

Brazilian Sources of Anthocyanins: Colored Pigments with Potential Health Benefits

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Abstract

The anthocyanins are a specific group of flavonoids responsible for the pink, red, blue and purple color of flowers and fruits, depending of the pH and the conjugation of these substances with other compounds. The anthocyanins - cyanidin, pelagomidine, delphinidin, peonidine, petunidine and malvidin - differs in the type and number of sugars bound to their structure. The food industry presents an interest in anthocyanins because it can be used as food natural coloring agent with a great potential for food application in order to obtain functional foods. The anthocyanins have shown some health benefits due to their biological activities, including antioxidant, anti-inflammatory, and antimicrobial potential. Studies describe a reduction in the oxidative stress, and cardiovascular, cancer and neurodegenerative diseases risks. Some fruits and vegetables are more know as anthocyanins sources, such grape, plum, blackberry, cherry, raspberry, eggplant, purple cabbage, beetroot, and others. However, the Brazil territory features a wide diversity of biomes, which present an interesting variety of plant species, highlighting peculiar native fruits. These review describes the characteristics of plant growth, major anthocyanin form and content, and the potential health benefits effects associated with 6 native Brazilian berries, açaí, araçá, camu-camu, guabijú, jabuticaba, and pitanga. The native species growth in different regions of Brazil and countries of South America, and the major anthocyanin form present in the berries evaluated is the cyanidin 3-glucoside, except for guabijú that malvidin 3-glucoside was present in higher concentrations than other anthocyanins. The whole fruits (peel and flesh) with grater content of cyanidin 3-glucoside is the açaí, purple pitanga, and jabuticaba. The jabuticaba peel present a large content of cyanidin 3-glucoside, justifying the studied conducted in animal with this portion of fruit. Inside the same species, fruits with purple color present higher content of anthocyanins than red colored and in the stage of total maturation of fruit the higher content of anthocyanins were detected. All fruits evaluated present biological activities, including antioxidant, anti-inflammatory, and antimicrobial potential, depending of the fruit. The Brazil is a large country with the potential production of differentiated berries with anthocyanins content that can to exercise biological activities.

Keywords: Açaí; Araçá; Cyanidin 3-Glucoside; Camu-Camu; Guabijú; Jabuticaba; Pitanga

Anthocyanins

The bioactives compounds have been highlighted due its potential health benefits. The group of flavonoids, represented by polyphenolic compounds, occurs in plants and are responsible for the pigmentation of flowers and fruits. Several studies have been described the flavonoids protective effect against UV rays, pathogens, oxidative stress and enzymes inhibition. These effects were associated with flavonoids chemical structure, characterized as a low molecular weight compounds with the presence of aromatic rings. The flavonoids classes - flavonas, flavonols, flavonones, iso-flavones, anthocyanins and catechins - differs from one another in one of the aromatic rings [1].

The anthocyanins are responsible for the pink, red, blue and purple color of flowers and fruits, depending of the pH and the conjugation of these substances with other compounds. The anthocyanin molecule is characterized by the presence of fifteen carbon atoms in the C6-C3-C6 form bound to glycosides; D-glucose, D-rhamnose and D-galactose being the most common sugars [2]. The main differences among anthocyanins - cyanidin, pelagomidine, delphinidin, peonidine, petunidine and malvidin - are the type and number of sugars bound to their structure, number of hydroxyl groups, aromatic groups attached to the sugar and the position of these bounds. Different from anthocyanidins, the presence of sug-

ars in the anthocyanins structure confers greater solubility, mainly in water, and stability of the pigment [3].

The food industry present an interest in anthocyanins because it can be used as food natural coloring agent. Furthermore, anthocyanins can be applied in the food industry in order to give functional characteristics for foods [4,5]. This bioactive compounds have shown great health benefits due to their biological activities, including antioxidant and anti-inflammatory potential that reduces the oxidative stress, and cardiovascular, cancer and neurodegenerative diseases risks decrease. Researches have been correlated the anthocyanins with LDL resistance increase to oxidation, with high potential compared to other compounds with antioxidant properties, such as L-ascorbic acid, reducing pro-inflammatory factors. Since inflammation and oxidation are characteristic processes of atherosclerosis, the effect of anthocyanins on the atherosclerotic process has been investigated and there is evidence that these compounds may decrease the development and progression of atherosclerotic lesions [1,2,6].

