Chapter

Natural vs Synthetic Colors

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Abstract

Anthocyanins are the most important group of water-soluble compounds responsible for the red, purple, and blue colors seen in flowers, fruits, and other parts of the plant. For centuries, these compounds have been consumed by man without obvious detrimental effects due to their bright colors and anti-inflammatory and antioxidant properties. Anthocyanins are an important alternative for synthetic food colorings that have been banned in foods, because they have been associated with certain diseases. Anthocyanins can be extracted from different plant tissues; the usual method of obtaining is solid-liquid extraction. However, it is worth mentioning the existence of other methods. Thus, Japanese scientists developed an alternative methodology that consists of extracting anthocyanins by fermenting the matrices that contain them. The stability of anthocyanins in processed products has been studied, and it has been shown that certain acid anthocyanins are stable after extraction. Anthocyanins are antioxidants that play an important role in reducing the risks of several human degenerative diseases.

Keywords: anthocyanins, antioxidants, natural colors

1. Natural versus synthetic colors

Anthocyanins are the most important group of water-soluble compounds responsible for the colors red, purple, and blue that appear in flowers, fruits, and other plant tissues. For centuries, these compounds have been ingested by humans due to their bright colors, anti-inflammatory, and antioxidant properties, without any evident harmful effects. Anthocyanins are a good potential replacement for synthetic food colorings, particularly for those that have been banned because of their association with disease.

Anthocyanins are extracted from vegetable tissues, and the most common method is liquid-liquid extraction. However, other methods are also available, such as an alternative methodology developed by Japanese scientists who extracted these compounds by fermenting vegetable matrices.

The stability of anthocyanins in processed products has also been a topic of research, and it has been shown that certain acid anthocyanins are highly stable after the extraction. Since these compounds are antioxidants, they play an important role in reducing the risk of several human degenerative diseases.

2. What are anthocyanins?

Anthocyanins belong to a class of substances known as flavonoids, one of the largest categories of phenolic compounds. The basic structure of anthocyanins is made up of a flavylium cation (C6-C3-C6), which may be attached to different sugars, as well as to hydroxyl and methoxy groups, resulting in over 635 different anthocyanins identified to date. The most common sugar associated to anthocyanins is glucose, although rhamnose, xylose, galactose, arabinose, and rutinose have also been found as part of these molecules [1]. Anthocyanins may be mono-, di-, or tri-glycosides, depending on the number of sugar molecules they contain. Durst and Wrolstad [2] reported that anthocyanins are glycoside groups that belong to the family of flavonoids; their structure contains two aromatic rings A and B, joined by a three-carbon link (**Figure 1**). The structural variations that occur in ring B result in six different anthocyanins as shown in **Table 1**.

Anthocyanins are the most important natural pigments, soluble in water, that give colors red, purple, and blue to flowers, fruits, and other parts of the plant. Besides coloring, these pigments play other roles in plants, such as attracting pollinizers in order to disperse pollen and seeds, as well as protecting the tissue against UV radiation and harmful virus and bacteria. Given the above, the scientific interest on anthocyanin pigments has increased in the past few years, particularly on their role in the reduction of heart disease, cancer, diabetes, anti-inflammatory effects, and improvement of visual acuity [3]. For centuries, these compounds have been a part of the human diet due to their attractive bright colors, anti-inflammatory, and antioxidant properties, without any evident harmful side effects. Anthocyanins are regarded as a potential alternative in the replacement of artificial food colorings, some of which have been associated to certain diseases. Several sources of anthocyanins have been studied in order to find acidified anthocyanins with greater stability at different pH conditions, at an affordable cost. Stability is a relevant factor since the color of these compounds is easily affected by several conditions, mainly pH [4]. Predominant structures of anthocyanins at different pH values are shown in **Figure 2**.

The color of anthocyanins depends on the number and orientation of hydroxyl and methoxy groups. Increases in hydroxylation produce color changes toward the blue side of the color spectrum, while increases in methoxylation produce red colorations [3].

