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REVIEW



## Could fruits be a reliable source of food colorants? Pros and cons of these natural additives

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### ABSTRACT

Color additives are important for the food industry to improve sensory quality lost during food process and to expand the variety of products. In general, artificial colorants have lower cost and better stability than the natural ones. Nevertheless, studies have reported their association with some health disorders. Furthermore, consumers have given greater attention to food products with health beneficial effects, which has provided a new perspective for the use of natural colorants. In this context, fruits are an excellent alternative source of natural compounds, that allow the obtainment of a wide range of colorant molecules, such as anthocyanins, betalains, carotenoids, and chlorophylls. Furthermore, in addition to their coloring ability, they comprise different bioactive properties. However, the extraction and application of natural colorants from fruits is still a challenge, since these compounds show some stability problems, in addition to issues related to the sustainability of raw-materials providing. To overcome these limitations, several studies have reported optimized extraction and stabilization procedures. In this review, the major pigments found in fruits and their extraction and stabilization techniques for uses as food additives will be looked over.

### KEYWORDS

Natural colorants; anthocyanins; betalains; carotenoids; chlorophylls; bioresidues

### Brief introduction to food additives

The use of food additives is fundamental to guarantee food quality and safety, and the introduction of chemical substances into food has been reported since the antiquity. However, with technological advances and population growth, an increasing number of food additives is generally used to confer various benefits to food such as microbiological safety and providing greater shelf-life, besides enabling the expansion of a variety of products with different sensory properties, such as color, flavor, and aroma (Carocho et al. 2014; Martins et al. 2016).

According to the *Codex Alimentarius* (WHO/FAO 2018), food additives are considered any substances that are not consumed as food and are not normally used as an ingredient in food, whether or not they contain nutritional value, whose addition is intentional in a food for technological purposes (including organoleptic) in the processing, packaging, storage and distribution of the product. About 230 food additives are often used by the food industry to confer technological, sensorial and/or preservation functionalities. These additives can be divided into 6 main groups: preservatives, nutritional additives, coloring agents, flavoring agents, texturizing agents, and miscellaneous agents (Carocho et al. 2014; Martins, Sentanin, and Souza 2019). The use of these compounds is controlled by regulations and legislations depending on the country in which the products are manufactured and/or marketed. The Joint FAO/WHO Expert

Committee on Food Additives (JECFA) is the international body responsible for evaluating the safety of food additives; however, some countries follow their own legislation determined by a competent agency. Food and Drug Administration (FDA) is the authority responsible for these legislation in the United States of America (USA), while in the European Union (EU), it is the European Food Safety Authority (EFSA). It is up to these authorities to determine which additives are allowed, as well as their Acceptable Daily Intake (ADI), which means the maximum amount of substance ingested per day by person, expressed by mg/kg body weight (bw)/day, throughout life that will not cause harm to the consumers health (Carocho et al. 2014).

In Europa, all food additives receive a specific code starting with the letter E following by three or four numbers, designated as E number, which facilitates consumers understanding when reading the label of foodstuff from different European countries, for example color additives correspond to the code range from E100 to E199; preservatives from E200 to E299, antioxidants from E300 to E399, structural additives (thickeners, stabilizers and emulsifiers) from E400 to E499; pH regulators and anti-caking agents from E500 to 599; flavor enhancers from E600 to E640; and sweeteners from E900 to E999. This code system has also been used by *Codex Alimentarius* (Carocho et al. 2014).

The use of food additives is common in several foods, from minimally processed to ultra-processed, but the

relationship between the consumer and the use of food additives, particularly the artificial, is not so friendly (Carocho et al. 2014). Acceptability and the use of artificial additives have been declining in recent decades, and this has been associated with issues of food safety and consumer concerns with the adverse effects of some of these substances on their health. Some additives, namely preservatives and colorants, have been associated with some health disorders, such as allergies and hyperactivity (Kamal and Fawzia 2018; Leo et al. 2018). However, it is still necessary to establish a worldwide consensus on the legislation of food additives. Thus, some substances are allowed to be added in foods in the USA, and banned in the EU, as is the case of the antimicrobials sodium sorbate (E201) and calcium sorbate (E203) and the colorants FD&C Green No. 3 (Fast Green (E143)) and citrus red No.2 (E121). On the other hand, the antimicrobial sodium methyl *p*-hydroxybenzoate (E219) and the colorants carmoisine (E122), amaranth (E123), and patent Blue (E131) are allowed in the EU and forbidden in the USA (Carocho et al. 2014; Martins, Sentanin, and Souza 2019). This shows a lack of harmony between legislations, which can become a barrier to international trade, in addition to making food safety uncertain to additives with contradictory evaluations.

### The class of colorants: natural vs artificial

Visual aspect, including color of food, is one of the first sensory characteristics to be evaluated by consumers. Colorants are important for the industry as they act to compensate for coloration due to exposure to light, air, humidity, processing and storage conditions; correct color variations, improve sensory aspects, and enable food diversification (Martins et al. 2016; Martins, Sentanin, and Souza 2019).

Differences in chemical structures, sources, and purpose of use, may make complex the classification of colorants. A simple way for their division could be based on their source, as natural and artificial. Natural colorants can be obtained from plant tissue (e.g. curcumin, carotenoids, anthocyanins, betalains, and chlorophylls), animal cell (e.g. carminic acid and kermesic acid), microorganism metabolism (e.g. carotenoids and chlorophylls), or mineral source (e.g. titanium dioxide and calcium carbonate). Artificial colorants are substances that are not found in nature and are obtained by chemical synthesis (Carocho et al. 2014). The use of artificial additives is preferred by industry, because they have a higher stability, attractive color, and lower costs (Martins et al. 2016). The artificial colorants used by the food industry are: (i) blue color: brilliant blue FCF (E133, ADI of 6 mg/kg bw/day), indigo carmine (E132, ADI of 5 mg/kg bw/day), and patent blue V (E131, ADI of 5 mg/kg bw/day); (ii) red-orange color: allura red AC (E129, ADI of 7 mg/kg bw/day), amaranth (E123, ADI of 0.15 mg/kg bw/day), carmoisine (E122, ADI of 4 mg/kg bw/day), erythrosine (E127, ADI of 0.1 mg/kg bw/day), litholrubine BK (E180, ADI of 1.5 mg/kg bw/day), and ponceau 4R (E124); (iii) yellow color: quinoline yellow (E104, ADI of 0.5 mg/kg bw/day), sunset yellow (E110, ADI of 2.5 mg/kg bw/day), and

tartrazine (E102, ADI of 7.5 mg/kg bw/day); and (iv) green color: fast green (E143, forbidden in the EU and allowed in the USA with ADI of 12.5 mg/kg bw/day) and green S (E142, 5 mg/kg bw/day) (Martins et al. 2016; Carocho et al. 2014). On the other hand, studies reported that the consumption of artificial colorants, especially nitrous derivatives, azo type (E102, E110, E122, E123, E124 and E129), can cause some health disorders. European Parliament in 2008 decreed that foods containing one or more of these color additives, should bear on their labels the name or E number information followed by the advertence: “may have an adverse effect on activity and attention in children” (Carocho et al. 2014). Tartrazine, a lemon yellow, used in candy, ice-cream, cereals, soup, jam, cake, soft-drink, and other foodstuffs, is one of the most contradictory color additives in relation to its safety. The ingestion of this colorant has been associated to obsessive-compulsive disturbances and hyperactivity in children (Kamal and Fawzia 2018). Several studies have investigated the ability of tartrazine to interact with human serum proteins and cause damage on DNA (Leo et al. 2018; Abo-EL-Sooud et al. 2018). In recent studies, the administration of tartrazine with ADI levels in mice, showed an increase of lipid oxidation and alterations in biochemical markers in the brain tissue (Bhatt et al. 2018), haematotoxin, immunotoxin effects, renal disorder, and increase of DNA abnormalities (Abd-Elhakim et al. 2018; Abo-EL-Sooud et al. 2018). Sunset yellow, produced from aromatic compounds derived from petroleum, has also been associated with the increase of pro-inflammatory activity, as well as carmoisine, allura red, and ponceau 4R, which are also reported to bind human and bovine serum albumin (Leo et al. 2018). Amaranth, a colorant that confers red color to food, such as in candy, ice-cream, and drinks, allowed in the EU, but banned in the USA due to carcinogenicity, has shown high genotoxic effect in cultured human lymphocytes (Carocho et al. 2014).

For these reasons and due to the changing lifestyle of consumers, natural colorants is a research area that is growing (Rodriguez-Amaya 2016). The interest in the use of natural colorants goes beyond their pigmentation capacity, since these compounds have bioactive properties that may be beneficial both for foodstuff preservation and for consumers health, such as antidiabetic, anti-neuro-disorder, anticancer and anti-cardiovascular effects (Rodriguez-Amaya 2016).

Natural colorants can also be classified by their chemical structure: flavonoid derivatives (anthocyanins, flavones and flavonols), isoprenoid derivatives (carotenoids), nitrogen-heterocyclic derivatives (betalains) or pyrrole derivatives (chlorophylls) (Sigurdson, Tang, and Giusti 2017). The occurrence of natural colorants is described in several vegetal tissues, for example, flowers are usually sources of anthocyanins, flavonols, and betalains (Leong et al. 2018; Sigurdson, Tang, and Giusti 2017), roots, such as beet root (*Beta vulgaris* L.) is the most known source of betalains (Khan 2016; Sigurdson, Tang, and Giusti 2017), and leaves are the mainly source of chlorophylls (Viera, Pérez-Gálvez, and Roca 2019). This review focuses on the colorants obtained from fruits and their bioresidues as alternatives to

artificial colorants, thus, the classes discussed below are based on the main pigments found in fruits: anthocyanins, betalains, carotenoids, chlorophylls, and other non-anthocyanin flavonoids.

## Colorant compounds from fruit sources

### Anthocyanins

Anthocyanins are the most important pigments between flavonoids; they are anthocyanidins bonded to one or more sugar molecules (glycoside form), in turn anthocyanidins consist of an aromatic ring bonded to an heterocyclic ring that contains oxygen, which is also bonded by a carbon-carbon bond at a third aromatic ring. Differences in the number of hydroxyl and methoxyl groups present in the structure characterize the various anthocyanins present in nature, interfering in their color and stability. Hydroxylation increases blue color and reduces stability, while methylation increases redness and increases stability. On the other hand, sugar moieties may be acylated with aromatic and/or aliphatic acids, which has positive effects on the stability (Leong et al. 2018; Rodriguez-Amaya 2018). Anthocyanins absorb light around 500 nm and are responsible for red, pink, violet, and blue colors of several fruits and flowers. There is an extensive number of these molecules present in nature and more than 600 types of anthocyanins have been identified (Zhang, Butelli, and Martin 2014), being the most common based anthocyanidins: cyanidin, pelargonidin, peonidin, delphinidin, petunidin, and malvidin, shown in Figure 1A (Zhang, Butelli, and Martin 2014; Li et al. 2017).

Anthocyanins are found mainly in fruits, vegetables, and flowers, and their production in plant tissues can be stimulated by stress conditions, pathological infection or by the plant protection system against oxidative damage (Zhang, Butelli, and Martin 2014). Red-purple fruits are usually the sources of these pigments. Table 1 shows anthocyanin-rich fruits.

According to data on Table 1, cyanidin 3-O-glucoside is the major anthocyanin in several fruits; this pigment is the most abundant anthocyanin in the plant kingdom and has been reported by its anti-obesity, anti-inflammatory, antioxidant and anti-tumor properties (Sun and Li 2018; You et al. 2018). Among the mentioned fruits, bay (*Laurus nobilis* L.), pagoda dogwood (*Cornus alternifolia* L.), mulberry (*Morus atropurpurea* Roxb.), juçara (*Euterpe edulis* M.) and haskap (*Lonicera caerulea* L.) show the highest concentration of anthocyanins (2170 mg/100 g of fresh fruit (ff), 1668 mg/100 g ff, 5472 mg/100 g of dry fruit (df), 2956 mg/100 g df and 2273 mg/100 g df, respectively) (Brito et al. 2007; Vareed et al. 2006; Celli, Ghanem, and Brooks 2015; Espada-Bellido et al. 2017). Curiously, bay plant is a very exploited species due to the aromatic and therapeutic properties of its leaves, but very few researches explore its potential as a source of colorants (Longo and Vasapollo 2005). An interesting amount of anthocyanins is also found in small fruits from shrubs, such as *Cornus* species (18–1668 mg/100 g ff (Vareed et al. 2006)) and wild madder (*Rubia peregrina* L.) (724 mg/100 g ff (Longo, Scardino, and Vasapollo 2007)), which are not usually appreciated for consumption. However,

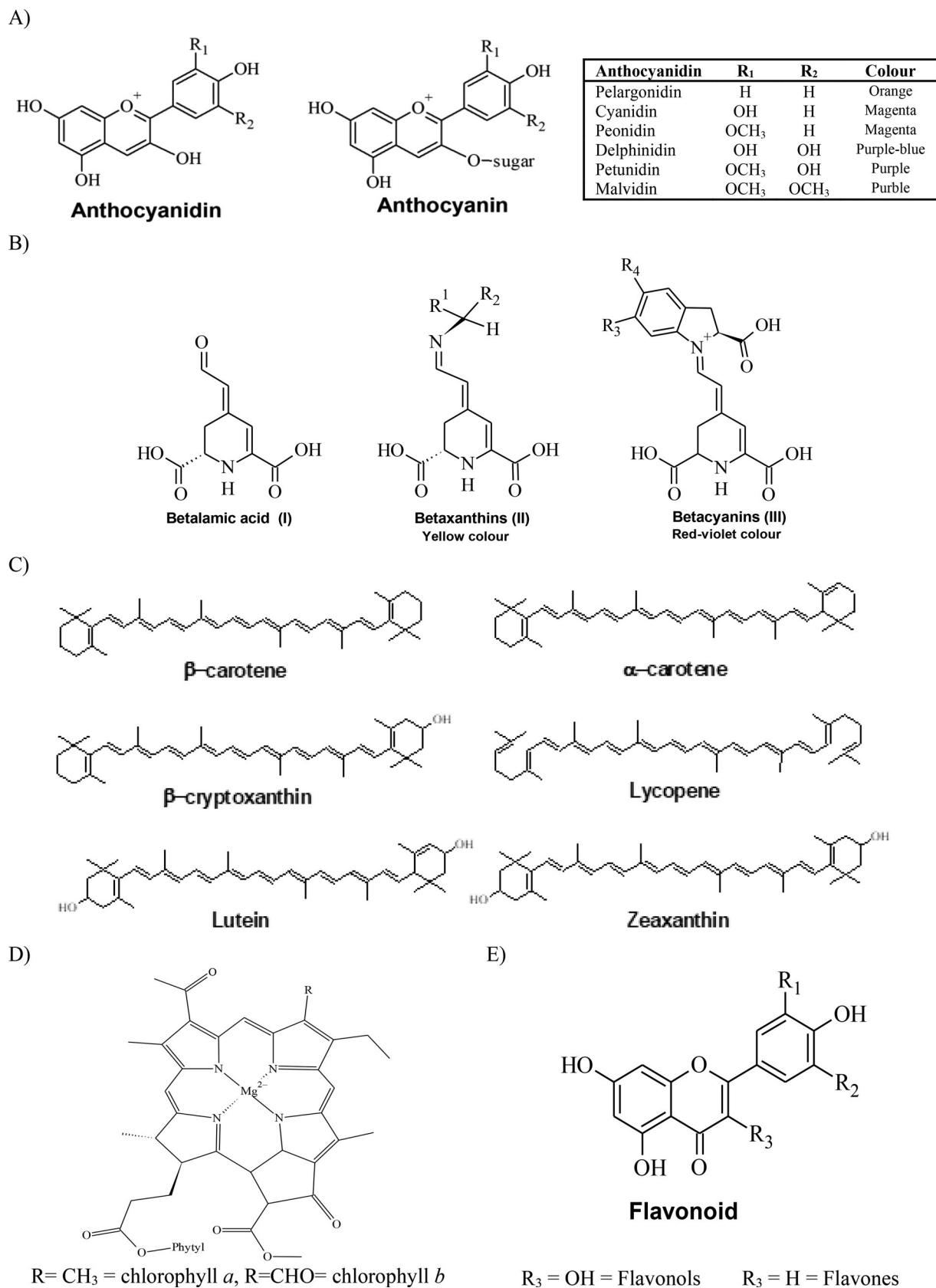
anthocyanins are found in various fruits that are ingested from the regular consumption, such as grapes (*Vitis vinifera* L.), blueberry (*Vaccinium ashei* Reade), blackberry (*Rubus fruticosus* L.) and strawberry (*Fragaria x ananassa* D). In this way, the use of anthocyanins from natural sources is permitted by EFSA and FDA. EFSA did not establish an ADI for this colorant due to the lack of characterization and toxicity data, thus, the intake as an additive should not exceed the normal intake of these compounds. JECFA has not allocated ADI for anthocyanin's from grape skin extract (EFSA 2013).

