactive compounds for cookies	(Chávez-Santoscoy et al., 2016)
Kefir	Grape skin	Enzymatic extraction and lyophilizate	2.61 mg/mL	Maintenance of 67% of total anthocyanins for 16 days Pn-3-glu was the most abundant Properties like kefir without additives	(Montibeller et al., 2018)
Yogurt	Whole seedless berry (*Berberis boliviana* Lechler) powder	Whole powder	10 and 20 mg Cy-3-glu eq/100g	Color like commercial blueberry yogurt at and 20 mg Cy-3-glu eq/100g High color, pigment, and phenolic stability No degradations in color during the first 2 months	(Wallace and Giusti, 2008)
Yogurt	Extracts of Cabernet Sauvignon, Chardonnay, Shyrah, and Merlot grape callus	Acidified extracts	1 mL extract/100 mL	Red grapes exhibited a higher content of anthocyanins than control White grape extracts content similar to the control	(Karaaslan et al., 2011)
Carbonated water	Grape skin	Enzymatic extraction and lyophilizate	6.69 mg/mL	Mv-3-glu had highest stability Light had adverse effects on the color	(Montibeller et al., 2018)
Model juice	*Solanum tuberosum* and *Raphanus sativus*	Solid-liquid extraction	15 mg monomeric anthocyanin/100 mL	Half-life of more than one year in refrigeration Desired orange-red colors Alternatives to FD&C Red #40 Radish extract was more stable	(Rodríguez-Saona et al., 1999).

Cy: cyanidin; Mv: Malvidin; Pn: peonidin. eq: equivalents. BHA: butylated hydroxyanisole.

parameters as necessary as the texture of the final products (Chávez-Santoscoy et al., 2016). According to the authors, the addition of 3 g extract/kg dough did not affect the rheological or functional properties of dough produced, although in the case of the addition of 7 g added extracts/kg dough did show some rheological changes. In the former, 60% of the bioactive compounds were obtained for both foods (Chávez-Santoscoy et al., 2016).

In a dairy product like kefir, Montibeller et al. (2018) found that fortification with anthocyanins from grape skin acted as a natural dye. In this study, the enriched kefir retained over 50% of total anthocyanins in the first 27 days and demonstrated about 67% on day 16. It is relevant to mention that the content of each anthocyanin was gradually reduced during storage at different extents. The anthocyanin that showed more excellent stability was peonidin-3-glucoside (had 87% anthocyanins on day 16) while the delphinidin-3-*b*-glucoside decreased to 50% on day 12. Furthermore, the kefir made with this natural dye showed properties similar to those of kefir without additives.

Also, Wallace & Giusti (2008) observed that the addition of a berry (*Berberis boliviana* L.) extract rich in non-acylated anthocyanins led to more attractive yoghurts. The investigators observed that the addition of 20 mg Cy-3-glu eq from blueberry powder to 100 g to yoghurt generated products with a color similar to commercial blueberry yoghurt. Besides, no significant color degradations were observed during the first two months, and the products displayed high phenolic stability, independent of the fat matrix. Moreover, Karaaslan, Ozden, Vardin, & Turkoglu (2011) fortified yoghurts with extracts of different red grapes varieties (Cabernet Sauvignon, Chardonnay, Shyrah, and Merlot) and grape callus rich in phenolic compounds with anthocyanins inclusive (1 mL of acidified extract/100 mL of yogurt). The products exhibited a higher content of anthocyanins and excellent antioxidant capacity when compared with control yoghurts. The yogurts fortified with extracts from white grapes had lower content of phenolic compounds.

Additionally, Giusti & Wrolstad (2003) found that black carrot (*Daucus carota* L.) and radish (*Raphanus sativus*) provided a satisfactory red color for dairy products such as yoghurt and sour cream, using low concentrations (5 mg of monomeric anthocyanins/100 g of product). Furthermore, these authors also observed the potential use of red cabbage, which caused purple hues in dairy products similar to that provided by blueberries. Beside prior reviewed products, beverages can also be easily reformulated to obtain anthocyanin-enriched products (McDougall, 2017).