Anthocyanins regular intake is recommended because the human body does not produce enough endogenous antioxidants to act against free radicals. Regarding foods, anthocyanins are found mainly in red wine, some types of cereals and certain vegetables such as eggplant, purple cabbage, beetroot, and fruits such grape, plum, blackberry, cherry, raspberry and others. In fruits and vegetables, the pigments are found in cells close to the surface and the amounts and distribution vary according to the species, maturation stage, and harvest and post-harvest conditions [7-9]. Some Brazilian native species have been studied due its antioxidant potential associated with the presence of anthocyanins, emphasizing the açai, araçá, camu-camu, guabijú, jabuticaba, and pitanga. These berries were detailed in the sequence considering the characteristics of plant growth, major anthocyanin form and content, and the potential health benefits effects.

Brazilian berries

Berries are small fruits, generally called fleshy, juicy berries, usually eaten fresh [10]. Dark colored fruit species have been associated with benefits for human health, mainly due to the bioactive composition of certain fruits. The families *Myrtaceae* and *Areaceae* are responsible for numerous native species found in Brazilian territory. The fruits have been gaining interest because some species present antioxidant activity and high content of phenolic and flavonoid compounds, such as anthocyanins [11]. These flavonoids group are present in vegetables, flowers and fruits, responsible for the appearance of red, blue or violet colors. Following is a summary of the literature on bioactive compounds present in the Brazilian native species of açai, araçá, camu-camu, guabijú, jabuticaba and pitanga.

Açai

The açai (*Euterpe oleracea* Martius) is a fruit from a plam tree belonging to the *Areaceae* family, native to South America, which

grows mainly in Brazilian Amazonia, Colombia and Suriname. The açai tree has bunches with hundreds of small berries of purple-black color with a yellowish seed. The edible fruits reach 10-12 mm in diameter when ripe. Fruit pulp is described for its nutritional value and is widely consumed as food [12-14]. In the chemical composition of açai fruits are present fatty acids, terpenes, fibers, minerals, flavonoids, phenolic compounds, lignoids, and anthocyanins [13,15,16].

The anthocyanins identified in açai were the cyanidin 3-glucoside and cyanidin 3-rutinoside. In the açai freeze-dried samples Carvalho, *et al.* [16] reported cyanidin 3-glucoside content between 2554 and 18942 mg kg⁻¹ and cyanidin 3-rutinoside content from 865 and 34397 mg kg⁻¹; moreover, the anthocyanin concentration rises in açai during maturity [17].

The biological potential of açai are reported in several studies, mainly related to the presence of flavonoid and phenolic compounds. In an *in vitro* analysis performed with açai hydroethanolic extract, Torma, *et al.* [18] observed that açai genotypes have an antioxidant effect against reactive species generated in SH-SY5Y cells (neural cells), suggesting a neuroprotective effect. The effect was associated with bioactive compounds present in the fruit, such as anthocyanins, which are able to form a protective barrier against reactive oxygen species through their cell membrane binding. Silveira, *et al.* [19] reported a protective effect of white açai juice on the formation of nitrous compounds present in red and processed meat, which are linked to the emergence of colorectal cancer. An *in vitro* analysis the authors verified the inhibition of the nitrosation reaction by the white açai juice. Results can be attributed to the presence of polyphenols that are capable of blocking and decreasing the nitrosamines endogenous formation.

Dias-Souza, *et al.* [20] evaluated the potential of açai ethanolic extracts against planktonic cells and *Staphylococcus aureus* biofilms. The extracts presented antimicrobial activity, biofilm destruction potential, and decreased the proliferation of hepatocellular carcinoma cells. The authors associate the results with the presence of fruit polyphenols with antioxidant potential and free radical scavenging activity.

