Original Article

Antioxidant capacity and bioactive compounds of four Brazilian native fruits

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ABSTRACT

The purpose of this study was to evaluate the bioactive compounds and antioxidant activity of extracts from araçá (*Psidium cattleianum*), butiá (*Butia eriospatha*), and pitanga (*Eugenia uniflora*) fruits with different flesh colors (i.e., purple, red, and orange), and blackberries (*Rubus sp.*; cv. Xavante and Cherokee) collected in the southern region of Brazil. The content of ascorbic acid, total carotenoids, and phenolics were determined. The profile of the phenolic compounds was assessed by high-performance liquid chromatography combined with diode array detection (HPLC-DAD). The antioxidant activity was determined using the ferric-reducing antioxidant power (FRAP) assay, 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) assay, total reactive antioxidant potential (TRAP) assay, and total antioxidant reactivity (TAR) assay. The Xavante blackberry and purple-fleshed pitanga showed the highest total phenolic content [816.50 mg gallic acid equivalents (GAE)/100g and 799.80 mg GAE/100g, respectively]. The araçá and red-fleshed pitanga showed the highest carotenoid content (6.27 ug β-carotene/g and 5.86 ug β-carotene/g, respectively). The fruits contained several phenolic compounds such as quercetin derivatives, quercitrin, isoquercitrin, and cyanidin derivatives, which may contribute differentially to the antioxidant capacity. The highest scavenging activity in the DPPH assay was found for purple-fleshed pitanga (IC_{50} 36.78 mg/L), blackberries [IC_{50} 44.70 (Xavante) and IC_{50} 78.25 mg/L (Cherokee)], and araçá (IC_{50} 48.05 mg/L), which also showed the highest FRAP, followed by orange- and red-fleshed pitanga. Our results revealed that some fruits grown in southern Brazil such as purple-fleshed pitanga, blackberries, and araçá are rich sources of phenolic compounds and have great antioxidant activity.

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1. Introduction

The relationship between nutrition and health has become a topic of great interest. There is substantial evidence of the beneficial effects of diets that are rich in fruits and vegetables. Brazil has a great biological diversity that can be explored to yield extracts for therapeutic application to control and/or prevent chronic diseases. Polyphenols from fruits and vegetables [1] can be divided into several classes (e.g., hydroxybenzoic acids, hydroxycinnamic acids, anthocyanins, proanthocyanidins, flavonols, flavones, flavanols, flavanones, isoflavones, stilbenes, and lignans). They contribute substantially to the antioxidant effect of many small-fruited species, and have potential healthful effects.

Psidium cattleianum Sabine (araçá; family Myrtaceae), which is also known as wild guava or Brazilian guava, is found from the state of Minas Gerais to the state of Rio Grande do Sul [2]. According to folk medicine, araçá is indicated to treat diarrhea, hemorrhages, and cramp. *Butia eriospatha* (Mart. ex Drude) Becc. (butiá; family Arecaceae) is a palm tree that is native to South America. In Brazil, it grows in the states of Parana, Santa Catarina, and Rio Grande do Sul. The ripe fruit can be eaten raw or used for preparing juices, wine, and liqueurs. We found no study in the literature that evaluated the content of phenolic compounds and antioxidant activity of araçá and butiá fruits.

Eugenia uniflora L. (Myrtaceae) is a widely distributed tree species in South America, primarily in Brazil, Argentina, Uruguay, and Paraguay. The leaves are used in popular medicine as an infusion for the treatment of fever, rheumatism, stomach diseases and digestive disorders, hypertension, yellow fever, and gout. It may also reduce weight, blood pressure, and serve as a diuretic [3,4]. Its fruit, which is known as pitanga, Brazilian cherry or Surinam cherry, also shows antioxidant activity by inhibiting lipid peroxidation and removing free radicals [5]. Pitanga fruits contain various volatile compounds that are also found in the essential oil from pitanga leaves [6]. Pitanga fruit (as well as its leaves) could also have healthful benefits. In the Brazilian food industry, the pitanga fruit has mostly been used to produce juice, which shows good economic potential because of consumer appeal arising from its high concentration of antioxidant compounds such as anthocyanins, flavonols, and carotenoids.