The colors conferred by anthocyanins are of great interest to industry, however their low stability may become a limitation to their application. Among all factors that cause instability, pH is mentioned as the main critical variable, since the color is dependent on the pH of the medium, due to the structural changes occurring in anthocyanins in the presence or absence of acid. In acid solution (pH 1–2), the structure of the anthocyanin is found in the form of flavylium cation ( $AH^+$ ), responsible by red color, becomes its stable form. As the pH increases, the cation can be hydrated, forming a carbinol pseudo base that is colorless at pH 4–5 or loses a proton at pH 6–6.5, assuming the form quinonal base of blue color. In solutions with pH 7–9 the tautomerization occurs, opening of the ring, resulting in the pale-yellow chalcone form. At pH > 9.0 it can occur the loss of another proton forming a dark blue ionized base (Zhang et al. 2014). Therefore, anthocyanins are more stable in values of low pH, however, Akogou et al. (2018) reported that alkaline pH provides a better extraction of anthocyanins, namely apigeninidin (3-desoxy-pelargonidin), from sorghum (*Sorghum bicolor*). An explanation for this effect, may be that the alkaline medium can release bounded phenolic compounds present in the plant tissue, and the absence of an hydroxyl group at the C-3 position of this pigment makes it more stable to changes of pH (Rodriguez-Amaya 2018).

Other factors that have effects on anthocyanins are temperature and the presence of co-pigments. Among the natural colorants from plant tissues, anthocyanins exhibit better stability in relation to heat. Thus, temperatures above 100°C can lead to their degradation to the chalcone form, which may limit some food processes with natural colorants based on anthocyanins. (Ngamwonglumlert, Devahastin, and Chiewchan 2017). Co-pigmentation reaction with other substances present in the food matrix, such as phenolic acids, metal ions, and proteins can help to improve their colorant capacity. For example, co-pigmentation with phenolic acids and other non-anthocyanin flavonoids increase the stability of these compounds during thermal processing (90°C) and storage (Fan et al. 2019; Kopjar, Jakšić, and Piližota 2012). Anthocyanins usually show high stability to light and to the presence of oxygen (Ngamwonglumlert, Devahastin, and Chiewchan 2017). However, there are various forms already mentioned in literature that stabilize this type of molecules; these aspects will be further discussed in section “Problems posed to natural colorants from fruit origin.”

In addition to the colorant capacity, anthocyanins are compounds that have antioxidant and other bioactive properties. Anthocyanins have also gained attention due to their potential health benefits, such as in diabetes

control (Leong et al. 2018; You et al. 2018), as also in the prevention of cardiovascular diseases, and prevention and treatment of neurological disorders (Li et al. 2017).



**Figure 1.** Structures of natural colorants found in fruits. (A) Anthocyanin; (B) betalains; (C) Carotenoids; (D) Chlorophylls; and (E) Flavonols and flavones.



**Table 1. (A)** Fruit sources of anthocyanins. **(B)** By-products and residues from fruit sources of anthocyanins.

(A) Fruit source	Major anthocyanin(s)	Content mg/100 g	Total anthocyanin content mg/100 g	References
Grape				
<i>Vitis vinifera</i> L.				
Garnacha	n.d	n.d	54 <sup>ff</sup>	(Gutiérrez-Gamboa et al. 2018)
Tempranillo	n.d	n.d	127 <sup>ff</sup>	
Graciano	n.d	n.d	173 <sup>ff</sup>	
Chinese bayberry	Cyanidin 3-O-glucoside	n.d	79 <sup>fp</sup>	(Bao et al. 2005)
<i>Myrica rubra</i> Seib & Zucc.				
Haskap	Cyanidin 3-O-glucoside	1675df	2273df	(Celli, Ghanem, and Brooks 2015)
<i>Lonicera caerulea</i> L.				
Aderno	Cyanidin 3-O-rutinoside	826 <sup>ff</sup>	949 <sup>ff</sup>	(Longo, Scardino, and Vasapollo 2007)
<i>Phillyrea latifolia</i> L.				
Wild madder	Cyanidin 3-O-rutinoside	606df	724 <sup>ff</sup>	(Longo, Scardino, and Vasapollo 2007)
<i>Rubia peregrina</i> L.				
Blackberry	Cyanidin 3-O-glucoside	55.3–191.3 <sup>ff</sup>	70.3–200 <sup>ff</sup>	(Chiang and Wrolstad 2006)
<i>Rubus fruticosus</i> L.				
Castilla Blackberry	Cyanidin 3-O-glucoside	n.d	157.8 <sup>fp</sup>	(Peñafiel et al. 2018)
<i>Rubus glaucus</i> Benth				
Raspberry	Cyanidin 3-O-sophoroside	n.d	43.42 <sup>ff</sup>	(Sun et al. 2007)
<i>Rubus idaeus</i> L.				
Black currant	Cyanidin 3-O-rutinoside	n.d	2040 <sup>dw</sup>	(Pap et al. 2013)
<i>Ribes nigrum</i> L.				
Black chokeberry	Cyanidin 3-O-galactoside	n.d	640.8df	(D'Alessandro et al. 2014)
<i>Aronia melanocarpa</i> Michx. Elliott				
Strawberry	Pelargonidin 3-O-glucoside	46.8 <sup>ff</sup>	20–60 <sup>ff</sup>	(Silva et al. 2007)
<i>Fragaria x ananassa</i> D.				
Maqui	Delphinidin 3-O-sambubioside-5-O-glucoside	125–364 <sup>df</sup>	614–984df	(Gironés-Vilaplana et al. 2014)
<i>Aristotelia chilensis</i> L.				
	Delphinidin 3,5-O-diglucoside	114–251df		
	Delphinidin 3-O-diglucoside	110–210df		
Açaí	Cyanidin 3-O-rutinoside	81–305df	49–348df	(Gironés-Vilaplana et al. 2014)
<i>Euterpe oleracea</i> L.				
Juçara	Cyanidin 3-O-rutinoside	1565df	2956df	(Brito et al. 2007)
<i>Euterpe edulis</i> M.	Cyanidin 3-O-glucoside	1358df		
Strawberry tree	Cyanidin 3-O-glucoside	24df	38df	(López et al. 2018)
<i>Arbutus unedo</i> L.				
Guajiru	Petunidin 3-O-(6''-succinyl)-rhamnoside	367 <sup>df</sup>	958df	(Brito et al. 2007)
<i>Chrysobalanus icaco</i> L.				
Guabiju	n.d	n.d	245.31df	(Seraglio et al. 2018)
<i>Myrcianthes pungens</i> (O. Berg) D. Legrand				
Jabuticaba	n.d	n.d	930.56df	(Seraglio et al. 2018)
<i>Myrciaria cauliflora</i> Berg.				
Acerola	Cyanidin 3-O-rhamnoside	359df	5.28df	(Brito et al. 2007)
<i>Malpighia emarginata</i> DC.				
Jambolan	Delphinidin 3,5-O-diglucoside	256df	771 <sup>df</sup>	(Brito et al. 2007)
<i>Syzygium cumini</i> L.				
	Petunidin 3,5-O-diglucoside	245df		
	Malvidin 3,5-O-diglucoside	166df		
Camu-camu	Cyanidin 3-O-glucoside	n.d	28.0–42.2 <sup>ff</sup>	(Neri-Numa et al. 2018)
<i>Myrciaria dubia</i> (HBK) McVaugh				
Mulberry	Cyanidin 3-O-glucoside	n.d	5472 <sup>dw</sup>	(Zou et al. 2012)
<i>Morus atropurpurea</i> Roxb.				
Black mulberry	Cyanidin 3-O-glucoside	n.d	14.99 <sup>ff</sup>	(Espada-Bellido et al. 2017)
<i>Morus nigra</i> L.				
Blueberry	Cyanidin 3-O-glucoside	n.d	2110df	(Piovesan et al. 2017)
<i>Vaccinium ashei</i> Reade				
Sweet cherry	Cyanidin-3-O-rutinoside	50.30 <sup>ff</sup>	57.09 <sup>ff</sup>	(Mikulic-Petkovsek et al. 2016)
<i>Prunus avium</i> L.				
Sohiong	Petunidin 3-O-glucoside	330.9df	989df	(Swier et al. 2018)
<i>Prunus nepalensis</i> L.				
Mahaleb cherry	Cyanidin 3-O-glucoside	89.85 <sup>ff</sup>	112.53 <sup>ff</sup>	(Mikulic-Petkovsek et al. 2016)
<i>Prunus mahaleb</i> L.				
Bird cherry	Cyanidin 3-O-glucoside	150.01 <sup>ff</sup>	207.11 <sup>ff</sup>	(Mikulic-Petkovsek et al. 2016)
<i>Prunus padus</i> L.				
Blackthorn	Cyanidin 3-O-glucoside	128.68 <sup>ff</sup>	233.55 <sup>ff</sup>	(Mikulic-Petkovsek et al. 2016)
<i>Prunus spinosa</i> L.				
Sanguinelli	Cyanidin 3-O-(6'-O-malonyl)glucoside	0.00421–0.01095 <sup>fp</sup>	0.097–0.025 <sup>fp</sup>	(Cebadera-Miranda et al. 2019)
<i>Citrus sinensis</i> (L.) cv. Sanguinelli				

(continued)

Table 1. Continued.

(A) Fruit source		Major anthocyanin(s)	Content mg/100 g	Total anthocyanin content mg/100 g	References
Tarocco		Cyanidin 3-O-(6'-O-malonylglucoside)	0.004–0.009 <sup>fp</sup>	0.011–0.0218 <sup>fp</sup>	(Cebadera-Miranda et al. 2019)
<i>Citrus sinensis</i> (L.) Osbeck					
Purple kiwifruit		n.d	n.d	25.0 <sup>ff</sup>	(Peng et al. 2019)
<i>Actinidia arguta</i> var. <i>purpurea</i> Rehder					
Tamarillo		Delphinidin 3-O-rutinoside	5.26 <sup>ff</sup>	8.50 <sup>ff</sup>	(Rosso and Mercadante 2007)
<i>Cyphomandra betacea</i> (Cav.) Sendt.					
Davyalis		Delphinidin 3-O-rutinoside	20.13 <sup>fw</sup>	42.00 <sup>fw</sup>	(Rosso and Mercadante, 2007)
<i>Dovyalis abyssinica</i> Warb.					
Elderberry		Cyanidin 3-O-sambubioside-5-O-glucoside	630.8 <sup>fw</sup>	1265 <sup>fw</sup>	(Veberic et al. 2009)
<i>Sambucus nigra</i> L.					
Red cabbage		Cyanidin 3-O-glucoside	586.4 <sup>fw</sup>		
<i>Brassica oleracea</i> L.		Cyanidin 3-O-diglucoside-5-O-glucoside	n.d	40*	(Ravanfar et al. 2018)
Cornelian cherry		Cyanidin 3-O-galactoside	176 <sup>fw</sup>	375 <sup>fw</sup>	(Vareed et al. 2006)
<i>Cornus mas</i> L.					
Kousa dogwood		Cyanidin 3-O-glucoside	16 <sup>fw</sup>	18 <sup>fw</sup>	(Vareed et al. 2006)
<i>Cornus kousa</i> F. Buerger ex Miq.					
Flowering dogwood		Cyanidin 3-O-galactoside	62 <sup>ff</sup>	65 <sup>ff</sup>	(Vareed et al. 2006)
<i>Cornus florida</i> L.					
Asiatic dogwood		Pelargonidin 3-O-galactoside	78 <sup>ff</sup>	114 <sup>ff</sup>	(Vareed et al. 2006)
<i>Cornus officinalis</i> Sieb. et Zucc.					
Giant dogwood		Delphinidin 3-O-rutinoside	592 <sup>ff</sup>	1369 <sup>ff</sup>	(Vareed et al. 2006)
<i>Cornus controversa</i> Hemsl.					
Pagoda Dogwood		Delphinidin 3-O-glucoside	775 <sup>ff</sup>		
<i>Cornus alternifolia</i> L.		Delphinidin 3-O-rutinoside	844 <sup>ff</sup>	1668 <sup>ff</sup>	(Vareed et al. 2006)
		Delphinidin 3-O-glucoside	821 <sup>ff</sup>		
Karanda		n.d	n.d	81*	(Sueprasarn, Reabroy, and Pirak 2017)
<i>Carissa carandas</i> Linn.					
Bay		Cyanidin 3-O-rutinoside	1160 <sup>ff</sup>	2170 <sup>ff</sup>	(Longo and Vasapollo 2005)
<i>Laurus nobilis</i> L.					
(B) Fruit		By-product/ residue			
Grape					
<i>Vitis vinifera</i> L.					
Teran	Pomace	Malvidin 3-O-glucoside	n.d	~370 <sup>dw</sup>	(Putnik et al. 2018)
Tintilla de Rota	Skin	Malvidin 3-O-glucoside	n.d	119 <sup>dw</sup>	(Liazid et al. 2011)
Eggplant	Peel	n.d	n.d	241 <sup>fw</sup>	(Dranca and Oroian 2016)
<i>Solanum melongena</i> L.					
Fig	Peel	Cyanidin 3-O-rutinoside	n.d	380 <sup>dw</sup>	(Backes et al. 2018)
<i>Ficus carica</i> L.					
Litchi	Peel	Cyanidin 3-O-rutinoside	770.6 <sup>dw</sup>	772.16 <sup>dw</sup>	(Liu et al. 2013)
<i>Litchi chinensis</i> Sonn					
Jabuticaba	Peel	Cyanidin 3-O-glucoside	490 <sup>dw</sup>	n.d	(Rodrigues et al. 2015)
<i>Myrciaria cauliflora</i> Berg.					
Purple passion	Peel	n.d	n.d	82.6 <sup>fw</sup>	(Liu et al. 2018)
<i>Passiflora edulis</i> Sims.					
Bilberry	Pomace	Delphinidin 3-O-glucoside	3970 <sup>dw</sup>	28495 <sup>dw</sup>	(Klavins et al. 2018)
<i>Vaccinium myrtillus</i> L.		Delphinidin 3-O-galactoside	3141 <sup>dw</sup>		
Cranberry	Pomace	Peonidin 3-O-galactoside	1241 <sup>dw</sup>	4353 <sup>dw</sup>	(Klavins et al. 2018)
<i>Vaccinium oxycoccos</i> L.					
Lingonberry	Pomace	Cyanidin 3-O-galactoside	1930 <sup>dw</sup>	2758 <sup>dw</sup>	(Klavins et al. 2018)
<i>Vaccinium vitis-idaea</i> L.					
Blueberry	Pomace	Peonidin 3-O-arabinoside	2582 <sup>dw</sup>	8412 <sup>dw</sup>	(Klavins et al. 2018)
<i>Vaccinium corymbosum</i> L.					
<i>Vaccinium ashei</i> Reade	Wine pomace	n.d	n.d	415 <sup>fw</sup>	(He et al. 2016)
Juçara	Residue	Cyanidin 3-O-rutinoside	n.d	92 <sup>dw</sup>	(Garcia-mendoza et al. 2017)
<i>Euterpe edulis</i> M.					
Avocado	Skin	n.d	n.d	57.3 <sup>fw</sup>	(Cox et al. 2004)
<i>Persea americana</i> Mill.					
Grumixama	Peel	Cyanidin 3-O-glucoside	3729 <sup>dw</sup>	4837.21 <sup>dw</sup>	(Nascimento et al. 2017)
<i>Eugenia brasiliensis</i> Lam.	Residue	Cyanidin 3-O-glucoside	1002 <sup>dw</sup>	1004 <sup>dw</sup>	(Machado et al. 2017)

(continued)

Table 1. Continued.