Araçá

The araçazeiro is a Brazilian native species with edible fruits, popularly known as araçá (*Psidium cattleianum* Sabine) found in the Brazil states from Bahia to Rio Grande do Sul and in Uruguay [21, 22]. With yellow or red in color when ripe, the araçá is an ovoid berry with a 2-5 cm size, translucent, juicy, slightly acid pulp and a high number of seeds in the center [23,24]. Characterized as a wild species, araçá has been highlighted due its pleasant sensory properties and for being a source of secondary metabolites, which have compounds of interest for presenting functional properties [25,26].

The major anthocyanins of red araçá were cyanidin 3-glucoside (354.7 mg kg⁻¹), malvidin-3-glucoside (243.6 mg kg⁻¹) and cyaniding (87.6 mg kg⁻¹) [27]. Vinholes., *et al.* [28] reported a greater content of cyanidin 3-glucoside for red araçá (293 mg kg⁻¹) if compared with yellow one (130 mg kg⁻¹). Similarly, the content of phenolic compounds in red fruit reach greater values (193.2 to 2130.4 mg kg⁻¹) than yellow one (402.6 to 672.2 mg kg⁻¹) in fresh pulp [29].

Phenolic compounds and anthocyanins have been related with the beneficial effects against diabetes, cardiovascular disease and cancer, due their antioxidant activity [26,28]. Nora., *et al.* [30] showed that ingestion of a lyophilized araçá and gubijú preparation had a protective effect against cisplatin, reducing the total cholesterol, LDL, glucose and fat levels in the liver of rats. Furthermore, the araçá extracts prevented the hyperglycemia and exert antioxidant action, preventing the formation of oxygen-reactive compounds and lipid peroxidation in the rat liver [31]. Araçá extracts showed antimicrobial effects against *Salmonella enteritidis* and effects against breast and colon cancer cells (MCF-7 and Caco-2, respectively) in *in vitro* analysis [29].

Camu-camu

The camu-camu (*Myrciaria dubia* Kunth McVaugh) is a native fruit shrub of *Myrtaceae* family found in the forests of the Amazonia, Venezuela, Colombia, Ecuador, Guyana and Bolivia. The camu camu grows in flood-flooded areas and on the banks of rivers, lakes or marshes [32,33]. Camu-camu produces globular berries with 1.0-3.2 cm in diameter, have white and gelatinous flesh with one to four seeds and red to purple outer color when ripe.

Due to its extremely acidic taste, the fruit is used to make jellies, ice cream and juices and is little consumed in natura. It also has a high content of ascorbic acid, phenolic compounds, anthocyanins and carotenoids, characterizing it as a fruit of high nutritional value [14,34].

Many factors are known to influence the camu-camu anthocyanin composition, such cultivars and environmental factors as light, rainfall index, and temperature. The prevalent compounds were cyanidin-3-glucoside and delphinidin-3-glucoside and a total content of anthocyanins of 540 and 303 mg kg⁻¹ were reported for peel and fruits, respectively [35]. According to Chirinos., *et al.* [7] the anthocyanin content were dependent of the maturation stage - higher in red fruits - and the cyanidin-3-glucoside and delphinidin-3-glucoside in red fruits (peel and flesh) were 224 and 22 mg kg⁻¹, respectively.

The biological activity of camu-camu is reported by some authors. The camu-camu juice administered in mice at different concentrations did not present toxicity. Moreover, an antigenotoxic and antioxidant activities of the fruit juice was observed in animal blood cells with DNA damage reduction; the flavonoids was one group associated with these effects [36]. Furthermore, Fujita., *et*

al. [37] evidenced that camu-camu has inhibitory potential against α -glucosidase - enzyme responsible for type 2 diabetes onset and its complications - and antimicrobial potential against *Staphylococcus aureus*. These effects were associated with the phenolic profile of camu-camu, including the cyanidin-3-glucoside.