Despite being native to Asia, Europe, and North and South America, *Rubus* sp. (blackberry; family Rosaceae) grows only in specific regions because most blackberry species are not adapted to regions with mild winters [7]. In Brazil, the blackberry was introduced in the 1970s by the Brazilian Agricultural Research Corporation (Embrapa Temperate Agriculture, Pelotas, Brazil). Embrapa has subsequently conducted a genetic improvement program that developed various blackberry cultivars (e.g., Guarani, Caingangue, Xavante, and Tupy) that are adapted to the southern region of Brazil. Because of its subtropical climate, Rio Grande do Sul was the first state in Brazil to produce blackberries, and it remains the main site [8].

Blackberry fruits are good sources of natural antioxidants. Extracts from blackberry fruits may have some health benefits such as antioxidant [9,10], anticonvulsant and muscle relaxant [11], and anti-inflammatory properties [12]. Their extracts are rich in secondary metabolites such as anthocyanins and phenolic acids [9]. However, little is known about the presence and antioxidant activity of these compounds in genotypes that grow in the state of Rio Grande do Sul (Brazil).

The phenolic compounds in fruit and vegetable-rich diets have attracted researchers' attention because of their health-promoting effects such as lowering the risk of cardiovascular disease, cancer, or other conditions associated with aging. The biological mechanisms behind these health-promoting effects include protection against free radicals, free radical-mediated cellular signaling, inflammation, allergies, platelet aggregation, ulcers, viruses, tumors and hepatotoxicity [13]. However, there are few studies on the identification of phytochemical compounds and antioxidant activity of extracts from Brazilian native fruits. Furthermore, knowledge concerning the antioxidant activity and content of bioactive compounds in different fruit genotypes may be useful for genetic improvement programs to select the varieties with higher nutritional values. Thus, the objective of this study was to evaluate the antioxidant activity and bioactive compounds of extracts from the fruits of araçá, butiá, pitanga (with purple, red, and orange flesh), and blackberry fruits (cv. Xavante and Cherokee) collected in different regions of Rio Grande do Sul (Brazil).

2. Materials and methods

2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox; 97%); 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH); 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ; $\geq$ 98%); 3-aminophthal-hydrazide (Luminol; 97%); 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH; 97%); Folin-Ciocalteu reagent (2N); *p*-hydroxybenzoic acid ($\geq$ 99%), chlorogenic acid ($\geq$ 95%), *p*-coumaric acid ($\geq$ 98%), caffeic acid $\geq$ 98%, ferulic acid (99%), syringic acid ($\geq$95%), gallic acid (97.5–102.5%), ellagic acid ($\geq$95%), and ascorbic acid ($\geq$ 99%); beta-carotene ($\geq$ 95%); rutin ($\geq$ 94%); kaempferol ($\geq$ 90%); kaempferol-3-glucoside ($\geq$ 97%); malvidin ($\geq$ 95%); delphinidin ($\geq$ 95%); pelargonidin ($\geq$ 95%); cyanidin ($\geq$ 95%); cyanidin-3-glucoside ($\geq$ 95%); quercetin-3-O-galactoside (hyperoside; $\geq$ 97%); quercetin-3-beta-D-glucoside (isoquercitrin; $\geq$ 90%); and quercetin-3-rhamnoside (quercitrin; $\geq$ 78%) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Quercetin ($\geq$ 98%) was purchased from Jassem Chemical (Beerse, Belgium). Vanillic acid ($\geq$ 97%) was purchased from Fluka Chemical (Bochus, Switzerland).

2.2. Preparation of the fruit extracts

Samples of orange-, red-, and purple-fleshed breeding lines of pitanga fruits (*Eugenia uniflora*) and the blackberry (*Rubus sp.*) cultivars Xavante and Cherokee were obtained from the 2009–2010 harvest at Embrapa Temperate Agriculture (Pelotas, Rio Grande do Sul, Brazil, 31°40′47″S, 52°26′24″W, 60 m) and immediately frozen. In January 2008, samples of araçá fruit (*Psidium cattleianum*) and butiá fruit (*Butia eriospatha*) were

collected in the municipalities of Tuparendi (Rio Grande do Sul, Brazil; 27°77′24″S, 54°49′76″W, 60 m) and Santa Maria (Rio Grande do Sul, Brazil; 29°42′27″S, 53°40′29″W, 318 m), respectively. Each species was sampled. A mixture of completely ripe fruits from various plants of the same genotype were gathered. Three independent samples were collected, frozen at −18°C, and transported to the Federal University of Santa Maria (Santa Maria, Brazil).