(A) Fruit source		Major anthocyanin(s)	Content mg/100 g	Total anthocyanin content mg/100 g	References
Pomegranate	Peel	n.d	n.d	8600 <sup>dw</sup>	(Alexandre et al. 2017)
<i>Punica granatum</i> L.					
Blackberry	Residue	Cyanidin-3-O-glucoside	2130 <sup>dw</sup>	2360 <sup>dw</sup>	(Machado et al. 2017)
<i>Rubus fruticosus</i> L.					
Purple kiwifruit	Skin	n.d	n.d	97.58 <sup>fw</sup>	(Peng et al. 2019)
<i>Actinidia arguta</i> var. <i>purpurea</i> (Rehder)					

<sup>df</sup>Values are based in dry fruit;<sup>fp</sup>Values are based in fresh pulp;<sup>ff</sup>Values are based in fresh fruit;<sup>dw</sup>Values are based in dry weight;<sup>fw</sup>Values are based in fresh weight ;

\*Values are based in extract;

n.d – not determined.

## Betalains

Betalains are vacuole pigments, water-soluble, composed of a nitrogenous core structure, formed from betalamic acid (Figure 1B). More than 75 betalains have already been described in plant tissues (Khan 2016). Basically the betalains can be divided into two groups according to their structure: a group responsible for red-violet color, denominated betacyanins, this group presents variations in their sugar and acyl groups (Figure 1B), and another group responsible for the yellow color, denominated betaxanthins, presenting amines and amino acids in their structure (Figure 1B) (Khan 2016; Rodriguez-Amaya 2016). The range of maximum absorption ( $\lambda_{\max}$ ) of betalains dependent on their structure, betalamic acid has  $\lambda_{\max}$  around 424 nm, betaxanthins at 471 nm, and betacyanins around 541 nm (Khan 2016).

Red beet (E162), a natural colorant obtained from *Beta vulgaris* L. (Amaranthaceae), is already an alternative used to as food colorant. This additive consists of several betacyanins obtained after the purification of mechanically processed beet juice. The ADI for this colorant has not been defined since the compounds present in E162 are commonly intake in the regular diet. Due to its sensitivity to heat and the presence of light, E162 is recommended for use in minimized processed products, but its application is released in a wide range of food products such as ice creams, cheeses and cereals (EFSA 2015a).

The presence of betalains in fruits occurs mainly in the plant order Caryophyllales, which include several cactus fruits, as shown in Table 2.

Table 2 shows that fruits of the genus *Stenocereus*, also known as pitayas of Mexican origin, are good sources of betalains, containing from 1770.6 to 2205.3 mg/100 g df. *Opuntia* spp. are also alternative sources of betalains, mainly betanin, as well as *Hylocereus* spp (Wybraniec and Mizrahi 2002).

In addition to the attractive color of betalains and its use as natural colorant, the antioxidant capacity of these compounds has been of great interest. Betalains have shown a high radical scavenging activity in a widely pH range; betanin exhibits a high radical scavenging activity at pH > 4, while the highest activity of betanidin was reported at pH values between 2 and 4 (Slimen, Najar, and Abderrabba 2017). In studies with mice, supplementation with betalains

(8 mL/kg) for 28 days decreased the lipid peroxidation and protein oxidation by oxidative stress; anti-inflammatory activity was also observed with doses of 25–250 mg/kg (Tan et al. 2015; Kaur et al. 2018). The authors have also reported effects on diabetes, cardiovascular and neurological diseases (Kaur et al. 2018).

Betalains color is stable between pH 3–7: betacyanins are most stable in acid pH and betaxanthins in neutral pH; at low pH values the color changes from red to violet-blue and in alkaline medium the conversion of betalains to betalamic acid occurs, which results in color change to yellow-brown. The instability of betalains also occurs in the presence of light, oxygen and metal ions (e.g.  $\text{Al}^{3+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Cu}^{2+}$ ), which may accelerate oxidation of betalains, resulting in color loss. However, heat is the most critical factor in the degradation of these compounds. At high temperature, the aldimine bond hydrolysis and decarboxylation occurs, which makes a color change to orange-yellow. In order to minimize the effect of the heat over degradation of betalains, it is important to cool down the food matrix after thermal processes and then apply the ingredient (Ngamwonglumlert, Devahastin, and Chiewchan 2017; Khan 2016). To overcome these obstacles, stabilization methods have been developed. For example, encapsulation with sorption protein and maltodextrin can increase the stability of these compounds at temperature of 60°C. Other methods of stabilization are presented in section “Problems posed to natural colorants from fruit origin.”

## Carotenoids

Carotenoids are lipid-soluble pigments that are synthesized by all photosynthetic organisms, which include plants, algae, and microorganisms. In vegetable tissues they are responsible for conferring yellow, orange or red colors. Its main sources are carrots (*Daucus carota* L.), tomatoes (*Solanum lycopersicum* L.) and peppers (*Capsicum* species) (Rodriguez-Amaya 2018; Saini and Keum 2018). In plants, carotenoids have primary functions, such as a photoprotection against photo-oxidation damage, and secondary functions due to their attractive coloration that attracts animals, which makes possible to disperse pollen from flowers and fruit seeds (Rodriguez-Concepcion et al. 2018). The main carotenoids



**Table 2.** (A) Fruits sources of betalains. (B) Residues from fruit sources of betalains.

(A) Fruit source	Major betalain	Content mg/100 g	Total betalain content (TBC) mg/100 g	References
Prickly pear <i>Opuntia robusta</i> Wendl.	Indicaxanthin	n.d	815dp	(Castellanos-Santiago and Yahia 2008)
<i>Opuntia robusta</i> Mill.	Betanin	n.d	304 <sup>dp</sup>	(Castellanos-Santiago and Yahia, 2008)
Purple <i>Opuntia ficus-indica</i> (L.) Miller	Betanin	n.d	50dp	(Castellanos-Santiago and Yahia, 2008)
Yellow <i>Opuntia ficus-indica</i> (L.) Miller	Indicaxanthin	27.0*	40.0*	(Fernández-López et al. 2018)
<i>Opuntia ficus-indica</i> var. Sanguigna	Betanin	195.3fp	n.d	(Melgar, Pereira, et al. 2017)
<i>Opuntia ficus-indica</i> var. gialla	Betanin	11.32fp	n.d	(Melgar, Pereira, et al. 2017)
<i>Opuntia engelmannii</i> Salm-Dyck ex Engelm	Betanin	225fp	n.d	(Melgar, Pereira, et al. 2017)
<i>Opuntia stricta</i> Haw.	Betanin	~50 FF	n.d	(Koubaa et al. 2016)
<i>Opuntia megacantha</i> Salm-Dyck	Betanin	n.d	23 <sup>dp</sup>	(Castellanos-Santiago and Yahia, 2008)
<i>Opuntia albi-carpa</i> Sheinvar	Betanin	n.d	17 <sup>dp</sup>	(Castellanos-Santiago and Yahia, 2008)
<i>Opuntia streptacantha</i> Lemaire	Betanin	n.d	308dp	(Castellanos-Santiago and Yahia 2008)
<i>Opuntia macrorhiza</i> Engelm.	Betaxanthins	0.42–0.45dp	n.d	(Moussa-Ayoub et al. 2011)
Xoconostle <i>Opuntia joconostle</i> F.A.C.Weber	Betalain	n.d	92.0ff	(Sanchez-Gonzalez et al. 2013)
Pitaya Red <i>Stenocereus pruinosus</i> (Otto) Buxb.	Indicaxanthin	1656.3dw	1770.6dw	(García-Cruz et al. 2017)
Orange <i>Stenocereus pruinosus</i> (Otto) Buxb.	Gomphrenin I	327.6dw		
Red <i>Stenocereus stellatus</i> (Pfeiffer) Riccobono	Indicaxanthin	2089.4dw	2205.3dw	(García-Cruz et al. 2017)
<i>Hylocereus polyrhizus</i> (Weber) Britton and Rose	Indicaxanthin	2097.0dw	2168.6dw	(García-Cruz et al. 2017)
	Betanin	23.78 <sup>fp</sup>	n.d	(Yong et al. 2017)
	Phyllocactin	27.17fp		
<i>Hylocereus purpusii</i> Britton and Rose	n.d	n.d	23ff	(Wybraniec and Mizrahi 2002)
<i>Hylocereus costaricensis</i> Britton & Rose	n.d	n.d	39ff	(Wybraniec and Mizrahi 2002)
<i>Hylocereus undatus</i> (Haw.) Britton and Rose	n.d	n.d	29ff	(Wybraniec and Mizrahi 2002)
Malabar spinach <i>Basella rubra</i> L.	Betacyanins	23.0ff	34.0 <sup>ff</sup>	(Kumar et al. 2015)
Pigeon berry <i>Rivina humilis</i> L.	Betaxanthins	n.d	170df	(Khan et al. 2012)
Jiotilla <i>Escontria chiotilla</i> (F.A.C.Weber) Rose	Betaxanthins	11.9ff	20.8ff	(Soriano-Santos et al. 2007)
Facheiro <i>Philosocereus pachycladus</i> Ritter	Iso-betanin	70fp	206fp	(Souza et al. 2015)

(B) Fruit	Residue				
Red Pitaya <i>Hylocereus polyrhizus</i> (Weber) Britton and Rose	Peel	n.d	n.d	73 <sup>fw</sup>	(Faridah, Holinesti, and Syukri 2015)
Prickly pear <i>Opuntia stricta</i> Haworth	Peel	Betanin (betanidin 5- O-glucoside)	n.d	~75fw	(Koubaa et al. 2016)
<i>Opuntia macrorhiza</i> Engelm.	Peel	Betacyanins	0.44–0.52dw	n.d	(Moussa-Ayoub et al. 2011)
<i>Opuntia ficus-indica</i> var gialla	Peel	Betanin	125*	n.d	(Melgar, Dias, et al. 2017)
<i>Opuntia ficus-indica</i> var sanguigna	Peel	Betanin	344*	397*	(Melgar, Dias, et al. 2017)
<i>Opuntia engelmannii</i> Salm-Dyck ex Engelm	Peel	Betanin	1490*	1940*	(Melgar, Dias, et al. 2017)
Xoconostles <i>Opuntia joconostle</i> cv. Cuaresmeño	Peel	Betanin	n.d	4.56 <sup>fw</sup>	(Osorio-Esquivel et al. 2011)
	Endorcap	Betanin	n.d	23.03fw	

(continued)

Table 2. Continued.

(A) Fruit source		Major betalain	Content mg/100 g	Total betalain content (TBC) mg/100 g	References
<i>Opuntia matudae</i>	Peel	Betanin	18*	31*	(Morales et al. 2015)
Scheinvar cv. Rosa		Isobetanin	19*		
<i>Opuntia matudae</i>	Endocarp	Betanin	11*	47*	(Morales et al. 2015)
Scheinvar cv. Rosa		Isobetanin	11*		
Rumpa	Peel	Betanaine	0.4–2.5fw	0.8–4.2fw	(Masson et al. 2011)
<i>Eulychnia acida</i> Phil.					

<sup>d</sup>PValues are based on dry pulp;<sup>d</sup>fValues are based on dry fruit;<sup>f</sup>PValues are based on fresh pulp;<sup>f</sup>fValues are based on fresh fruit;<sup>d</sup>wValues are based on dry weight;<sup>f</sup>wValues are based on fresh weight;

\*Values are based on extract;

n.d – not determined.

found in food are:  $\beta$ -carotene,  $\alpha$ -carotene,  $\beta$ -cryptoxanthin, lycopene, lutein, and zeaxanthin (Figure 1C) (Rodriguez-Amaya 2016). Carotenoids can also be found in some species of fish (e.g., salmon) and crustaceans (e.g., crab and shrimp) (Rodriguez-Amaya 2018). A large variety of fruits are considered as sources of carotenoids, and some examples are presented in the Table 3.

$\beta$ -carotene is the main carotenoid found in most fruits, according to data present in Table 3. Buriti (*Mauritia flexuosa* L.) and tucumã (*Astrocaryum aculeatum* Meyer) show the highest amounts of this pigment, 31.13 and 20.97 mg/100 g ff, respectively. Pitanga (*Eugenia uniflora* L.), and tomato are rich in lycopene, with 361 mg/100 g df and 511 mg/100 g of df, respectively (Eh and Teoh 2012; Filho et al. 2008).

More than 1000 carotenoids are known, however only 40–50 types are consumed in the human diet (Leong et al. 2018). All carotenoids have a long chain structure, generally they are  $C_{40}$  tetraterpenoids, with double carbon bonds and bilateral symmetry around the central double bond. Carotenoids are distinguished mainly by the formation of the ring at the ends (cyclic carotenoids) or not (acyclic carotenoids), and by the presence of oxygen atoms. When constituted only by carbon and hydrogen atoms, they are known as carotenes (e.g.  $\beta$ -carotene and lycopene) and when they have oxygen in the structure, they are known as xanthophylls (e.g.  $\beta$ -cryptoxanthin and lutein) (Ngamwonglumlert, Devahastin, and Chiewchan 2017; Saini and Keum 2018). This diversity of carotenoids occurs due to reactions of hydrogenation, dehydrogenation, cyclization of the end groups, and oxygen insertion, providing their color characteristics and antioxidant properties (Saini and Keum 2018). The range of maximum absorption ( $\lambda_{max}$ ) of carotenoids in the ultraviolet and visible spectra covers values between 286 nm (phytoene) and 470 nm (lycopene), due to their different structures. For carotenoids that are mainly found in vegetal tissue, the  $\lambda_{max}$  of  $\beta$ -carotene, zeaxanthin, and  $\beta$ -cryptoxanthin corresponds to 450 nm; for lutein  $\lambda_{max}$  is 445 nm, and for lycopene 470 nm. These values correspond to the use of ethanol or petroleum ether as solvents (Rodriguez-Amaya 2001).