The color changes in anthocyanins given by variations of pH are due to the glycoside substitutions (mono-, di-, or tri-saccharides) in positions 3 and/or 5 of the B ring (**Figure 1**), and this also helps to increase solubility. Some examples of glycosylated saccharides are glucose, galactose, xylose, arabinose, rutinose, sambubiose, and

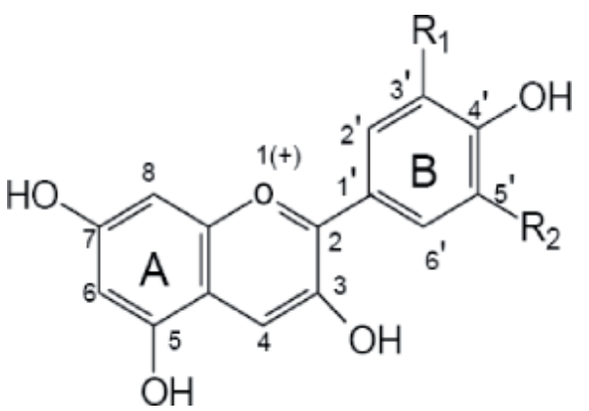

Figure 1.
Structure of anthocyanins [2].

Aglycone	Substitution R1	Substitution R2	Absorbance (nm) visible spectrum
Pelargonidin	H	H	494 (orange)
Cyanidin	OH	H	506 (orange-red)
Delphinidin	OH	OH	508 (blue-red)
Peonidin	OCH_3	H	506 (orange-red)
Petunidin	OCH_3	OH	508 (blue-red)
Malvidin	OCH_3	OCH_3	510 (blue-red)

Table 1.
Substituents of the six types of anthocyanins.