Fruit extracts were prepared from the edible portions of fruits. In brief, fresh fruit samples were homogenized with an Ultra-Turrax homogenizer (IKA (SP, Brazil)) for 5 minutes in 95% ethanol (1:3 w/v). The homogenates were blended for 30 minutes at room temperature, and then centrifuged at 1500g for 5 minutes. The supernatant was collected and the extraction procedure was repeated. The pooled supernatants were concentrated in a rotary evaporator at 40°C. The samples were reconstituted in water and stored at −80°C. The extracts obtained from each fruit were always adjusted to the same final volume so that the yield of extraction was 0.15 mL of the extract per gram of fruit.

2.3. Ascorbic acid, total carotenoids, and phenolic content

The ascorbic acid content of sampled extracts was assessed as described by Sánchez-Mata et al [14] with some modifications. Ethanolic extracts were filtered through a 0.22 mm Millipore filter (EMD Millipore Bedford, MD, USA) and 10 μL were analyzed using an Intralab (Varian, 5100 model, USA) high-performance liquid chromatography (HPLC) system (5100 model) coupled with a UV-visible detector (Intralab 5100) and reverse phase Microsorb—MW C18 column (4.6 mm × 250 mm, particle size 5 μm; Varian (Varian, USA)). The flow rate was 0.9 mL/min (i.e., the isocratic gradient) and the mobile phase was a solution of sulfuric acid (0.01%) in Milli-Q water (final pH 2.8). The total run time was 8 minutes and the wavelength of detection was set at 245 nm. The quantification of ascorbic acid was achieved using calibration curves with seven concentrations of ascorbic acid ($R^2 = 0.9992$).

The carotenoids were exhaustively extracted from the fruits with ethyl acetate, by vortexing for 1 minute. The organic phases were transferred to a separatory funnel, washed with water until obtaining a neutral pH, dried under a nitrogen (N_2) stream, and then dissolved in petroleum ether [15]. The total carotenoid content was subsequently measured spectrophotometrically at 450 nm using an extinction coefficient of 2590. The results were expressed as β-carotene equivalents (μg/g of fresh weight).

Total phenolic content was measured, based on the Folin-Ciocalteu method adapted from Swain and Hillis [16]. The extract (100 μL) was mixed with 1600 μL of Milli-Q water, and 100 μL of 0.25N Folin-Ciocalteu reagent. The mixture was allowed to react for 3 minutes, 200 μL of 1N sodium carbonate (Na_2CO_3) was added, and the solution was incubated at room temperature (23 ± 1°C) in the dark for 2 hours. The absorbance was measured at 725 nm, and the results were expressed as gallic acid equivalents (GAE; mg/100 g fresh weight) using a gallic acid standard curve (0–0.4 mg/mL).

2.4. HPLC combined with diode array detection analysis

Samples were filtered through a 0.45 μm filter (Millipore). The HPLC analysis was performed on a Waters 2695 HPLC system (Waters Corp., Milford, MA) equipped with a Luna C18 reversed-phase silica 100 Å column (250 mm × 4.6 mm; particle size, 3 μm; Phenomonex, Torrance, CA, USA), a Waters 996 diode array detection (DAD) detector (Waters Corp.), and Empower Software (Waters Corp.). The solvents were 2.5% aqueous formic acid (pH 2.4; solvent A) and acetonitrile (solvent B). Anthocyanins were analyzed in the ethanolic extracts (10 μL injection volume) at 520 nm using the following gradient: from 12% to 50% B in 20 minutes and from 50% to 12% B in 22 minutes, and isocratically with 12% B up to 30 minutes at a flow rate of 0.5 mL/min. For other phenolic compounds, the gradient conditions were the following: from 0% to 30% B in 80 minutes, from 30% to 50% B in 90 minutes, from 50% to 100% B in 95 minutes, and then isocratically with 100% B up to 98 minutes at a flow rate of 0.5 mL/min. The column was thereafter washed for 5 minutes and equilibrated for 8 minutes. The injection volume was 10 μL, and the detection was performed between 200 nm and 600 nm. Peak identification and quantification was based on comparison with the retention times and UV visible spectra of authentic standards.