Carotenoids are known to have antioxidant capacity and as vitamin A precursors, namely  $\beta$ -carotene, have the ability to reduce cardiovascular disease and display anti-cancer effects (Leong et al. 2018). Lutein and zeaxanthin have been reported as having beneficial properties for vision problems, preventing the Age-Related Macular Degeneration (AMD) and cataracts; these pigments are accumulated in ocular cells and have shown efficient quenching of both singlet oxygen and lipid peroxy radicals (Roberts and Dennison 2015). Lycopene is well-known for its prevention of the prostate cancer, but it has also shown promising effects in other tumor cell lines, such as breast, ovarian, gastric, and cervical, besides to its positive effect on cardiovascular disease and other disorders caused by oxidative stress, such as osteoporosis and neurodegenerative damage (Holzapfel et al. 2017; Leong et al. 2018).

The use of carotenoids as a colorant additive is allowed by EFSA and FDA, being coded as: mixed carotenes (E160a (i)),  $\beta$ -carotene (E 160a (ii)), annatto (E160b), paprika extract (E160c), and lycopene (E160d (i) and (ii)). Mixed carotenes are composed by  $\beta$ - and  $\alpha$ -carotene obtained from plant tissues, mainly palm fruit (*Elaeis guineensis* Jacq.), carrots (*Daucus carota* L.), and algae (namely *Dunaliella salina* and *Dunaliella bardawil*).  $\beta$ -Carotene can also be obtained from fermentative processes of fungus, namely *Blakeslea trispora*, but due to the presence of synthetic  $\beta$ -carotene, it is not considered a natural obtained colorant. The ADI for these additives is not determined due to the lack of studies proving their toxicity, however their consumption as food colorant should not exceed the normally ingestion of these compounds (EFSA 2012). Annatto is a natural colorant obtained from seeds of *Bixa Orellana* L. composed by bixin or norbixin carotenoids. The ADI for annatto depends of the carotenoid content: for bixin is 0.6 mg/kg bw/day and for norbixin is 0.3 mg/kg bw/day (EFSA 2016). Paprika is the colorant obtained from *Capsicum annum* L. fruit, also known as pepper or sweet pepper, being composed by capsanthin and capsorubin carotenoids. The ADI for paprika extract is 24 mg/kg bw/day or 1.7 mg of carotenoids/kg bw/day from paprika extract (EFSA 2015 b). Lycopene colorant can be natural obtained from tomato fruit (*Lycopersicon*

**Table 3. (A)** Fruits sources of carotenoids. **(B)** By-products and residues from fruit sources of carotenoids.

(A) Fruit source	Major Carotenoid(s)	Content mg/100 g	Total carotenoid content (TCC) mg/100 g	References
Pitanga	Lycopene	361df	547.4df	(Filho et al. 2008)
<i>Eugenia uniflora</i> L.				
Camu-camu	n.d	n.d	0.4ff	(Neri-Numa et al. 2018)
<i>Myrciaria dubia</i> (HBK) McVaugh				
Pumpkin	n.d	n.d	33.9–379.7df	(Nawirska-Olszańska, Stepień, and Biesiada 2017)
<i>Cucurbita moschata</i> Duch.				
Pepper	$\beta$ -carotene	13.9–66.6df	55.3 – 98.8df	(Navarro et al. 2006)
<i>Capsicum annuum</i> L cv Orlando				
Acerola	n.d.	n.d.	0.94–3.00ff	(Lima et al. 2005)
<i>Malpighia emarginata</i> D.C.				
Mango	Lycopene	0.553ff	1.043ff	(Setiawan et al. 2001)
<i>Mangifera indica</i> L.				
Mangosteen	Lycopene	0.177ff	0.221ff	(Setiawan et al. 2001)
<i>Garcinia mangostana</i> L.				
Orange	$\beta$ -carotene	0.275ff	0.419ff	(Setiawan et al. 2001)
<i>Citrus nobilis</i> Lour				
Papaya	Lycopene	5.750ff	6.370ff	(Setiawan et al. 2001)
<i>Papaya Carica</i> L.				
Jackfruit	$\beta$ -carotene	0.36ff	0.43ff	(Setiawan et al. 2001)
<i>Artocarpus heterophyllus</i> Lam.				
Salak	$\beta$ -carotene	2.997ff	4.127ff	(Setiawan et al. 2001)
<i>Salacca edulis</i>				
Buriti	$\beta$ -carotene	n.d.	31.13ff	(Neri-Numa et al. 2018)
<i>Mauritia flexuosa</i> L.				
Pistachio kernels	Lutein	0.812 <sup>ff</sup>	n.d	(Pumilia et al. 2014)
<i>Pistacia vera</i> L.				
Apricot	$\beta$ -carotene	4.75 <sup>dw</sup>	6.62dw	(Zaghdoudi et al. 2015)
<i>Prunus armeniaca</i> L.				
Peach	$\beta$ -carotene	1.22dw	3.98dw	(Zaghdoudi et al. 2015)
<i>Prunus persica</i> L.				
Kaki	$\beta$ -cryptoxanthin	3.32dw	7.76dw	(Zaghdoudi et al. 2016)
<i>Diospyros kaki</i> L.				
Tomato	Lycopene	511 <sup>dp</sup>	–	(Eh and Teoh 2012)e
<i>Solanum lycopersicum</i> L				
Grumixama	$\beta$ -cryptoxanthin	2.23ff	3.29ff	(Nascimento et al. 2017)
<i>Eugenia brasiliensis</i> Lam.				
Tucumã	$\beta$ -carotene	20.97ff	n.d	(Sagrillo et al. 2015)
<i>Astrocaryum aculeatum</i> Meyer				
Sanguinelli	$\beta$ -carotene	0.366–0.418fp	n.d	(Cebadera-Miranda et al. 2019)
<i>Citrus sinensis</i> (L.) cv. Sanguinelli				
Tarocco	$\beta$ -carotene	0.261–0.588fp	n.d	
<i>Citrus sinensis</i> [L.] Osbeck				
Gardenia	Crocin	841 <sup>dw</sup>	n.d	(Yang, Liu, and Gao 2009)
<i>Gardenia jasminoides</i> Ellis.				
Dovyalis	$\beta$ -cryptoxanthin	3.17ff	6.60 <sup>ff</sup>	(Rosso and Mercadante 2007)
<i>Dovyalis abyssinica</i> Warb.				
Tamarillo	$\beta$ -cryptoxanthin	1.97ff	4.40ff	(Rosso and Mercadante 2007)
<i>Cyphomandra betacea</i> (Cav.) Sendt.				
Grape				
<i>Vitis vinifera</i> L.				
Garnacha	$\beta$ -carotene	174.7ff	216.4ff	(Gutiérrez-Gamboa et al. 2018)
Tempranillo	$\beta$ -carotene	133.6 <sup>ff</sup>	171.9ff	
Graciano	$\beta$ -carotene	300.3.ff	341.3ff	
(B) Fruit	By-product/ residue			
Apple	Peel	n.d	n.d	(Delgado-Pelayo, Gallardo-Guerrero, and Hornero- Méndez 2014)
<i>Malus × domestica</i> Borkh				
Mango	Peel	n.d	n.d	(Ajila, Bhat, and Prasada Rao 2007)
<i>Mangifera indica</i> L.				
Melon	Rind	n.d	n.d	(Tadmor et al. 2010)
<i>Cucumis melo</i> L.				
Ponkan	Peel	n.d	n.d	(Wang, Chuang, and Hsu 2008)
<i>Citrus reticulata</i> B.				
Murcott	Peel	n.d	n.d	(Wang, Chuang, and Hsu 2008)
<i>Citrus</i> <i>reticulata</i> × <i>C. sinensis</i>				

(continued)

Table 3. Continued.

(A) Fruit source		Major Carotenoid(s)	Content mg/100 g	Total carotenoid content (TCC) mg/100 g	References
Tomato	Peel	Lycopene	13.59*	n.d	(Ho et al. 2015)
<i>Lycopersicon esculentum</i> L.	Pasta residue	Lycopene	41.11dw	n.d	(Xi 2006)
Peach palm	Peel	n.d	n.d	164.8dw	(Ordóñez-Santos, Pinzón-Zarate, and González- Salcedo 2015)
<i>Bactris gasipaes</i> Kunch					(Viganó et al. 2016)
Yellow Passion	Bagasse	$\beta$ -carotene + $\beta$ -cryptoxanthin	1.4fw	n.d	(Nascimento et al. 2017)
<i>Passiflora edulis</i> Sims					
Grumixama	Peel	$\beta$ -cryptoxanthin	3.96*	5.59*	
<i>Eugenia brasiliensis</i> Lam.					

<sup>df</sup>Values are based on dry fruit;

<sup>ff</sup>Values are based on fresh fruit;

<sup>fp</sup>Values are based on fresh pulp;

<sup>dw</sup>Values are based on dry weight;

<sup>fw</sup>Values are based on fresh weight;

\*Values are based on extract;

n.d – not determined.

*esculentum* L.) (E160d (i)), by the fermentation process of *Blakeslea trispora* fungi (E160d (ii)). The ADI for both is 0.5 mg/kg bw/day (EFSA 2008).

Despite of the approval of the main world authorities, EFSA and FDA, the stability of carotenoids may be a limitation for their application in food products. The presence of oxygen, light and temperature may cause oxidation of these compounds due to the amount of unsaturated bonds in their structure, resulting in color change (Ngamwonglumlert, Devahastin, and Chiewchan 2017). The coloration of carotenoids is also dependent on their aggregations or interactions with other substances, such as proteins and fat (Rodríguez-Concepcion et al. 2018; Saini and Keum 2018). In order to reduce the problems with oxidation it is possible to inactivate oxidative enzymes by bleaching with hot water/steam, however, the heat can cause changes in carotenoid structure, and for this reason the addition of an antioxidant agent, such as citric acid,  $\alpha$ -tocopherol, and butylated hydroxyanisole, may be more feasible for the preservation of oxidation (Ngamwonglumlert, Devahastin, and Chiewchan 2017). In relation to other pigments, such as anthocyanins, carotenoids reveal a greater stability to changes of pH (Ngamwonglumlert, Devahastin, and Chiewchan 2017).

### Chlorophylls

Among all pigments present in nature, chlorophylls are the ones that are most abundant in the plant kingdom, being the only ones responsible for the green color found in plant tissues; however, they have been the least studied natural colorants (Rodríguez-Amaya 2016). Five classes of chlorophylls can be found in the plant kingdom, which are chlorophyll *a*, *b*, *c*, *d* and *e*; chlorophyll *a* and *b* are the most found in several plant tissues and in fruits. The differences found between these compounds are the substituent group, chlorophyll *a* has a methyl ( $\text{CH}_3$ ) which gives a blue-green color, while chlorophyll *b* has an aldehyde ( $\text{CHO}$ ), that confers a yellow-green color. Usually these two forms of

chlorophyll may be found together in plants in a ratio of 3:1 (Ngamwonglumlert, Devahastin, and Chiewchan 2017). Leaves are generally the main sources of chlorophyll, nevertheless, their presence in fruits may occur before maturation process and synthesis of other pigments (Viera, Pérez-Gálvez, and Roca 2019). Moreover, green fruits have significant amounts of chlorophyll throughout their lifetime. Table 4 shows some fruits that can be alternative sources of this pigment.

Cucumber fruit (*Cucumis sativus* L.) (69–109 mg/100 g ff) has the highest content of chlorophyll, as well as olive (*Olea europaea* L.) (35 – 92 mg/100 g ff) (Shao, Tan, and Li 2016; Gandul-Rojas, Cepero, and Mínguez-Mosquera 1999). Usually, the content of chlorophyll *a* found in fruits is higher than the content of chlorophyll *b*, however, chayote has a higher content of chlorophyll *b* (24.58 against 22.30 mg/100 g of ff). Changes in the proportion of chlorophylls (and other pigments) can be induced by cultivation conditions, temperature and soil factors, so to standardize the yield of natural colorants from fruits, a control of the cultivation conditions is fundamental (Shao, Tan, and Li 2016; Pumilia et al. 2014; Iñiguez et al. 2011).

The chemical structure of chlorophylls comprises porphyrins or closed ring tetrapyrroles chelated with a centrally bound magnesium atom (Figure 1D) (Ngamwonglumlert, Devahastin, and Chiewchan 2017).