In vitro cytotoxic effect of camu-camu seed extracts was analyzed by for cells A549 (lung adenocarcinoma epithelial cells), Caco-2 (colorectal adenocarcinoma epithelial cells), HepG2 (human hepatoma carcinoma cells), HCT8 (human colon carcinoma), and IMR90 (human lung fibroblast). The study indicated that none of the extracts tested exerted toxicity to normal IMR90 cells, while for all cancer cell lines there was a large cytotoxic effect showing inhibition of cell growth and antioxidant activity. The phenolic compounds are associated with these effects [38].

Guabiju

The guabiju (*Myrcianthes punges*) is a Brazilian native fruit found especially in the southern states and in Argentina, Paraguay and Uruguay [39,40]. The fruit is a round, yellow-fleshed, with a thick velvety peel, with one or two seeds, and juicy berry with a pleasant sweet taste; when ripe the peel acquire dark purple color [8,27]. The guabiju tree are found in domestic environments, used in the ornamentation of cities or in mixed planting for conservation purpose [41].

The malvidin 3-glucoside (7223.51 mg kg⁻¹, 60% of the total anthocyanin content), delphinidin 3-glucoside (4249.31 mg kg⁻¹), cyanidin chloride, (232.74 mg kg⁻¹), peonidin chloride (195.20 mg kg⁻¹), cyanidin 3-glucoside (134.57 mg kg⁻¹), and malvidin chloride (111.21 mg kg⁻¹) were found in red guabijú (peel and flesh) [27]. Furthermore, the content of anthocyanin are dependent of maturation stage, rising the higher concentration for the fully mature fruit [8].

Guabijú extracts were investigated for their effects on polymorphonuclear leukocytes, showing significant inhibitory activity from 83.32% to 92.95% even in extracts concentrations ≤ 12 μ g mL⁻¹. Moreover, the extracts had potent antioxidant, being compared to vitamin E. The guabiju fruits presented notable antichemotactic activities, related to their polyphenol contents [39]. Nora., *et al.* [30] described that araçá and gubijú preparation had a protective effect against cisplatin, reducing the total cholesterol, LDL, glucose and fat levels in the liver of rats.

Jaboticaba

The jaboticaba tree is native from South and Southeast regions of Brazil, however it is found in Paraguay and Argentina. It belongs to the family *Myrtaceae* and the genus *Myrciaria cauliflora* - known as "Paulista" - and *Myrciaria jaboticaba* - known as "Sabará" - are widespread and produced in commercial scale in Brazil [14,34,42,43]. The jaboticaba fruit are born directly from the branches and trunks of the tree. When ripe, the globular berry are dark purple or black

in size and 3-4 cm in size. The bark is bright, white and gelatinous pulp with 1-4 seeds and its taste is slightly acidic sweet, which provides much of its consumption in a fresh way [14,34,42].

The cyanidin 3-glucoside and delphinidin 3-glucoside were the anthocyanins forms present in jabuticaba, and a higher content of these anthocyanins were found in jabuticaba peel (25820-25980 mg kg⁻¹, 2710-3090 mg kg⁻¹) if compared with pulp (70-180 mg kg⁻¹, 0 mg kg⁻¹) [44]. Similarly, Alezandro. [4] identified the cyanidin 3-glucoside and delphinidin 3-glucoside in *jabuticaba*, which represented a total of 1230 and 235 mg kg⁻¹.