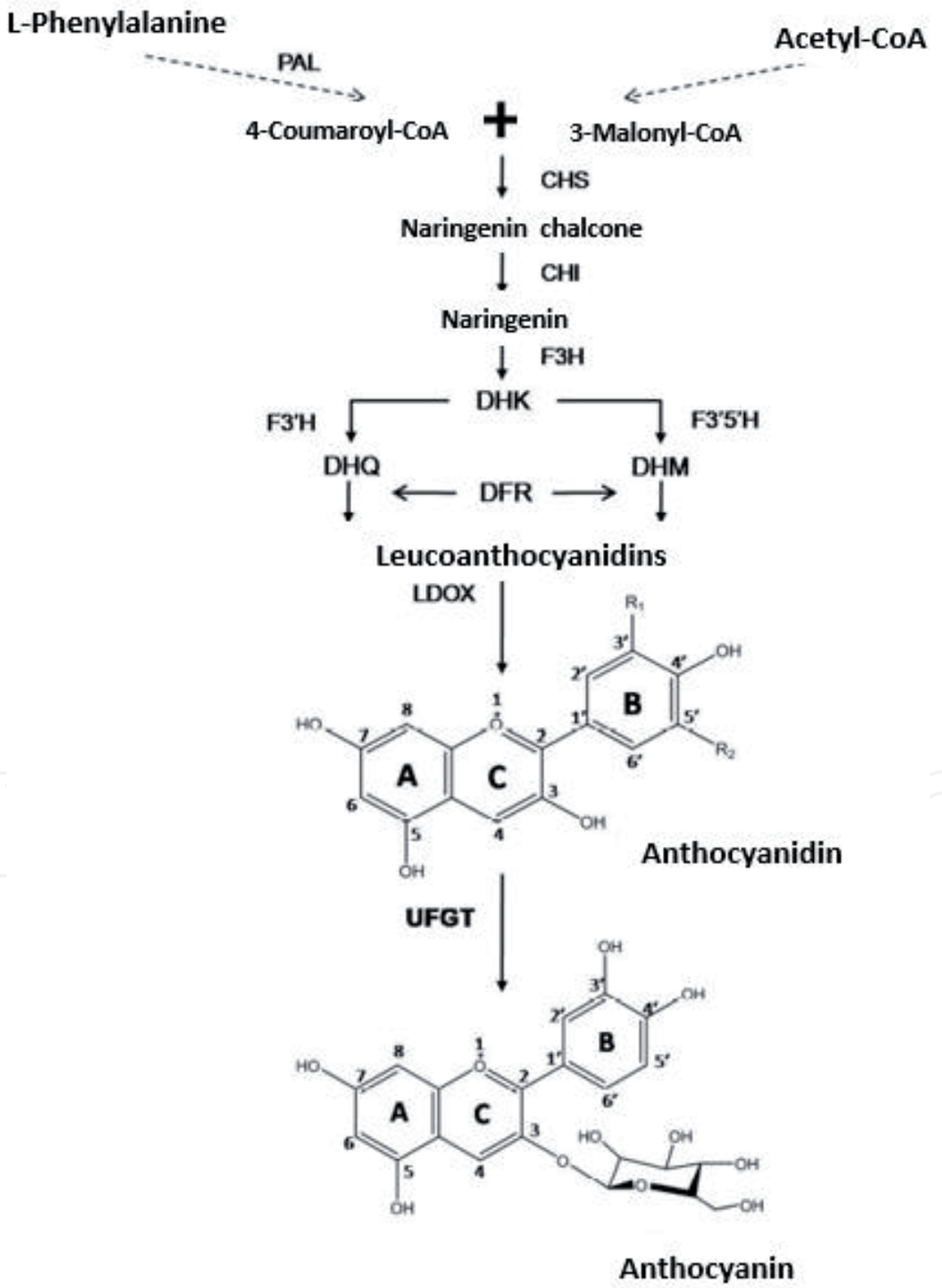

Figure 2.
Anthocyanins biosynthesis pathway [5].

gentiobiose. Another cause of the color displacement toward purple in the molecule is the aromatic acylations in the position 5 of carbon B in the structure [6].

Figure 2 shows the biosynthesis of anthocyanins as established experimentally, where ring A is synthesized via the malonic acid pathway, by condensing three molecules of the malonyl-CoA. On the other hand, ring B is synthesized via the shikimic acid pathway. The enzyme phenylalanine ammonia lyase (PAL) reacts with phenylalanine, which converts into *p*-coumaric acid by the loss of NH_3. Afterward, a condensation reaction of three molecules of malonyl-CoA results in an intermediate 15-carbon compound, which is transformed into a flavanone. Then, the flavanone is converted into an anthocyanin by the hydroxylation of carbon 3 and the subsequent dehydration. Finally, the molecule is stabilized by glycosylations of the heterocycle, and the reaction is catalyzed by the glycosyl transferase enzyme and then by methylation reactions followed by acylations [7].

The use of natural anthocyanin pigments as food colorings in processed products is getting increasing attention, since they are very attractive to consumers, while having beneficial health effects. Anthocyanin pigments are permitted as natural food colors in the United States under the fruit (21 CFR 73.250) and vegetable categories (21 CFR 73.260) [8].

3. Anthocyanin sources

Hibiscus flower (*Hibiscus sabdariffa* L.) is a source of vitamins C and E, polyphenolic acids, anthocyanins, and flavonoids, all of which are known to have antioxidant activity, as they are capable of reducing free radicals [9].

Different varieties of *Hibiscus sabdariffa* L. are available in Mexico, and each one is characterized by its anatomy, color, and physicochemical properties. The content of active compounds varies according to the chalice, as well as to the extraction method. These pigments may be an alternative to the industry of colorings, cosmetology, and processed food, while providing extra health benefits [10].

Nowadays, the food and cosmetic industries demand an ample variety of additives and colorings, in order to improve the appearance of a product and make it attractive to consumers. Industrial food colorings are found in many products that we use or buy on a daily basis, such as juice, jellies, pastries, soft drinks, paints, cosmetics, and more. Most of these additives are synthetically produced and may cause adverse health consequences: allergic reactions, digestive problems, cancer, and asthma, among others [11].

Natural food colors are found in fruits such as acai, cherries, cranberries, elderberries, raspberries, blueberries, black and blue grapes, plums, strawberries, figs, pomegranate, and red apple [12]. Other important sources are found in vegetables: beets, purple lettuce, green onion, radish, purple cabbage, red bell peppers, eggplant, as well as cereals such as blue corn (*Zea mays* L.) [13], blue wheat [14], and rice [15]. Recently, other plants and flowers have been studied as potential sources of antioxidants [16, 17], such as elder (*Sambucus nigra*) [18], perilla fruit from Japan (*Perilla frutescens*) [19], and flower petals: iris (*Iris dichotoma*, *Iris domestica*) from China [20], Damascus rose (*Rosa damascena*) [21], cyani flower (*Centaurea cyanus*) [22], dahlia (*Dahlia mignon*) [23], and viola (*Viola tricolor*) [16].