As a colorant, chlorophylls obtained from edible plant material or not (e.g. grass, lucerne (*Medicago sativa* L.) or nettle (*Urtica dioica* L.) can be used as natural colorants, such as chlorophylls (E140 (i)) and chlorophyllins (E140 (ii)), the latter being the colorant produced by saponification of the chlorophyll extract (EFSA 2015c; EFSA 2015d). The color additives copper complexes of chlorophylls, also known as Cu-chlorophylls E141(i), and copper complexes of chlorophyllins, also known as Cu-chlorophyllins E141 (ii), and sodium copper chlorophyllin in the USA, are manufactured from chlorophylls obtained from natural sources with addition of a copper, which causes the replacement of  $\text{Mg}^{2+}$  ions in the center of tetrapyrroles in chlorophyll with  $\text{Cu}^{2+}$  ions, and makes the pigment more stable, but due to this

**Table 4.** (A) Fruits sources of chlorophylls. (B) By-products and residues from fruit sources of chlorophylls.

(A) Fruit source	Major chlorophyll	Content mg/100 g	Total chlorophyll content mg/100 g	References
Bell pepper <i>Capsicum annuum</i> L.	n.d	n.d	103 <sup>df</sup>	(Pal, Khan, and Mohanty 2008)
Pistachio kernels <i>Pistacia vera</i> L.	Chlorophyll <i>a</i>	0.443 <sup>ff</sup>	1.59 <sup>ff</sup>	(Pumilia et al. 2014)
Olive <i>Olea europaea</i> L.	n.d	n.d	35–92 <sup>ff</sup>	(Gandul-Rojas, Cepero, and Mínguez-Mosquera 1999)
Pumpkin <i>Cucurbita moschata</i> Duch.	n.d	n.d	5.66–85.52 <sup>df</sup>	(Nawirska-Olszańska, Stępień, and Biesiada 2017)
Cucumber <i>Cucumis sativus</i> L.	Chlorophyll <i>a</i>	51–71 <sup>ff</sup>	69–109 <sup>ff</sup>	(Shao, Tan, and Li 2016)
Tomato <i>Lycopersicon esculentum</i> L.				(Manoharan et al. 2017)
Orange-brown	n.d	n.d	0.20 – 0.32 <sup>ff</sup>	
Brown	n.d	n.d	0.20 – 0.58 <sup>ff</sup>	
Red	n.d	n.d	0.01–0.28 <sup>ff</sup>	
Assam Lemon <i>Citrus limon</i> Burm.	n.d	n.d	2–86 <sup>ff</sup>	(Mukhim et al. 2016)
Thai lime <i>Citrus aurantifolia</i> Swingle cv. Paan	Chlorophyll <i>a</i>	~ 25 <sup>ff</sup>	n.d	(Kaewsuksaeng et al. 2015)
Grape <i>Vitis vinifera</i> L.				
Garnacha	n.d	n.d	.6.29 <sup>ff</sup>	(Gutiérrez-Gamboa et al. 2018)
Tempranillo	n.d	n.d	15.30 <sup>ff</sup>	(Gutiérrez-Gamboa et al. 2018)
Graciano	n.d	n.d	20.10 <sup>ff</sup>	(Mikulic-Petkovsek et al. 2016)
Mahaleb cherry <i>Prunus mahaleb</i> L.	Chlorophyll <i>a</i>	1.0 <sup>ff</sup>	1.13 <sup>ff</sup>	(Mikulic-Petkovsek et al. 2016)
Bird cherry <i>Prunus padus</i> L.	Chlorophyll <i>a</i>	4.25 <sup>ff</sup>	6.50 <sup>ff</sup>	(Mikulic-Petkovsek et al. 2016)
Blackthorn <i>Prunus spinosa</i> L.	Chlorophyll <i>a</i>	1.563 <sup>ff</sup>	2.99 <sup>ff</sup>	(Mikulic-Petkovsek et al. 2016)
Carambola <i>Averrhoa carambola</i> L.	n.d	n.d	~2.7 <sup>ff</sup>	(Gol, Chaudhari, and Rao 2015)
Chayote <i>Sechium edule</i> (Jacq.) Sw.	Chlorophyll <i>b</i>	24.58 <sup>ff</sup>	46.80 <sup>ff</sup>	(Iñiguez et al. 2011)
Daraesoon <i>Actinidia arguta</i> Planchon	Chlorophyll <i>a</i>	178.4 <sup>df</sup>	293.2 <sup>df</sup>	(Ahn and Choe 2015)
Guava <i>Psidium guajava</i> L.	n.d	n.d	9.9 <sup>ff</sup>	(Hong et al. 2012)
Apple <i>Malus × domestica</i> Borkh				(Delgado-Pelayo, Gallardo-Guerrero, and Hornero-Méndez 2014)
Granny Smith	n.d	n.d	6.03 <sup>dp</sup>	
Green Doncella	n.d	n.d	~4 .0 <sup>dp</sup>	
Green Golden Delicious	n.d	n.d	2.76 <sup>dp</sup>	

(B) Fruit	By-product/residue				
Kiwi	Skin	n.d	n.d	12.13 <sup>dw</sup>	(Soquetta et al. 2016)
<i>Actinidia chinensis</i> Planch	Pomace	n.d	n.d	2.02 <sup>dw</sup>	
Lemon <i>Citrus limon</i> L. Osbeck	Skin	Chlorophyll <i>a</i> and <i>b</i>	0.02–0.142 <sup>fw</sup>	n.d	(Conesa et al. 2019)
Apple <i>Malus × domestica</i> Borkh	Peel	Chlorophyll <i>a</i> and <i>b</i>	14.8–26.8 <sup>dw</sup>	n.d	(Delgado-Pelayo, Gallardo-Guerrero, and Hornero-Méndez 2014)
Melon <i>Cucumis melo</i> L.	Peel	n.d	n.d	15–85 <sup>fw</sup>	(Tadmor et al. 2010)
Avocado <i>Persea americana</i> Mill.	Skin	Chlorophyll <i>a</i>	43 <sup>fw</sup>	59 <sup>fw</sup>	(Cox et al. 2004)

<sup>df</sup>Values are based on dry fruit;<sup>ff</sup>Values are based on fresh fruit;<sup>dp</sup>dry pulp;<sup>dw</sup>Values are based on dry weight;<sup>fw</sup>Values are based on fresh weight;

n.d. – not determined.



**Table 5. (A)** Fruits sources of flavonoids. **(B)** Residues from fruit sources of flavonoids.

(A) Fruit source		Natural colorant	Total content mg/100 g	References
Raspberry		Total quercetin content	0.32–1.55 <sup>ff</sup>	(Anttonen and Karjalainen 2005)
<i>Rubus idaeus</i> L.				
Tucumã		Quercetin	4.97 <sup>ff</sup>	(Sagrillo et al. 2015)
<i>Astrocaryum aculeatum</i> Meyer		Quercetin 3-O-rutinoside	14.51 <sup>ff</sup>	
Papaya		Total quercetin content	81.0 <sup>df</sup>	(Miean and Mohamed 2001)
<i>Carica papaya</i> L.				
Bell pepper		Total quercetin content	79.9 <sup>df</sup>	(Miean and Mohamed 2001)
<i>Capsicum annum</i> L.		Total apigenin content	27.2 <sup>df</sup>	
Belimbi		Total apigenin content	45.8 <sup>df</sup>	(Miean and Mohamed, 2001)
<i>Averrhoa belimbi</i> L.				
Guava		Total apigenin content	57.9 <sup>df</sup>	(Miean and Mohamed, 2001)
<i>Psidium guajava</i> L.				
Malabar spinach		Total quercetin content	0.82 <sup>ff</sup>	(Kumar et al. 2015)
<i>Basella rubra</i> L.		Total apigenin content	0.42 <sup>ff</sup>	
Goji		Quercetin 3-O-rutinoside	1660 <sup>df</sup>	(Pires et al. 2018)
<i>Lycium barbarum</i> L.				
Sanguinelli		Total quercetin content	29.4–40.0 <sup>fp</sup>	(Cebadera-Miranda et al. 2019)
<i>Citrus sinensis</i> (L.) cv. Sanguinelli				
Tarocco		Total quercetin content	31.8–39.7 <sup>fp</sup>	
<i>Citrus sinensis</i> (L.) Osbeck				
Olive		Quercetin 3-O-rutinoside	6.19 <sup>ff</sup>	(Deng et al. 2017)
<i>Olea europaea</i> L.				
Apple		Total quercetin content	1.19 <sup>ff</sup>	(Sultana and Anwar, 2008)
<i>Malus pumila</i> Mill.				
Apricot		Total quercetin content	32.2 <sup>ff</sup>	(Sultana and Anwar, 2008)
<i>Prunus armeniaca</i> L.				
Wild cherry		Quercetin 3-O-glucoside	0.89 <sup>ff</sup>	(Mikulic-Petkovsek et al. 2016)
<i>Prunus avium</i> L.		Quercetin 3-O-rutinoside	2.36 <sup>ff</sup>	
Mahaleb cherry		Quercetin 3-O-sophoroside-7-O-rhamnoside	10.54 <sup>ff</sup>	(Mikulic-Petkovsek et al. 2016)
<i>Prunus mahaleb</i> L.				
Bird cherry		Quercetin 3-O-hexosyl-pentoside	22.3 <sup>ff</sup>	(Mikulic-Petkovsek et al. 2016)
<i>Prunus padus</i> L.		Quercetin 3-O-rutinoside	6.47 <sup>ff</sup>	
		Quercetin 3-O-galactoside	5.29 <sup>ff</sup>	
		Apigenin rhamnoside	2.45 <sup>ff</sup>	
Blackthorn		Quercetin 3-O-pentoside	7.06 <sup>ff</sup>	(Mikulic-Petkovsek et al. 2016)
<i>Prunus spinosa</i> L.		Quercetin 3-O-hexosyl-pentoside	5.23 <sup>ff</sup>	
		Quercetin 3-O-rutinoside	0.37 <sup>ff</sup>	
Tomato		Quercetin pentosyl-rutinoside	0.470 <sup>ff</sup>	(Barros et al. 2012)
<i>Solanum lycopersicum</i> L.		Quercetin 3-O-rutinoside	0.468 <sup>ff</sup>	
Elderberry		Quercetin 3-O-rutinoside	35.59–52.02 <sup>ff</sup>	(Veberic et al. 2009)
<i>Sambucus nigra</i> L.		Quercetin 3-O-glucoside	6.38–26.52 <sup>ff</sup>	
Strawberry tree		Quercetin 3-O-rutinoside	1.70 <sup>df</sup>	(Guimarães et al. 2013)
<i>Arbutus unedo</i> L.		Quercetin 3-O-glucoside	2.34 <sup>df</sup>	
		Quercetin rhamnoside	2.10 <sup>df</sup>	
		Quercetin pentoside	1.32 <sup>df</sup>	
Dog rose		Quercetin 3-O-rutinoside	0.47 <sup>df</sup>	(Guimarães et al. 2013)
<i>Rosa canina</i> L.		Quercetin 3-O-glucoside	0.66 <sup>df</sup>	
		Quercetin hexoside	0.78 <sup>df</sup>	
		Quercetin rhamnoside	0.46 <sup>df</sup>	
Wild rose		Quercetin 3-O-rutinoside	0.32 <sup>df</sup>	(Guimarães et al. 2013)
<i>Rosa micrantha</i> Borrer ex Sm.		Quercetin 3-O-glucoside	0.97 <sup>df</sup>	
		Quercetin hexoside	0.90 <sup>df</sup>	
		Quercetin rhamnoside	0.71 <sup>df</sup>	
Jabuticaba		Total quercetin content	5.22–6.79 <sup>df</sup>	(Seraglio et al. 2018)
<i>Myrciaria cauliflora</i> Berg.				
Guabiju		Total quercetin content	7.88–8.24 <sup>df</sup>	(Seraglio et al. 2018)
<i>Myrcianthes pungens</i> (O. Berg) D. Legrand				
Jambolan		Total quercetin content	0.44 – 0.98 <sup>df</sup>	(Seraglio et al. 2018)
<i>Syzygium cumini</i> L.				
Camu-camu		Total quercetin content	3.0 <sup>fp</sup>	(Neri-Numa et al. 2018)
<i>Myrciaria dubia</i> (HBK) McVaugh				
(B) Fruit		Residue		
Avocado	Peel	Quercetin 3-O-glucoside	117.5*	(Melgar et al. 2018)
<i>Persea americana</i> Mill.		Quercetin 3-O-rhamnoside-hexoside	121.1*	
Xoconostle	Peel	Quercetin O-(di-deoxyhexosyl-hexoside)	10.0*	(Morales et al. 2015)
<i>Opuntia matudae</i> Scheinvar cv. Rosa				

(continued)

Table 5. Continued.

(A) Fruit source		Natural colorant	Total content mg/100 g	References
<i>Opuntia joconostle</i> cv. Cuaresmeño	Peel	Quercetin O-(di-deoxyhexosyl-hexoside))	38 <sup>fw</sup>	(Morales et al. 2014)
		Quercetin 3-O-rutinoside	21 <sup>fw</sup>	
		Quercetin 3-O-glucoside	19 <sup>fw</sup>	

<sup>df</sup>Values are based on dry fruit;<sup>ff</sup>Values are based on fresh fruit;<sup>fp</sup>Values are based on fresh pulp;<sup>dw</sup>Values are based on fresh weight;

\*Values are based on extract.

process these additives are not considered natural. These four colorants are allowed in the EU, yet, EFSA, in the last reevaluation of color additives, finished in 2016, considered that there were a few studies on the toxicity and absorption by the human body of these substances and therefore, it was not possible to establish a value of ADI safe for the human consumption (EFSA, 2015f). In the USA, the FDA allows the use of sodium copper chlorophyllins with ADI of 7.5 mg/kg bw/day.

The acidic pH and the high temperature are crucial factors over the stability of chlorophylls, in which it can occur the formation of several distinct derivatives, changing the color from green to brown; this reaction is known as pheophytinization, and results into the conversion of chlorophylls to Mg<sup>2+</sup> free derivatives, such as pheophytins and pyropheophytin (Pumilia et al. 2014).

Moreover, chlorophylls and the colorants derived from it have shown anti-carcinogenic activity and protective effect against DNA damage (Kang et al. 2019).

### Others colorant compounds

In addition to the compounds mentioned above, other molecules found in fruits can promote color, more specifically the yellowness. A promising class of natural food colorants is non-anthocyanin phenolic compounds, namely flavonols (myricetin, quercetin and kaempferol) and flavones (luteolin and apigenin) (Figure 1E). In fact, non-anthocyanin phenolic compounds do not have legislation as food additives; despite their wide recognition as prominent antioxidants, health promoters and functional food ingredients, the use of this large group of bioactive molecules as food colorants remains little investigated (Martins et al. 2016).

Briefly a contextualization of the main flavonols and flavones found in fruits will be stated. Flavonols (3-hydroxyflavones) are known to confer a yellow coloration in plant tissues. In general, they are sensitive to the presence of light and easily photo-oxidized, which promotes the appearance of a darker coloration. Quercetin, kaempferol, and myricetin are the most common flavonols in nature. In fruits, they are present mainly as quercetin (Sultana and Anwar 2008; Mian and Mohamed 2001). Thus, this review will give more focus on this compound.

The quercetin aglycone has a crystal form with brilliant yellow color and poorly water soluble. The glycosylation with sugar molecules, mainly glucose, rhamnose and rutinoside, promote the formation of quercetin glycoside derivatives, which increases its solubility in water. Natural

occurrence of quercetin in fruits and vegetables is in its glycoside form. However, the acid present in the composition of vegetal tissue, may cause its hydrolysis into quercetin and the sugar moieties (Kumar, Vijayalakshmi, and Nadanasabapathi 2017; Li et al. 2016). The term quercetin refers only to the aglycone, thus in research, it is typically used to quantify the total quercetin-type molecules, including its glycoside (Li et al. 2016). These compounds are widely distributed in fruits and some examples can be seen in Table 5. Onions (*Allium cepa* L.) are known as the main source of quercetin and its derivatives (~14 mg/100 g of fresh weight) (Kumar, Vijayalakshmi, and Nadanasabapathi 2017). Elderberry (*Sambucus nigra* L.) presents a high content of quercetin derivatives (35.59 mg/100 g ff) (Veberic et al. 2009); surpassing the content found in onions and apples (*Malus pumila* Mill.) (1.19 mg/100 g ff) (Sultana and Anwar 2008), that are considered a source of these flavonols. Other berries, such as *Prunus* species, are also rich in quercetin.

Quercetin and its derivatives have been widely reported for their beneficial properties in health; generally they are known due to their antioxidant, anti-obesity, anti-inflammatory, anti-proliferative effects, and its usage in the prevention of neurodegeneration and cardiovascular diseases, and cytoprotective activity (Kumar, Vijayalakshmi, and Nadanasabapathi 2017; Li et al. 2016). However, its colorant capacity has been little exploited by the food industry. On the other hand, there are several studies directed to the production of natural colorants for the textile industry based on quercetin (Yan, Pan, and Ji 2018).

In food products, Cavin et al. (2013) used quercetin and its derivatives to promote the browning in foodstuffs heated by microwave oven, to simulate the color developed by Maillard reaction, that occurs during the baking/cooking with high temperature. The yellow-brownish color obtained by quercetin after heat can be intensified with the use of a chemical base, such as sodium bicarbonate, which effects on the breakdown of quercetin glycosides.