In a recent study, Montibeller et al. (2018) studied the influence of the addition of anthocyanins from grape skins over-carbonated water. They demonstrated that anthocyanins had a longer half-life and better thermal stability when protected from light, with malvidin-3-glucoside displaying greater stability. The authors proposed the use of anthocyanins as a natural colorant in drinks stored in the dark, as this could have beneficial effects on health (Montibeller et al., 2018). Besides water, different juices could experience an increase in anthocyanin content as they are fortified with juices and berry purees or by-products of these processed fruits (McDougall, 2017; Shahidi and Alasalvar, 2016). For instance, in a model juice, the suitability of the addition of acylated pelargonidin-based anthocyanins from red fleshed potatoes (*Solanum tuberosum*) and red radishes (*Raphanus sativus*) was observed.

Concurrently, the natural dyes imparted desired orange-red colors and showed adequate stability under refrigeration, with a half-life over one year (Rodríguez-Saona et al., 1999). According to the investigators, both pigments could be used as natural alternatives to FD&C Red #40 in food systems, although only red radish showed greater stability (Rodríguez-Saona et al., 1999).

It is also relevant to mention that the age group that can have the beneficial effects from the consumption of foods fortified with anthocyanins are adults due to scientific evidence accumulated using the information form subjects older than 20–30 y such as reported by Guo and Ling (2015) and Kimble et al. (2019) in clinical trials and cohort studies. Another relevant aspect that must be considered in the context of food fortified with anthocyanins is their potential modification and metabolism in the human body. It is known that anthocyanins can undergo several reactions after being ingested prior to achieve the circulatory system and internal organs. Once the anthocyanins achieve the small intestine, these compounds are hydrolyzed by enzymes released in lumen and lost their sugar moiety, which facilitates the absorption of the aglycone (anthocyanidin) by passive diffusion in the epithelium cells. Alternatively, a sodium-dependent glucose transporter can be used for the transport of anthocyanins into intestine cell, which causes an increase in the circulatory levels of anthocyanins. In this case, the original structure of the anthocyanin is preserved. Once the anthocyanins/anthocyanidins are absorbed, these compounds can undergo the conjugation reactions in order to be converted into more hydrophilic compounds and be transported via circulatory system. Once these reactions take place diverse products can be generated depending on the enzyme involved in the reaction: glucuronidated, sulfated, and methylated derivates. It is also relevant to mention that gut microbiota can metabolize anthocyanins and produce several compounds that are further observed by the host (Eker et al., 2020).

Conclusions

Due to the health benefits reported by anthocyanins, its use as a natural dye and/or bioactive compound is attractive for the food industry. However, the recovery of these biomolecules from different matrices requires the employment of distinct extraction methods, which are conditioned by the sensitivity of said compounds. Due to this, it has been demonstrated that new green technologies such as pulsed electric field, ultrasounds, microwave, high hydrostatic pressurized, and supercritical fluids extraction can be used to obtain high-quality extracts. This reduces/minimize the use of toxic solvents and high temperatures that may compromise the stability of anthocyanins. These extraction methods

complemented with purification works with resins and membrane processes yield improved anthocyanins-rich extracts, since they help to minimize the presence of impurities. Furthermore, regarding the recovery of anthocyanins, it should be noted that encapsulation has been identified as one of the most critical methods for stabilizing these molecules, especially due to the decay in anthocyanin concentration in fortified products during thermal treatment and storage period.

On the other hand, it has been seen that the utilization of anthocyanins in the fortification and/or coloring of certain foods have yielded functional food with health benefits. Specifically, it has been determined that in the case of baked products such as cookies, biscuits, macarons, among others, the addition of extracts rich in anthocyanins can protect the food from damage caused during baking, while improving its antioxidant capacity surpassing even synthetic additives, and all without affecting the foodstuff acceptability. Also, in products such as kefir, yoghurt, and different beverages, anthocyanins have been found to have high stability during storage and can be considered as suitable foods for anthocyanin fortification. Finally, it should be noted that the addition of anthocyanins worked to improve the color of processed foods, representing a viable alternative to synthetic colorants.

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