Considering the greater content of anthocyanin in the jabuticaba peel, several studies have been conducted to evaluate the effect on health of this fruit portion. Leite-Legatti., *et al.* [45] evaluated freeze-dried jabuticaba peel extracts in rats and found no DNA damage and no mutagenic effects and the extract also showed antiproliferative effects against leukemia and prostate cancer. Batista., *et al.* [46] evaluated obese rats fed with freeze-dried jabuticaba peel at different concentrations and found that saturated fatty acids decreased and plasma antioxidant defenses increased; moreover 1, 2 and 4% freeze-dried jabuticaba peel supplemented in the feed prevented lipid peroxidation in the liver, increasing antioxidant defenses. Batista., *et al.* [47] supplemented the hyperlipid diet of rats with jabuticaba peel powders and evaluated a reduction in proinflammatory cytokines in the adipose tissue, weight gain prevention, increased triglyceride excretion, reduction of hepatic steatosis area, and stimulation of short chain fatty acids production. Several researchers analyzed the effects of the consumption of jabuticaba in the animal organism and it can be concluded that there are benefits associated with the consumption due to the presence of bioactive compounds of the fruit, such as anthocyanins, which are reported in higher concentration in the fruit peel. Furthermore, Oliveira., *et al.* [48] evaluated that the aqueous, methanolic and ethanolic extracts of jabuticaba peel, which were effective against *Staphylococcus aureus* and *Listeria monocytogenes*, showing antimicrobial.

Pitanga

The pitanga (*Eugenia uniflora* L.) is a Brazilian native fruit found in all national territory, Argentina, Paraguay and Uruguay. Belonging to the *Myrtaceae* family, pitangueira adapts in various environments, growing in the wild and cultivated [34,49]. Is a globular berry with one to two seeds and colors ranging from orange, red and purple when ripe. Its shape resembles a small pumpkin, due to the presence of longitudinal grooves, up to 3 centimeters in diameter. It has an exotic flavor ranging from sweet and sour, being appreciated the consumption in natura and of economic interest to produce juices and jams [34,49].

The main anthocyanin determined by Celli., *et al.* [9] in red and purple pitanga was the cyanidin 3-glucoside. The highest content was in the stage of total maturation and in higher concentration

for the purple variety (1689.67 mg kg⁻¹) if compared with red one (31.04 mg kg⁻¹). These authors still detect small concentrations of myricetin 3-O-hexoside, myricetin 3-O-pentoside, myricetin 3-O-rhamnoside, quercetin 3-O-hexoside, quercetin 3-O-pentoside, quercetin 3-O-rhamnoside, and myricetin deoxyhexosidegallate. Similarly, Chaves., *et al.* [50] determined a higher content of cyanidin 3-glucoside in purple pitanga (582.9 mg kg⁻¹) in relation to red ones (69.1 mg kg⁻¹). The cyanidine-3-glycoside was also the anthocyanin with highest concentration (5120.1 mg kg⁻¹) in lyophilized purple pitanga evaluated by Tambara., *et al.* [51], followed by delphinidin 3-glucoside (996.5 mg kg⁻¹).

The red pitanga extract present no histopathological changes in the rat liver and adipose tissue of the animals, since it did not trigger any inflammatory or degenerative responses. Furthermore, the pitanga extract showed an antihyperglycemic, antihyperlipidemic, and a neuroprotective role since it presented antioxidant and antidepressant-like effects, associated with the phenolic compounds present in *pitanga* fruits [52]. Tambara., *et al.* [51] investigated the antioxidant effect of purple pitanga extracts on the nematode *Caenorhabditis elegans*. The extract of purple pitanga had no toxic effects and increased the life time of nematode. They also found improved life time after oxidative stress-causing agents exposure. Purple pitanga extract has a high anthocyanin content, which may be associated with the result found.

Final considerations

Considering the native species of Brazilian berries evaluated, the major anthocyanin form present in is the cyanidin 3-glucoside, except for guabijú that malvidin 3-glucoside was present in higher concentrations. The açaí, purple pitanga, and jabuticaba presented the higher content of cyanidin 3-glucoside (considering the whole fruit, peel and flesh). The jabuticaba peel present a large content of cyanidin 3-glucoside, justifying the studies conducted in animal with this portion of fruit. Even in the same species, purple color fruits present higher content of anthocyanins than red ones and in the stage of total maturation of fruit the higher content of anthocyanins were detected. All fruits present biological activities, highlighting the antioxidant, anti-inflammatory, and antimicrobial potential, depending of the fruit. The Brazil is a large country with the potential production of differentiated berries with anthocyanins content that can to exercise biological activities.

Conflict of Interest

The authors declare that they have not conflict of interest.

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