The extraction of quercetin from plant matrices is benefited by alkaline media, due to quercetin glycoside breaking down and the rupture of cell wall. In relation to quercetin stability, acid or alkaline solutions cause the conversion of quercetin glycoside into its aglycone form, and light has oxidative effect on them. Quercetin and its derivatives have shown stability during thermal processing (Li et al. 2016). The use of quercetin as a colorant additive has not yet been evaluated by regulatory authorities. However, these compounds are consumed daily. In Spain, for example, the consumption of these compounds is

about 18.48 mg/day and in the USA is 9.75 mg/day (Li et al. 2016). These values are lower than the daily doses of quercetin ingestion recommended of 250–600 mg per day for the promotion of their health benefits (Kumar, Vijayalakshmi, and Nadanasabapathi 2017).

Another group of non-anthocyanin flavonoids, known for their colorant capacity, are flavones and can be found in many plants, such as vegetables and in different plant parts, such as flowers. They are known for their antioxidant, anti-carcinogenic, anti-inflammatory, and anti-estrogenic activities, among other properties (Ali et al. 2017).

There is an immense range of flavones, thus the best-known and occurring in higher amounts in plants is apigenin. Apigenin is a yellow crystalline powder, being the aglycone of several naturally occurring glycosides. Glycosylation of apigenin may occur by hydroxyl substitutions at positions 4', 5, and 7 of the basic flavonoid skeleton (Ali et al. 2017). It is insoluble in water but soluble in organic solvents. This pigment occurs mainly in flowers, where one of the most common sources is chamomile (*Chamomilla matricaria* L.), with about of 320 mg/100 g of dry weight, but vegetables and fruits can also contain this flavone. Table 5 presents the content of apigenin and its derivatives found in some fruits. Guava (*Psidium guajava* L.), belimbi (*Averrhoa belimbi* L.) and bell pepper (*Capsicum annum* L.) are the fruits with the highest apigenin content, with 57.9, 45.8 and 27.2 mg/100 g df, respectively. A relative high amount of this pigment can also be found in some berries, such as blackthorn (*Prunus spinosa* L.) and sweet cherry (*Prunus avium* L.).

Apigenin and its derivatives have also shown antioxidant and anti-inflammatory activities, and are considered potent therapeutic agents to overcome diseases such as rheumatoid arthritis, autoimmune disorders, neurological diseases, as Parkinson and Alzheimer, and also exhibit anti-proliferative activity in several cancer types, including breast, prostate, melanoma, and leukemia (Ali et al. 2017).

The research conducted using apigenin is mainly related to its pharmacological potential, being its use as food colorant scarcely explored. However, as previously mentioned apigenin and its derivatives are used in the textile industry for dyeing wool (Ali et al. 2017).

### Extraction of natural colorants from fruits

The extraction of the pigments from the plant matrix is a crucial step for the success of obtaining natural colorants, since these substances, such as anthocyanins, betalains, carotenoids, and chlorophylls, are sensitive to temperature, presence of oxygen, light, among other factors. Therefore, several studies evaluate different methods of extraction of these colorant compounds (Saini and Keum 2018; Ngamwonglumlert, Devahastin, and Chiewchan 2017; Martins and Ferreira 2017). Moreover, it is necessary to consider the peculiarities of the plant matrix that contain the desired pigment. In this section we will discuss the main methods and extraction variables used to obtain natural colorants from fruits.

### Extraction methods of natural colorants from fruits

Several extraction techniques are applied to obtain natural colorants; the choose of the best method to extract these compounds is essential to make a process viable and sustainable. These techniques are defined as conventional, such as is the case of Soxhlet (Sox) and maceration (MA), or non-conventional, such as microwave assisted extraction (MAE), ultrasound assisted extraction (UAE), supercritical fluid extraction (SFE), pressurized liquid extraction (PLE) and high pressure extraction (HPE). Non-conventional techniques have been referred to as “green extraction methods” due to the use of less energy and solvent, being considered environmentally friendly.

Conventional techniques are simple methods that do not require great skills. Sox is traditionally known as a method for lipid extraction; however, it has also been used to obtain bioactive compounds. In the case of pigments, Sox has shown good yields for carotenoids and anthocyanins. For example, this conventional method was around 13% more efficient in carotenoids extraction from kaki (*Diospyros kaki* L.), apricot (*Prunus armeniaca* L.) and peach (*Prunus persica* L.) fruits than PLE (Zaghdoudi et al. 2015). For anthocyanins extraction from bilberry (*Vaccinium myrtillus* L.) residue, Sox presented almost double the yield obtained under conditions optimized for PLE, 457 and 254 mg of anthocyanins/100 g of extract, respectively (Paes et al. 2014). However, this method is generally applied only in order to evaluate the efficiency of other methods, not being employable at industrial level due the long extraction time and amount of solvent that are required to process (Saini and Keum 2018).

Extraction by MA does not require great technology and can be conducted at room temperature, which is interesting for obtaining thermosensitive pigments, such as betalains (Ngamwonglumlert, Devahastin, and Chiewchan 2017). However, MA with heat (also known as heat assisted extraction (HAE)) can increase the solubility of target compounds and reduce the extraction time. The efficiency of MA is dependent on the solubility of the pigment from the fruit matrix in the extraction solvent (Ngamwonglumlert, Devahastin, and Chiewchan 2017; Saini and Keum 2018). Although these methodologies facilitate mass transfer between the different phases of the system, it consumes a lot of energy and time, which can be unviable for an industrial scale production, and can also result in the degradation of thermosensitive compounds, except in the extraction by maceration without heat (Ngamwonglumlert et al. 2017). Optimized conditions can make this method more efficient in relation to the obtained yield versus extraction time. According to data shown Table 6, the optimum extraction of anthocyanins from strawberry tree (*Arbutus unedo* L.) by MA consumed only 5 min (yield of 38.2 mg of anthocyanins/100 g of dry weight (López et al. 2018), and to obtain optimum yield of betalains from xocostle (*Opuntia joconostle* cv.) it was only necessary 10 min (yield of 92 mg of betalains/100 g of fresh weight (Sanchez-Gonzalez et al. 2013).

UAE is a non-conventional method which has shown an actual effect in obtaining compounds. This method is

**Table 6.** Optimized methods for extraction of natural colorants from fruits.

Extraction conditions								
Method	Fruit	Part	Natural colorant	Solvent/cosolvent	Added Substance/ pH	Temp Time frequency (°C) (min.) (W or kHz)	Pressure Yield (bar or MPa) (mg/100 g)	References
Maceration (MA)	Strawberry tree	Whole fruit	Anthocyanin's	Ethanol 80%	0.05% Hydrochloric acid	90 5	–	(López et al. 2018)
	Sweet cherry	Whole fruit	Anthocyanin's	Methanol 100%	0.1 % hydrochloric acid	37 90	–	(Blackhall et al. 2018)
	Fig	Peel	Anthocyanin's	Ethanol 100%	Citric acid - pH 3	35.6 13.7	–	(Backes et al. 2018)
	Raspberry	Whole fruit	Anthocyanin's	Ethanol 95%	1.5 M Hydrochloric acid	71 53	–	(Chen et al. 2007)
	Pitaya	Pulp	Betalains	Methanol 80%	1% Trifluoroacetic acid	– 30	–	(García-Cruz et al. 2017)
	Prickly pear	Pulp	Betalains	Water	Citric acid - pH 6.9	46 115	–	(Maran, Manikandan, and Mekala 2013)
	Prickly pear	Pulp	Betalains	Water	–	5 20	–	(Castellanos-Santiago and Yahia, 2008)
	Xoconostle	Whole Fruit	Betalains	Methanol 20 %	1% Citric acid	15 10	–	(Sanchez-Gonzalez et al. 2013)
	Gardenia	Whole fruit	Carotenoids	Ethanol 51.3%	–	70.4 28.6	–	(Yang, Liu, and Gao 2009)
Ultrasound Assisted Extraction (UAE)	Tomato	Pulp	Carotenoids	hexane/acetone/ ethanol (2:1:1)	–	40 40	–	(Eh and Teoh 2012)
	Black chokeberry	Pomace	Anthocyanin's	Ethanol 34%	–	70 17	–	(D'Alessandro et al. 2014)
	Blueberry	Pomace	Anthocyanin's	Ethanol 70%	–	61 26	–	(He et al. 2016)
	Blueberry	Residue	Anthocyanin's	Ethanol 70%	–	80 90	–	(Machado et al. 2017)
	Grumixama	Residue	Anthocyanin's	Ethanol 70%	–	80 90	–	(Machado et al. 2017)
	Black Mulberry	Whole fruit	Anthocyanin's	Methanol 76 %	Hydrochloric acid -pH 3	48 10	–	(Espada-Bellido et al. 2017)
	Eggplant	Peel	Anthocyanin's	Methanol 54.4%	0.01% Hydrochloric acid	55.1 44.85	–	(Dranca and Oroian 2016)
	Haskap	Whole fruit	Anthocyanin's	Ethanol 80%	0.5% Formic acid	35 20	–	(Celli, Ghanem, and Brooks 2015)
	Fig	Peel	Anthocyanin's	Ethanol 100 %	Citric acid - pH 3	– 21	–	(Backes et al. 2018)
	Raspberry	Whole fruit	Anthocyanin's	Ethanol 95%	1.5 M Hydrochloric acid	– 3.33	–	(Chen et al. 2007)
	Blackberry	Residue	Anthocyanin's	Ethanol 70 %	–	80 90	–	(Machado et al. 2017)
	Red cabbage	Whole fruit	Anthocyanin's	Water	–	15 90	–	(Ravanfar et al. 2018)
	Jabuticaba	Peel	Anthocyanin's	Ethanol 46%	Hydrochloric acid - pH 2	30 10	–	(Rodrigues et al. 2015)
	Purple passion	Peel	Anthocyanin's	Ethanol 100%	0.01% Hydrochloric acid	– 50	–	(Liu et al. 2018)
	Strawberry tree	Whole fruit	Anthocyanin's	Ethanol 60%	0.05% Hydrochloric acid	– 27	–	(López et al. 2018)
Haskap	Whole fruit	Anthocyanin's	Ethanol 80%	0.5% Formic acid	35 20	–	(Celli, Ghanem, and Brooks 2015)	
Malabar spinach	Pulp	Betalains	Water	–	54 32	–	(Maran and Priya 2015)	
Peach palm	Residue	Carotenoids	Sunflower oil	–	35 30	–	(Ordóñez-Santos, Pinzón-Zarate, and González-Salcedo 2015)	

	Tomato	Pulp	Carotenoids	Hexane/acetone/ ethanol (2:1:1)	47.6	45.6	340 W	–	511dw	(Eh and Teoh 2012)
Microwave Assisted Extraction (MAE)	Grape	Skin	Anthocyanin's	Methanol 40%	100	5	500 W	–	119dw	(Liazid et al. 2011)
	Blueberry	Whole fruit	Anthocyanin's	Ethanol 60%	60	20	800 W	–	2110dw	(Piovesan et al. 2017)
	Mulberry	Whole fruit	Anthocyanin's	Methanol 56%	–	2.2	425 W	–	5472dw	(Zou et al. 2012)
	Fig	Peel	Anthocyanin's	Citric acid - pH 3	62	5	400 W	–	411dw	(Backes et al. 2018)
	Raspberry	Whole fruit	Anthocyanin's	1.5 M Hydrochloric acid	–	12	366 W	–	43.42 <sup>fw</sup>	(Sun et al. 2007)
Supercritical Fluid Extraction (SFE)	Black currant	Whole fruit	Anthocyanin's	Hydrochloric acid - pH 2	–	10	700 W	–	2040 <sup>dw</sup>	(Pap et al. 2013)
	Pitaya	Peel	Betalains	Water	35	8	100 w	–	9*	(Thirugnanasambandham and Sivakumar 2017)
	Pitaya	Peel	Betalains	Water	45	20	400 W	–	520*	(Nazeri and Zain 2018)
	Tomato	Peel	Carotenoids	Ethyl acetate	–	1	400 W	–	13.59 *	(Ho et al. 2015)
	Tomato	Whole fruit	Quercetin	Ethanol 100%	60	20	200 W	–	1858 *	(Pinela et al. 2016)
Pressurized Liquid Extraction (PLE)	Juçara	Residue	Anthocyanin's	90% CO <sub>2</sub> + 5% H <sub>2</sub> O + 5% ethanol	60	46	–	20 MPa	2200dw	(Garcia-mendoza et al. 2017)
	Bilberry	Residue	Anthocyanin's	90% CO <sub>2</sub> + 5% H <sub>2</sub> O + 5% ethanol	40	–	–	20 MPa	1071 *	(Paes et al. 2014)-
	Jambulan	Pulp	Anthocyanin's	CO <sub>2</sub> + ethanol (2 g/min)	50	–	–	162 bar	231.13dw	(Maran, Priya, and Manikandan 2014)
	Prickly pear	Pulp	Betalains	CO <sub>2</sub>	40	60	–	100 bar	89 <sup>dw</sup>	(Nunes, Carmo, and Duarte 2015)
	Kaki	Whole fruit	Carotenoids	75% CO <sub>2</sub> + 25% ethanol	60	30	–	300 bar	7.6dw	(Zaghdoudi et al. 2016)
High Pressure Extraction (HPE)	Tomato	Residue	Carotenoids	95 % CO <sub>2</sub> + 5% ethanol	55	120	–	300 bar	17.3dw	(Baysal, Ersus, and Starmans 2000)
	Passion	Pomace	Carotenoids	CO <sub>2</sub>	60	–	–	35 MPa	1.16dw	(Viganò et al. 2016)
	Pitanga	Whole fruit	Carotenoids	CO <sub>2</sub>	60	120	–	250 bar	547.4dw	(Filho et al. 2008)
	Juçara	Residue	Anthocyanin's	Water	40	30	–	10 MPa	920dw	(Garcia-mendoza et al. 2017)
	Açaí	Pulp	Anthocyanin's	Ethanol 50%	30	20	–	20 bar	700dw	(Alcázar-Alay et al. 2017)
High Pressure Extraction (HPE)	Grumixama	Residue	Anthocyanin's	Ethanol 70%	80	30	–	10 MPa	44dw	(Machado et al. 2017)
	Blackberry	Residue	Anthocyanin's	Ethanol 70%	80	30	–	10 MPa	184dw	(Machado et al. 2017)
	Blueberry	Residue	Anthocyanin's	Ethanol 70%	80	30	–	10 MPa	101dw	(Machado et al. 2017)
	Bilberry	Residue	Anthocyanin's	Water	40	15	–	20 MPa	254 *	(Paes et al. 2014)-
	Kaki	Whole fruit	Carotenoids	Methanol:tetrahydrofuran (80:20)	40	5	–	103 bar	6.78dw	(Zaghdoudi et al. 2015)
High Pressure Extraction (HPE)	Peach	Whole fruit	Carotenoids	Methanol:tetrahydrofuran (80:20)	40	5	–	103 bar	3.98dw	(Zaghdoudi et al. 2015)
	Apricot	Whole fruit	Carotenoids	Methanol:tetrahydrofuran (80:20)	40	5	–	103 bar	6.62dw	(Zaghdoudi et al. 2015)
	Grape	Skin	Anthocyanin's	Ethanol 100 %	50	30	–	600 Mpa	~3200dw	(Corrales et al. 2009)
	Grape	Pomace	Anthocyanin's	Ethanol 75 %	29.5	3.39	–	268 Mpa	~370dw	(Putnik et al. 2018)
	Pomegranate	Peel	Anthocyanin's	Ethanol 80 %	20	23	–	385 Mpa	8600dw	(Alexandre et al. 2017)
High Pressure Extraction (HPE)	Tomato	Residue	Carotenoids	Ethyl lactate	25	10	–	700 Mpa	16.52dw	(Strati, Gogou, and Oreopoulou 2015)
	Tomato	Residue	Carotenoids	Ethanol 75 %	–	1	–	500 Mpa	41.11dw	(Xi 2006)
	Tomato	Pulp	Carotenoids	Hexane 75 %	–	10	–	450 Mpa	2.01 <sup>fw</sup>	(Briones-Labarca, Giovagnoli-Vicuña, and Canas- Sarazúa 2019)