2.5. Antioxidant activity

2.5.1. Ferric-reducing antioxidant power assay
The ferric-reducing antioxidant power (FRAP) of each ethanolic extract (i.e., three different dilutions of the sample) was estimated according to the procedure described by Pulido et al [17]. In brief, 2.7 mL of the FRAP reagent, which was prepared freshly and warmed at 37°C, was mixed with 270 μL of Milli-Q water and 90 μL of the test sample, water, or methanol as appropriate for the reagent blank. The FRAP reagent contained 2.5 mL of a 10-mM TPTZ solution in 40mM HCl plus 2.5 mL of 20mM iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) and 25 mL of 0.3M acetate buffer at pH 3.6. The reaction mixture was incubated at 37°C for 30 minutes and the absorption maximum was assessed at 595 nm. An intense blue color formed when the ferric—tripyridyltriazine (Fe^{3+}-TPTZ) complex was reduced to the ferrous (Fe^{2+}) form. Aqueous solutions of known Fe^{2+} concentrations in the range of 500–1500μM iron(II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) were used for calibration.

The total antioxidant activity was defined as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1mM $FeSO_4 \cdot 7H_2O$/g of fruit. Total antioxidant activity was calculated as the concentration of antioxidant that produces an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1mM $FeSO_4 \cdot 7H_2O$ solution, which was determined using the corresponding regression equation.

2.5.2. DPPH assay
The capacity to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was assessed using the method of Brand-Williams et al [18] with some modifications. Fruit extracts (100 μL) were allowed to react with 3.9 mL of the DPPH solution for 90 minutes in the dark. The blank sample consisted of

0.1 mL of methanol added to 3.9 mL of DPPH. The absorbance was then obtained at 515 nm. The radical scavenging activity was calculated as follows:

$$I\% = [(Abs_0 - Abs_1)/Abs_0] \times 100, \tag{1}$$

in which Abs_0 was the absorbance of the blank and Abs_1 was the absorbance in the presence of the test compound at different concentrations. The concentration providing 50% inhibition of DPPH absorbance (IC_{50}) was calculated graphically using a calibration curve in the linear range by plotting the extract concentration versus the corresponding scavenging effect.

2.5.3. Total reactive antioxidant potential and total antioxidant reactivity

Total reactive antioxidant potential (TRAP) assay has been described by Lissi et al [19]. We used this test as an index of the nonenzymatic antioxidant capacity of each ethanolic extract, based on the peroxyl radical scavenge by the sample compounds. The peroxyl radical was generated by mixing 2,20-azobis[2-amidinopropane] (AAPH) solution with Luminol (i.e., system). The first reading of chemiluminescence emission was performed 2 hours after the system preparation to allow stabilization. After adding the sample, readings were obtained for nearly 30 minutes. The results were transformed as a percent of the first reading and plotted against time. The area under curve (AUC) was calculated using GraphPad 5.0 software (GraphPad Software Inc, San Diego, CA, USA). The total antioxidant reactivity (TAR) was also analyzed for each ethanolic extract, and TAR is based on the same technical principles of TRAP; however, TAR is more correlated with the quality of the antioxidant samples. The TAR results were calculated as the ratio of light in the absence of sample (I_0) divided by the light intensity immediately after the addition of the sample (I) [19].

2.6. Statistical analysis

Data were reported as the mean ± the standard deviation (SD) of the three replicates for each sampled species. The results were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's test ($p < 0.05$). The relationship between the antioxidant compounds (i.e., phenolics, carotenoids, or ascorbic acid) and antioxidant activity was evaluated by Pearson's correlation. All analyses were performed using the statistical software SPSS (SPSS 16.0, SPSS Inc., Chicago, USA).