4. Anthocyanins as natural colorings

The growing concern about the use of synthetic colorings in processed foods, cosmetics, and pharmaceuticals is caused by their potential harmful effects. Countries like Australia, Japan, Norway, and Switzerland have banned the use of

some synthetic colorings, such as Red No. 20 and 40, since they have been related to hyperactivity in children of school age. This effect may be considered as an acute neuronal illness; however, these food additives are still being used in the United States [24].

Regulatory policies dealing with the use of colorings derived from anthocyanins vary from country to country. The United States is the most restrictive country on the use of anthocyanins as natural colorings, where four out of the 26 colorings that are approved for their use in foods are derived from grape peel, vegetable, and fruit juice [25]. In Mexico, there is no regulator policy for natural colorings at this point.

In the European Union, Chile, Colombia, Iran, Israel, South Korea, Malta, Peru, Saudi Arabia, and the Emirates, all colorings derived from anthocyanins are regarded as natural [26].

5. Functional properties of anthocyanins

The stability of anthocyanins in processed products has been studied, demonstrating that some acid anthocyanins are stable after extraction. These compounds have antioxidant properties and play an important role in reducing the risk of developing several human degenerative diseases [27].

The interest in anthocyanin pigments is not only due to a potential replacement of artificial food colorings, but is also due to their pharmacological and therapeutic properties. **Table 2** shows different investigations on the biological properties of anthocyanins from several substrates.

Biological property	Studies	Authors
Therapeutic	Reduction of coronary heart disease, anticarcinogenic effects, antitumor, anti-inflammatory, and antidiabetic	Miyazawa et al. [28]
Antioxidant activity	Stabilization of oxygen reactive species, inhibition of lipoprotein oxidation, and platelet aggregation (wine anthocyanins)	Ghiselli et al. [29]
Antioxidant activity	Anthocyanin-rich foods show a high antioxidant activity against hydrogen peroxide (H_2O_2), peroxide radicals (ROO), superoxide (O_2), hydroxyl (OH), and singlet oxygen (O_2)	Wang and Jiao [30]
Antitumor and anticarcinogenic activities	Sweet purple potatoes and blue cabbage were fed to lab rats causing tumor suppression	Hagiwara et al. [31]
Antitumor effects	Soy red bean extract, containing cyanidin conjugated with glucose and rhamnose, was fed to rats	Koide et al. [32]
Anticarcinogenic activity	Fractions of red wine anthocyanins suppressed cancer HCT-15 cells from human colon and carcinogenic gastric AGS cells.	Kamei et al. [33]
Anticarcinogenic activity	Essays demonstrate that cranberries inhibit the initiation, promotion, and progression stages of carcinogenesis	Chang et al. [34]
Anti-inflammatory activity	Concentrated anthocyanin extracts showed inhibitory effect in the production of nitrous oxide in activated macrophages	Wang and Mazza [35]
Anti-inflammatory activity	Anthocyanin extracts from raspberry inhibited EG2 prostaglandin, a synonym of anti-inflammatory activity	Vuorela et al. [36]

Table 2.
Functional properties attributed to anthocyanins.

6. Anthocyanin extraction methods

Anthocyanins may be extracted from different vegetable tissues, the most common method being the solid-liquid extraction. However, novel methods have been developed, such as the methodology developed by Japanese researchers where anthocyanins are extracted after fermenting the vegetable matrices that contain them [37].

Nowadays, anthocyanin extracts are usually applied without separating the individual components, since all the compounds have shown antioxidant activity, not only those with color [38].

On the other hand, the polar nature of the anthocyanin molecule permits solubility in a variety of solvents, such as alcohols, acetone, and water. However, their stability is easily affected by structural modifications by hydroxyl and methoxy groups, glycosides, and particularly acyl groups, as well as by environmental factors such as temperature and light [25].

Amongst those technologies, those currently available for anthocyanin extraction are the use of polar organic solvents, such as ethanol and methanol, and sometimes acidified media. In many cases, the solvents or chemical synthesis involved in the extraction are derived from petroleum, which leaves a strong carbon print on the environment [39].

Extraction of natural colorings by organic solvents has been the method of choice for decades. The toxicity of these solvents complicates the marketing of the final product due to their toxicity and environmental concerns. Many technologies for exploitation of agro-industrial residues have been developed out of the need of solving the problem of accumulation of solid organic residues. Green and clean technologies focus on lessening the environmental impact, while helping the processing and marketing of the final products [40].