<sup>dw</sup>Values are based on dry weight; <sup>fw</sup> Values are based on flesh weight; \* values for mg/L of extract.



usually applied in either solid/fluid media and consists in the transmission of ultrasound waves by cavitation, allowing the rupture of the plant tissue, facilitating the extraction of intracellular molecules, for this reason, the extraction by UAE may be faster than by conventional extraction and require less solvent, and in addition, UAE can be applied without heat (Saini and Keum 2018; Ngamwonglumlert, Devahastin, and Chiewchan 2017). Thus, this method is an interesting methodology to be applied in the extraction of natural colorants, since it allows the extraction of molecules at lower temperatures and can be used for both hydro and lipid soluble pigments depending on the solvent. According to data shown in Table 6, efficient results by UAE have been obtained in the extraction of anthocyanins, betalains, and carotenoids. For example, UAE was more efficient than ME and MAE for anthocyanins extraction from fig (*Ficus carica* L.) peel (Backes et al. 2018). For carotenoids extraction, namely lycopene from tomato, the yield by UAE was higher in 26% than the one obtained by conventional method (MA) (Eh and Teoh 2012). However, the same success of UAE was not obtained with fruits of strawberry tree (*Arbutus unedo* L.); for this plant matrix HAE showed a slight increase in the yield with less time (López et al. 2018). This shows that the specificity of the extraction can change according to the characteristics of the fruit matrix.

MAE is a simple technique that allows the extraction of hydro and liposoluble compounds. Generally, the extraction by MAE requires less time and solvent than that carried out by conventional methods. These advantages are consequence of the rupture of vegetal cells caused by microwaves radiation, allowing a great transference of the targeted compounds to the solvent (Ngamwonglumlert, Devahastin, and Chiewchan 2017; Saini and Keum 2018). For pigments extraction, MAE has been reported by several authors as the most efficient technology, as shown in Table 6. MAE provides the possibility to obtain lycopene from tomato in relatively short time under optimum conditions. The same can be observed for anthocyanins extraction from mulberry (*Morus atropurpurea* Roxb.) (Zou et al. 2012). In comparison with the UAE method for anthocyanins extraction from fig peel, MAE was slightly less efficient in the extraction yield (432 and 411 mg/100 g dry weight by UAE and MAE, respectively); however, the UAE required an extraction time 4 times longer than the MAE to achieve optimal results (21 and 5 min, respectively) (Backes et al. 2018), thus the extraction efficiency per time may also be an interesting parameter for the employment of this method at industrial scale.

The SFE consists in the use of a solvent at its critical point with a defined temperature and pressure, at which point the solvent has gas and liquid properties (Supercritical fluid), allowing a greater efficiency in the extraction due to the higher penetration of the solvent into the matrix (Ngamwonglumlert, Devahastin, and Chiewchan 2017). Carbon dioxide (CO<sub>2</sub>) is normally used as the solvent for several reasons: (i) its critical temperature and pressure are 31.2°C and 74 bars, respectively, which are not so high, therefore the extraction of heat-sensitive compounds is possible; (ii) as a solvent it is harmless to the environment and

to human health; iii) the oxidation reactions are limited due to the absence of air (Saini and Keum 2018). Due to the CO<sub>2</sub> polarity, the SFE methodology is mainly applied to the extraction of non-polar compounds, such as carotenoids (Ngamwonglumlert, Devahastin, and Chiewchan 2017; Saini and Keum 2018). However, with the addition of a polar co-solvent, also known as modifier, such as ethanol and water, it is possible to obtain other hydro-soluble pigments, such as betalains and anthocyanins. Table 6 shows examples of optimized SFE of these compounds. According to Table 6, ethanol has also been used as co-solvent for carotenoids extraction, which can be explained by the presence of slightly polar carotenoids in the xanthophyll class (Baysal, Ersus, and Starmans 2000). However, it is important to emphasize that the efficient use of SFE with co-solvent requires a massive study about the proportion that provides the best solubility of the target compounds and yields, as reported by Seabra et al. (2010). In their study, the extraction of anthocyanins from elderberry (*Sambucus nigra* L.) pomace was evaluated, using 30 different combinations of CO<sub>2</sub> and co-solvents (ethanol (EtOH) and water (H<sub>2</sub>O)). They found lower yield (1.7%) using 90% CO<sub>2</sub>:8% EtOH:2% H<sub>2</sub>O, and higher yield (21.3%) with 20% CO<sub>2</sub>:40% EtOH:40% H<sub>2</sub>O. Also, according with data present in Table 6, the proportion CO<sub>2</sub>, ethanol and water, 90%, 5% and 5% respectively, was more efficient in the extraction of anthocyanins from juçara (*Euterpe edulis* M.) (Garcia-mendoza et al. 2017; Garcia-mendoza et al. 2017), and bilberry residue (Paes et al. 2014).

Another method based on high pressure extraction is PLE, which is also known for its efficiency and for being more ecological. PLE, also known as accelerated solvent extraction (ASE), is a method employed to the use of a liquid solvent at high pressure (10.3–13.8 MPa) combined with high temperature. Non-polar and polar solvents can be used in PLE, allowing the extraction of several compounds. However, this technique is not recommended for heat-sensitive molecules (Ngamwonglumlert, Devahastin, and Chiewchan 2017). The penetration of the solvent into the vegetal cell is favored by high pressure, that cause deformation or cell membrane damage, meanwhile the heat decreases the solvent viscosity, which provides a favorable condition for the mass transfer (Saini and Keum 2018; Ngamwonglumlert, Devahastin, and Chiewchan 2017).

Examples of colorants optimization processes using PLE can be visualized in Table 6. The optimized yield of carotenoids extracted from kaki (*Diospyros kaki* L.), apricot (*Prunus armeniaca* L.) and peach (*Prunus persica* L.) was obtained by applying a low temperature (40°C) and a short-time (5 min) extraction period, due to the application of an adequate pressure (103 bar) and most appropriate solvents, in this case tetrahydrofuran (moderate polar and aprotic solvent for non-polar carotenoids) and methanol (polar carotenoids, such as xanthophylls) (Zaghdoudi et al. 2015). Temperature also had no effect on the yield of anthocyanins extraction from açai (*Euterpe oleracea* Mart.) pulp, which contradicts the effect of high temperatures on the extraction efficiency of these compounds (Alcázar-Alay et al. 2017).

Meanwhile, the extraction efficiency of anthocyanins from blueberry (*Vaccinium ashei* Reade), blackberry (*Rubus fruticosus* L.) and grumixama (*Eugenia brasiliensis* Lam.) residues using PLE in relation to UAE was lower (Machado et al. 2017); according to the authors, high pressure and high temperature may have occasioned the degradation of anthocyanins.

HPE, also known as high hydrostatic pressure (HHP), was developed with the aim of extracting bioactive molecules. As well as in PLE method, the high pressure (100–900 MPa) causes damage in plant cells, facilitating the extraction of targeted compounds. As opposed to PLE, this method does not require the use of heat, which avoids the thermal degradation and loss of bioactivity of the compounds (Ngamwonglumlert, Devahastin, and Chiewchan 2017; Saini and Keum 2018). Thus, this method is interesting in the obtainment of natural colorants, since it does not require high temperatures, thus the extraction of pigments using HPE from fruits has not yet been investigated. However, according to Table 6, HPE has been mainly applied for the recovery of pigments from tomato and grape residue. The low time observed for the recovery of anthocyanins (~ 3.5 min) and lycopene (1–10 min) from grape and tomato, respectively, may be a consequence of the effect of high pressure on the solvent permeability in the fruit cells (Putnik et al. 2018; Xi 2006). This method may also be of interest in the selectivity of the pigment. Corrales et al. (2009) reported that optimum anthocyanins' extraction conditions are dependent on the compounds structure, being the extraction of anthocyanin monoglucosides from grape skin more efficient applying lower pressures (200 MPa), whereas pressures of 600 MPa were optimal for the extraction of acylglucosides. Thus, the pressure variation may be important to obtain more stable molecules (acylglucoside).

### Extraction variables for natural colorants from fruits

The success of a natural colorant extraction depends on several factors, such as the solubility of the target compound in the solvent employed, the solid/solvent ratio, the temperature, and other factors specific to each extraction method (Ngamwonglumlert, Devahastin, and Chiewchan 2017; Saini and Keum 2018; Pinela et al. 2016).

The solubility of the compounds is an important factor in the choice of the extraction solvent. Anthocyanins and betalains are water-soluble compounds, therefore they have greater affinity with water; however, several studies present a better yield in the extraction of these compounds with the use of methanol (Dranca and Oroian 2016; Sanchez-Gonzalez et al. 2013) and ethanol solution (Celli, Ghanem, and Brooks 2015). Carotenoids and chlorophylls are oil-soluble, therefore they are soluble in organic solvents, such as acetone, chloroform, methanol and N,N-dimethylformamide (Saini and Keum 2018; Delgado-Pelayo, Gallardo-Guerrero, and Hornero-Méndez 2014). In fruits with a high water content, a step of dehydration is recommended prior to the extraction of carotenoids to increase the efficiency of the process (Saini and Keum 2018).

The solid/solvent ratio is another very important variable that may implicate the transfer of colorant compounds from the plant matrix to the solvent. For anthocyanins extraction from haskap (*Lonicera caerulea* L.) fruit the increase of the proportion of liquid positively improved the yield effect, indicating that in proportions lower than 25:1 (mL/g), solvent saturation may occur (Celli, Ghanem, and Brooks 2015). On the other hand, the same observation was not detected in the extraction of betalains from prickly pears (*Opuntia ficus-indica* Mill.) fruit, in which the authors concluded that the increase of the mass of the fruit caused greater extraction yield (Maran, Manikandan, and Mekala 2013).

The heat in the extraction process is a responsible variable that can increase the solubility of the organic compounds in the solvent. However, colorant compounds tend to be degraded at high temperature. In general, betalains are more sensitive to heat, followed by carotenoids and chlorophylls, thus anthocyanins exhibit higher resistance to temperature (Ngamwonglumlert, Devahastin, and Chiewchan 2017). Sanchez-Gonzalez et al. (2013) determined that increasing the temperature from 5 to 30°C reduced the extraction yield of betalains from Xoconostle (*Opuntia jocosostle* cv.) in about 10%. On the other hand, Maran, Manikandan, and Mekala (2013) observed that increasing the temperature to 46°C provided greater solubility of the betalains present in prickly pear (*Opuntia ficus-indica* Mill) during the extraction process. Similar results were also found for carotenoids, namely crocin, from gardenia (*Gardenia jasminoides* Ellis) fruit, where the optimum extraction condition was reached at 70°C (Yang, Liu, and Gao 2009) and for anthocyanins from strawberry tree (*Arbutus unedo* L.) at 90°C (López et al. 2018). However, the binomial time and temperature are usually crucial for the success of an extraction, since the heat has effect on the solubility of the compounds, but can cause their degradation.

Some classes of colorants have sensitivity to changes in pH, which may be a negative factor for extraction. For example, chlorophylls may be easily degraded to pheophytin in an acidic medium, which causes a change of color to brown, therefore the addition of alkaline substances help kept the stability of these compounds. On the other hand, anthocyanins are more stable in acidic pH, and the increase of pH to alkaline (pH 8–9) results in the degradation of these compounds. The adjustment of pH with acid, such as citric, hydrochloric and trifluoroacetic acids, is a frequent practice for the preservation of these compounds during the extraction process. Nevertheless, betalains and carotenoids are more stable to pH changes (Khan 2016; Ngamwonglumlert, Devahastin, and Chiewchan 2017). Moreover, for the extraction of betalains (namely betanin) and anthocyanins, it is commonly used an acidic solution with citric, ascorbic or hydrochloric acids to prevent the degradation of the compounds (Ngamwonglumlert, Devahastin, and Chiewchan 2017).

### Problems posed to natural colorants from fruit origin

For the production and use of natural colorants from fruits at industrial scale there are several challenges to be overcome, ranging from growing the fruit to the approval by

food legislation and by consumers. Some of the major problems encountered in obtaining and applying natural colorants from fruits are described below.

**Fruit sources:** Natural colorants from fruits can be found in species that do not have a cultivation system, which can hinder access and eliminate the availability of the material. This point is crucial and needs to be considered for these matrices to be a possible source of colorant compounds for the industrial extraction (Bernal-Mercado et al. 2018). Alternative sources of natural colorants may be a good option to increase the demand for natural compounds as food additives; however, the new source of pigments would require a safety assessment by regulatory agencies, which are expensive and require a long time (Martins et al. 2016). Another problem accounted to the use of these matrices as natural colorants is the differences found in the composition between cultivars and the maturation periods, which can also influence the direct extraction yield and the stability of the compounds (Rodriguez-Amaya 2016).

**Stability of natural colorants:** In general, natural colorants are less stable than artificial. Several factors, as mentioned above, such as heat, pH changes, presence of light, oxygen, and metal ions may cause the instability during the extraction, food processing and shelf-life of the product (Rodriguez-Amaya 2016; Ngamwonglumlert, Devahastin, and Chiewchan 2017; Martins et al. 2016). The use of more appropriate extraction technologies may require a higher investment cost and the extraction process requires higher control of the conditions at an industrial scale. Normally the use of natural colorants is more difficult than the use of the artificial counterparts. However, natural compounds may have a positive influence on the conservation of food product. For example, the addition of anthocyanins' rich extract from Karanda fruit in fermented pork sausage, provided an increase of 100% in its shelf-life, from 10 to 20 days, in relation to the control product without preservative additive, which may be beneficial to the production chain and for alternative substitution of artificial preservatives (Sueprasarn, Reabroy, and Pirak 2017).

The stability conditions of natural pigments limit their application in food matrices, such as anthocyanins, that are more stable in foodstuff with low pH. Montibeller et al. (2018) applied anthocyanins from by-products of grape into kefir (pH 4.5) and carbonated water; as result, the authors concluded that better color stability was achieved for the kefir product, since half-live time of the total anthocyanins was of 27 days and for the carbonated water stored in the dark, half-life time was shorter (only 6 days), which could be influenced due to the type of food matrix over the anthocyanins' stability. Betalains are more sensible to the increase of temperature, therefore their application is limited to foods produced with a minimum heat treatment, such as cooled and frozen food products (Martins et al. 2017). Carotenoids are lipid soluble pigments, except crocin, consequently they are selected for the inclusion in foodstuffs with higher fat content (Rodriguez-Amaya 2001; Martins et al. 2016). In addition to their low stability, natural colorants from fruits have a more limited range of colors than artificial colorants, and are more expensive (Martins et al. 2016).

However, some of the mentioned problems with instability and solubility can be overcome by employing different stabilization techniques, such as thus shown in Table 7. Stabilization methods have effect mainly on the thermal degradation by heat-process and storage. Betalains, which are the most sensitive pigments to heat, have shown higher stability during storage at 60°C when encapsulated with a protein and maltodextrin/insulin (Robert et al. 2015). In addition, according to data in Table 7, stabilization methods can have effect on the solubility of the compound; for example, the stabilization by molecular inclusion with  $\beta$ -cyclodextrin or nanoencapsulation with gelatin, provided solubility of up to 98–100% of carotenoid compounds in aqueous solution (Horuz and Belibağlı 2018; Lobo et al. 2018).

**Consumer acceptance:** The addition of natural colorants from fruits to enhance the color attribute may reduce the consumer's sensory acceptance, for example, the addition of anthocyanins from jabuticaba (*Myrciaria cauliflora* Berg.) peel into fresh sausage, decreased the liking score to the color attribute in relation to the sausage without colorant or with carmine colorant (E120) (Baldin et al. 2016). Effects over other attributes, such as flavor and odor, may also be observed with the addition of natural compounds. Kaimainen et al. (2015) evaluated the acceptability of natural colorants based on betalains from beetroot and anthocyanins from grapes in different concentrations in model juice; as a result, it was observed that the increase in the concentration of beetroot power as a colorant reduced significantly the acceptability of the attributed flavor, being the product characterized as unpleasant and strange, which did not occur with the addition of anthocyanins. However, the same effect was observed with the incorporation of grape marc power into fettuccini pasta (Sant'Anna et al. 2014). On the other hand, the addition of the natural colorant from beetroot in flavored milk, increased the sensory scores of color, flavor and acceptability in relation to product control without colorant (Kavitkar et al. 2017). The same was observed in the incorporation of 2 to 3% of lycopene from tomato peels in ice cream, which increased acceptability, also presenting a higher texture score (Rizk, El-Kady, and El-Bialy 2014). Other studies have shown that the use of natural pigments in foodstuff have been very well acceptable, such as banana fruit spread with the addition of betalains from pigeon berry (*Rivina humilis* L.) (Khan et al. 2015).

Regarding these obstacles, there has been massive research in the development of natural colorants from wastes and by-products obtained from the fruit processing system, with more effective and sustainable extraction technologies, which seek to understand and increase the stability of these compounds for food processing and for substitution of artificial colorants in food without affecting their attractiveness for the consumers.

### Using fruits as colorant sources in a circular economy perspective

The use of natural additives by the food industry has been increasing in the last decades, due to their beneficial effects

**Table 7.** Stabilization methods for natural colorants from fruits.

Natural colorant	Specific compound(s)	Source	Method	Result	References
<i>ANTHOCYANINS</i>					
	Not specified	Blueberry and elderberry	Copigmentation with catechin and encapsulation with polyelectrolyte complexes composed of chondroitin sulfate and chitosan.	Intensified red color, higher stability in the presence of ascorbic acid, reduction of about 40% in oxidation during storage for one month, and greater resistance to heat treatment (80 °C/5h).	(Tan et al. 2018)
	Cyanidin 3-O-glucoside	Blackberry	Molecular inclusion complexes of $\beta$ -cyclodextrin.	Increase in the half-life of anthocyanin's from 14 to 41 hours at 60 °C and from 3.6 to 4.4 h at 90 °C in aqueous solution.	(Fernandes et al. 2018)
	Not specified	Blackberry juice	Addition of sugar and/or chlorogenic acid.	Lower degradation after 90 days of storage at 4 °C when compared to the control product.	(Kopjar, Jakšić, and Pilizota 2012)
	Not specified	Blackberry residue	Copigmentation with phenolic acids.	Increase in half-life in thermal treatment at 50 and 70 °C and greater stability in the presence of light.	(Fan et al. 2019)
	Not specified	Blackberry residue	Copigmentation with flavonoids.	Increase in half-life in thermal treatment at 90 °C.	(Fan et al. 2019)
	Cyanidin 3-O-glucoside and delphinidin 3-O-glucoside	Black currant	Complexation with pectin.	Increase in the half-life of anthocyanins from 53 to 144 days at room temperature and aqueous solution.	(Buchweitz et al. 2013)
	Not specified	Jaboticaba skin	Encapsulation with calcium-alginate.	Increased stability to light and temperature. Preservation of the compound for at least 14 days at 4 °C.	(Santos et al. 2013)
	Not specified	Jaboticaba skin	Encapsulation in polyethyleneglycol using supercritical CO <sub>2</sub> .	Increased stability to light and temperature.	(Santos et al. 2013)
	Cyanidin 3-O-glucoside	Jaboticaba pomace	Encapsulation with maltodextrin, pectin and soy protein isolate by freezing-dried.	Preservation of ~94% of the anthocyanin's and of ~99% of antioxidant activity after 90 days of storage exposed to ultraviolet light. Increase in the half-life of color.	(Souza, Gurak, and Marczak 2017)
	Not specified	Bilberry	Microencapsulation with whey protein gel.	Preservation of 92% of the anthocyanins in solution with pH 1.5 stored at 4 °C e preservation of 80% of the anthocyanins in solution with pH 3 stored at 20 °C for 28 days.	(Betz and Kulozik 2011)

(continued)

Table 7. Continued.

Natural colorant	Specific compound(s)	Source	Method	Result	References
	Delphinidin 3-O-sambubioside-5-O-glucoside, delphinidin 3-O-glucoside and cyanidin 3-O-glucoside	Maqui	Microencapsulation with inulin by spray-drying.	Increased half-life values of del-3-sa, del-3-glu, and cy-3-glu to $98 \pm 10$ , $173 \pm 8$ , and $150 \pm 9$ days, respectively.	(Fredes et al. 2018)
	Not specified	Tamarillo	Microencapsulation with n-octenyl succinic anhydride starch.	Degradation of only 0.59% of the anthocyanin's after storage at 4 °C for 84 weeks and better light protection.	(Ramakrishnan et al. 2018)
	Not specified	Elderberry	Encapsulation with water-in-oil-in-water emulsion by glass microfluidic.	Retention of 100% of the color after 30 days of storage at 4 °C and pH 3. Increase of the stability to high pH values.	(Comunian et al. 2018)
	Not specified	Raspberry	Encapsulation with gelatin and gum Arabic.	Increased stability up to 23.6% for 2 months of storage at 37 °C.	(Shaddel et al. 2018)
BETALAINS	Betacyanins and Betaxanthins	Jiotilla and Pitaya	Microencapsulation with cactus ( <i>Opuntia. ficus-indica</i> Mill) mucilage by spray-drying.	Retention of more than 90% of betalains after 3 months of storage.	(Delia et al. 2019)
	Betacyanins and Betaxanthins	Pitaya	Microencapsulation with potato native starch by spray-drying.	Stability during storage of 32 days at 4 °C in yogurt with pH 4.6.	(Vargas-Campos et al. 2018)
	Betanain, isobetanain and indicaxanthin	Prickly pear	Encapsulated by ionic gelation with calcium alginate.	Greater retention of the pigments after 25 days of storage in different conditions (Humidity 34.6%, 57.6%, 74.8%, and 84.3% and temperature 25 and 50 °C) .	(Otálora et al. 2016)
	Betanain, isobetanain and indicaxanthin	Prickly pear	Microencapsulation by spray-drying using cactus ( <i>Opuntia. ficus-indica</i> Mill) cladode mucilage and maltodextrin.	Increase in half-life for up to 103.4 and 117.4 days in storage between 75 and 57% relative humidity, respectively.	(Otálora et al. 2015)
	Indicaxanthin	Prickly pear	Encapsulation by spray-drying with maltodextrin.	Preservation of the compound and coloration after 6 months of storage until 20 °C in the absence of light.	(Gandía-Herrero et al. 2010)
	Betacyanins and betaxanthins	Prickly pear	Encapsulation with soybean protein isolate and maltodextrin or insulin by spray-drying.	Higher stability in 35 days of storage at 60 °C when compared to encapsulation with only protein isolate.	(Robert et al. 2015)

(continued)



Table 7. Continued.

Natural colorant	Specific compound(s)	Source	Method	Result	References
<i>CAROTENOIDS</i>					
	Not specified	Tamarillo	Microencapsulation with n-octenyl succinic anhydride starch.	Degradation of only 1.12% of the carotenoids after storage at 4 °C for 84 weeks and better light protection.	(Ramakrishnan et al. 2018)
	Norbixin	Annatto seeds	Encapsulation with water-in-oil-in-water emulsion by glass microfluidic.	Retention of 82% of the color after 30 days of storage at 4 °C and pH 3.	(Comunian et al. 2018)
	Bixin	Annatto seeds	Nanoencapsulation with lipid-core.	Increased light and heating stability.	(Lobato et al. 2015)
	Bixin	Annatto seeds	Encapsulation with sodium caseinate by spray-drying.	Reduced the thermal decomposition of bixin in the powder and light degradation in aqueous dispersions.	(Zhang and Zhong 2013)
	Bixin	Annatto seeds	Encapsulations with gum arabic or maltodextrin by spray-drying.	Greater stability in aqueous solution than free bixin. The encapsulated with gum Arabic was 3 to 4 times more stable than that encapsulated with maltodextrin.	(Barbosa, Borsarelli, and Mercadante 2005)
	$\beta$ -carotene	Mango	Complexion with sunflower oil.	Stabilization and protection against degradation during heating at cooking temperatures for at least 30 min (100 °C).	(Guzman et al. 2015)
	Lycopene	Tomato peels	Nanoencapsulation into zein fibers by electrospinning.	Increased stability at different temperatures (−20–25 °C). Total preservation of the lycopene content in storage at −20 °C in the dark for 14 days.	(Horuz and Belibağlı 2018)
	Lycopene	Tomato peels	Nanoencapsulation by electrospinning with gelatin.	Greater stability to temperature than free lycopene. Total preservation of the lycopene content in storage at −20 °C in the dark for 14 days. 98% improvement of the water solubility.	(Horuz and Belibağlı 2018)
	Lutein, zeaxanthin, $\beta$ -cryptoxanthin, $\beta$ -carotene and $\alpha$ -carotene	Pepper	Inclusion complex with $\beta$ -cyclodextrin.	Provided greater color stability for up to 21 days when added in isotonic drink. Resulted in complete solubilization in the beverage.	(Lobo et al. 2018)
	Lycopene	Tomato	Microencapsulation by spray drying with maltodextrin and Capsul®.	Retention of up to 33% lycopene in the particles after 30 days storage at 25 °C.	(Souza et al. 2018)
	Lycopene	Pink grapefruit	Encapsulation with alginate and $\beta$ -cyclodextrin or arabic gum.	Higher stability to freezing thermal treatments and drying.	(Calvo and Santagapita 2018)

(continued)

**Table 7.** Continued.

Natural colorant	Specific compound(s)	Source	Method	Result	References
CHLOROPHYLL	Chlorophyll	Commercial	Microencapsulation in maltodextrin.	Microencapsulation enhanced thermal and storage stability. Retention of more than 95% of chlorophylls after 10 days of storage at 20 °C.	(Kang et al. 2019)

on food preservation (antioxidant/antimicrobial properties), as also due to the consumers' demand, seeking for the application of these natural molecules (Leong et al. 2018). Consumers have become increasingly demanding not only in the sensory characteristics of food, but also regarding the effects of consumption associated to health, promoting the need to adapt the industry to these requirements (Martins et al. 2016).

Recently, there is a new direction in the use of wastes and by-products, rich in bioactive and pigment compounds from the food industry, to produce natural additives, including coloring agents (Martins and Ferreira 2017). Food wastes can be defined as "any food or inedible part of food removed from the food supply chain to be recovered or dispose" and by-product as "a substance or object, resulting from a production process, the primary aim of which is not the production of that item" (Stenmarck et al. 2016). In Europe, food waste production is estimated to reach 88 million tonnes per year, which costs around 148 billion euros per year, the primary production and food processing corresponding about 9 and 17 million tonnes, respectively (Stenmarck et al. 2016). By-products and wastes of the food processing based on fruit may account for a large percentage of the fruit weight; these estimations included edible and inedible parts of food. It has been estimated that around 45% of the fruit is converted into waste and losses during the production chain, for example the by-product and waste of mango, namely pomace, seeds and peels, may correspond to 10-60% of the fruit weight, the by-product from apple juice processed represents about 25% of the processed fruit, and in the winery it is generated a waste corresponding to 30% of the production (Bernal-Mercado et al. 2018).

Part B of Tables 1–5, show the amount of pigment recovery from fruit residues, where it can be noticed that fruit peels, which are generally rejected from processing and/or consumption, are promising sources of natural colorants. This is the case of litchi (*Litchi chinensis* Sonn.) (772.16 mg of anthocyanins/100 g dry peel) (Liu et al. 2013); avocado (*Persea americana* Mill.) (57.3 mg of anthocyanins/100 g of fresh peel, 238.6 mg of quercetin/100 g of extract peel, and 59 mg of chlorophylls/100 g of fresh peel) (Cox et al. 2004; Melgar et al. 2018); jabuticaba (*Myrciaria cauliflora* Berg.) (490 mg of anthocyanins/100 g of dry peel) (Rodrigues et al. 2015); and melon (*Cucumis melo* L.) (78 mg of chlorophylls/100 g of fresh peel) (Shao, Tan, and Li 2016). Moreover, fruit residues from the food industry are already used as natural colorants, such as the case of grape and blueberry

pomace from winery (~370 and 415 mg of anthocyanins/100 g of dry weight, respectively) (Putnik et al. 2018; He et al. 2016); and tomato residues from production of sauce (30.9 mg of lycopene/100 g of dry weight) (Baysal, Ersus, and Starmans 2000).

The use of waste and by-products not only benefits the development of additives for the food industry, but may also be interesting for other industrial areas, such as the case of natural pigments for the textile industry obtained from by-products, namely peels, from eggplant (Mazeyar 2009). Therefore, there is an extensive market that can benefit from the recovery of natural pigments from fruit bio-residues, as well as the environmental.

In this context, there is a better use and valorization of natural sources, less economic losses, and decrease of the food industry impact in the environment, thus contributing to a circular economy (Bernal-Mercado et al. 2018; Martins and Ferreira 2017).

## Concluding remarks and future perspectives

In recent years, a new perspective on the use of food additives has gained strength due to several studies pointing some problems related to the use of artificial additives, including colorants. Otherwise, consumer habits have tended to search for functional foods, which promote healthier benefits. In parallel with the worldwide problems of food security, which are associated with political, social, and economic factors, it is required a greater attention to minimize losses and to better use the products of great biological value. Fruits and their residues are a great source of different pigments that provide an attractive range of colorations for application in food products, in addition to their richness in bioactive compounds, being alternatives to artificial colorants. However, studies are still needed to better understand the behavior of these natural compounds during extraction and stabilization processes for their incorporation into food matrices, as well as further research for the purposes of regulation approvals.

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