Novel methods for the extraction of anthocyanins are ultrasound-assisted extraction [41] and extraction using supercritical fluid CO_2 [42].

A viable method for anthocyanin extraction is the use of hydrolytic enzymes, which accelerate the reaction at which a substance is broken down into simpler components when reacting with water. This is the case of cellulase and pectinase that hydrolyze cellulose and pectin, respectively; both are found in the cell wall of fruits and vegetables [43].

A response surface methodology based on the Box-Behnken design may also be used to optimize an extraction method [44]. Identification and quantification of anthocyanins are based on the use of chromatographic methods, mainly the HPLC and UHPLC liquid analyses; mass spectrophotometry is also very helpful for the identification of individual compounds [44].

7. Antioxidant capacity highlights

Antioxidants are molecules that inhibit or delay the oxidation in two ways: by trapping free radicals, in which case they are known as primary antioxidants (phenolic compounds) and are destroyed in the induction process, or by mechanisms such as chelation with heavy metals, capture of oxygen, conversion of hyperoxides into nonradical species, absorption of UV radiation, or inactivation of singlet oxygen; substances exhibiting these properties are known as secondary antioxidants [45].

Antioxidant activity is defined as the capacity of one or several compounds within a substance to inhibit oxidative degradation of another compound, acting mainly on free radicals [46].

8. Free radicals

Most of the chemical compounds of biological relevance are made by atoms joined together by covalent bonds, where two different atoms share a pair of electrons in the same orbital, and each electron rotates in the opposite direction to its pair. In the cells, chemical reactions that break these bonds heterolytically take place continuously, making one of the parts take two electros and generating unstable nucleophilic or electrophilic compounds, known as anions and cations. However, some chemical reactions, electromagnetic radiation, and other factors may break bonds homolytically, resulting in two parts that have one electron each; these are known as free radicals [47].

Generally speaking, a free radical is an atom or molecule that has one or more unpaired electrons in the external orbitals and is capable of existing independently. It is very reactive and tends to reduce in order to stabilize, which means that subtracts an electron from stable atoms or molecules that are in turn oxidized. Once the free radical has obtained its missing electron, the stable molecule is oxidized and is left with an unpaired electron, which makes it a new free radical that initiates a chain reaction [6].

9. Mechanisms of antioxidant defense

Figure 3 is a summary of the oxidation mechanisms of a cell, as well as the action that the antioxidant exerts to prevent oxidation. Cell respiration is shown, where molecular oxygen is converted into a superoxide anion, followed by hydrogen peroxide, then a hydroxyl radical and finally water, while the central illustration explains how cell metabolism may form free radicals (superoxide anion and hydroxyl) [48].

According to Londoño [46], when the superoxide anion suffers a mutation catalyzed by the superoxide dismutase enzyme, it becomes less reactive but is still

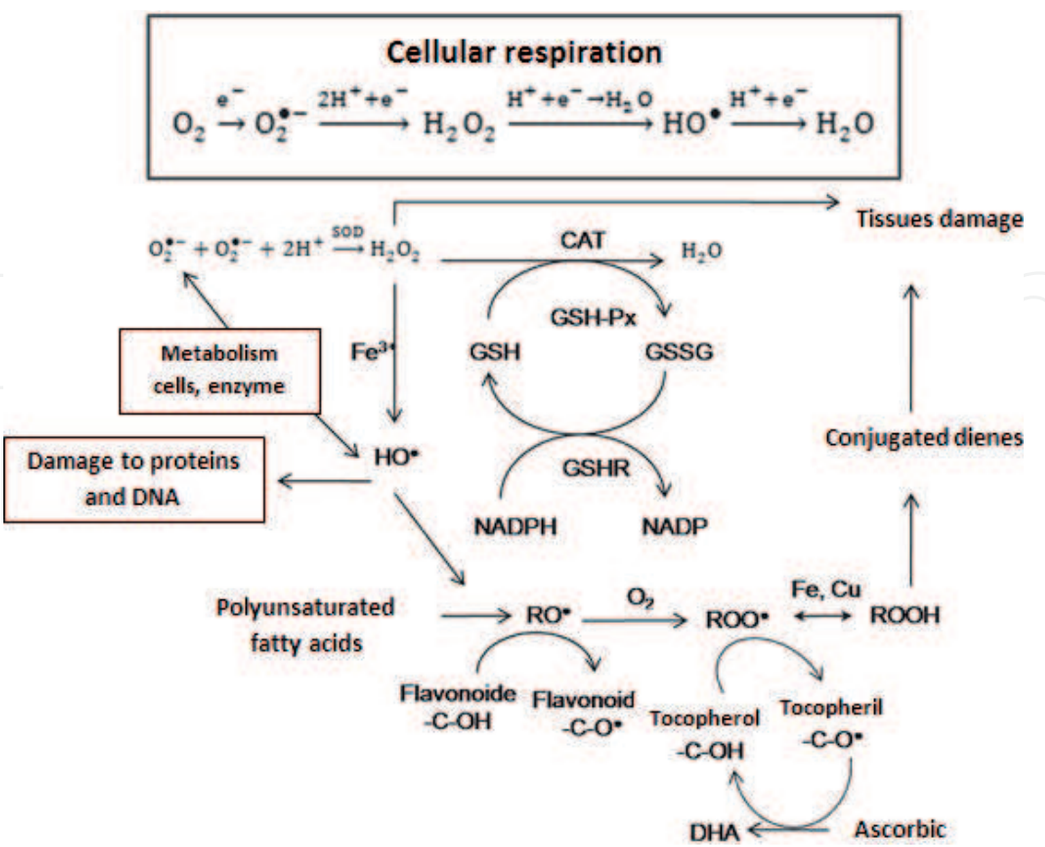

Figure 3.
Pathways of free radical production and action of antioxidants [46].

toxic for tissues; therefore, it is converted into water by the action of the catalase enzymes, which reduce hydrogen peroxide by oxidizing glutathione; this in turn is generated by the action of glutathione reductase, which uses NADPH as a cofactor. Hydrogen peroxide may also be converted into a hydroxyl radical via a Fenton-type reaction, which is catalyzed by iron. Once produced, the hydroxyl radical attacks proteins, nucleic acids, and mainly polyunsaturated fatty acids, thus generating lipid radicals that quickly react with oxygen to produce peroxyl radicals.

Stabilization of free radicals derived from lipids may be accomplished by phenolic antioxidants such as flavonoids and tocopherols, which stabilize the free radicals (phenoxyl and tocopheryl, respectively). Stabilization may happen inside the molecule by displacement or by reaction with ascorbic acid to generate a reduced compound [49].

10. Safety and toxicology of anthocyanins

Consumption of anthocyanins is generally recognized as safe for humans, since they have been a part of our diets for generations, and so far, no harmful effects have been reported. This may be associated with their low absorption and bioavailability. Nevertheless, the use of nutritional supplements based on anthocyanins is a growing trend among consumers, and this has raised some concern because the doses recommended by manufacturers are generally much higher than that given by natural foods. Furthermore, no regulation is available for such dietetic supplements in the United States, among other countries, which may result in fraudulent/adulterated products. It is also likely that people looking to benefit from anthocyanins are also using other supplements or pharmaceuticals. Anthocyanins are treated as xenobiotics [50] and, therefore, are able to modulate biochemical activities or compete for several enzymes that metabolize or transport medications [51]. This increases the risk for potential adverse effects and toxicity due to interactions with pharmaceuticals. However, so far, no reports have demonstrated adverse effects on anthocyanins in levels associated to a healthy diet.

11. Perspectives of inclusion of anthocyanins in processed foods

Several studies show the possibility of replacing artificial food colorings for anthocyanins, such as those derived from flowers. When mixed into dairy matrices such as yogurt, there are some improvements in the production and final product [17]. Furthermore, the addition of anthocyanins is not only recommended for their color and bioactivity, since recent studies propose their use during processing and/or storage of the final product by their inclusion into intelligent films based on biodegradable polymers, which work as biosensors due to their high sensitivity to pH changes. In this manner, freshness of meat and fish may be monitored [52, 53]. Both studies used *Hibiscus* flower extract due to its low cost. Other researchers have studied anthocyanins from purple cabbage (*Brassica oleracea*) as temperature indicator when incorporated into chitosan films; this study also suggests its application in the production of smart food packages [54].

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