3. Results and discussion

3.1. Total phenolics, carotenoids, and ascorbic acid

Significant differences ($p < 0.05$) were found among the fruit extracts for the content of total phenolics, carotenoids, and ascorbic acid (Table 1). The total phenolic content ranged 359.5–816.5 mg GAE/100 g fresh weight. The Xavante blackberry and the purple-fleshed pitanga showed the highest total phenolic content, followed by Cherokee blackberry, araçá, orange- and red-fleshed pitanga, and butiá (Table 1). The high content of phenolic compounds in blackberry reported in the present study has also been reported by several authors for different cultivars in the United States, Brazil, and Italy with values ranging 192.8–499.0 mg GAE/100 g [9,10,20]. Jacques et al [21] also observed a higher content of phenolic compounds in purple pitanga (420.8 mg GAE/100g), compared to red pitanga (239.2 mg GAE/100 g) and orange pitanga (201.8 mg GAE/100 g). Butiá showed a similar content (328.6 mg GAE/ 100 g). The total phenolic content for araçá was higher than the content reported by Biegelmeyer et al [22] for the same yellow araçá species (292.03 mg/100 g). This is probably because other methods were used for the determination of phenolic compounds.

Phenolic compounds are secondary products of plant metabolism that constitute a large and complex group. These molecules are essential for the growth and reproduction of plants, and their synthesis is induced under conditions of biotic and abiotic stress such as infections, injury, UV radiation, ozone, salinity, water stress, and heat. They are partially responsible for the color, astringency, aroma, and oxidative stability in foods [1].

The total carotenoids ranged 6.27–0.87 μg β-carotene/g fresh weight. The araçá and red-fleshed pitanga showed the highest carotenoid content, followed by orange-fleshed pitanga, butiá, purple-fleshed pitanga, and Xavante and Cherokee blackberries (Table 1). Sixteen carotenoids have been isolated from guava (*Psidium guajava* L.) and identified

Fruit	Total phenolics content (mg GAE/100 g fw)	Total carotenoids (ug β-carotene/g)	Ascorbic acid (mg/100 g)
Butia	359.50 ± 45.2[d]	3.85 ± 0.74[b]	9.351 ± 0.06[a]
Araça	660.19 ± 47.6[b]	6.27 ± 0.06[a]	0.095 ± 0.01[b,c]
Orange pitanga	457.43 ± 15.2[c]	4.02 ± 0.05[b]	0.128 ± 0.03[b]
Red pitanga	433.84 ± 60.5[c,d]	5.86 ± 0.03[a]	0.086 ± 0.00[b,c]
Purple pitanga	799.80 ± 54.7[a]	3.04 ± 0.06[b]	0.101 ± 0.01[b,c]
Blackberry Xavante	816.50 ± 63.6[a]	1.04 ± 0.04[c]	0.010 ± 0.00[c]
Blackberry Cherokee	718.65 ± 59.0[b]	0.87 ± 0.05[c]	0.004 ± 0.00[c]

Table 1 — The content of total phenolics, total carotenoids, and ascorbic acid in some Brazilian native fruits.

The results are presented as mean ± standard deviation ($n = 3$).
[a–e] The values with the same letters in the same column are not significantly different (Tukey's test, $p < 0.05$).
araça = *Psidium cattleianu*; blackberry = *Rubus sp.*; butia = *Butia eriospatha*; fw = fresh weight; pitanga = *Eugenia uniflora*.

as phytofluene, β-carotene, γ-carotene, lycopene, β-cryptoxanthin, rubixanthin, cryptoflavin, lutein, and neochrome [23]. We found no studies that evaluated the carotenoid content in araçá. According to Coimbra and Jorge [24], the Brazilian palm species guariroba (*Syagrus oleraces*), jerivá (*Syagrus romanzoffiana*), and macaúba (*Acrocomia aculeata*) contain high levels of carotenoids (158.44μg β-carotene/g, 1219 μg β-carotene/g, and 300.01 μg β-carotene/g, respectively); however, in this study the concentration of carotenoids in butiá was only 3.8 μg β-carotene/g. Among the three selections of pitanga, the red-fleshed variety showed higher total carotenoids (5.9 μg β-carotene/g) than the orange-fleshed pitanga (4.0 μg β-carotene/g), and purple-fleshed pitanga (3.0 μg β-carotene/g). This finding is in agreement with results obtained in the literature [21]. During the ripening process, the pitanga fruit changes from green to yellow to orange to red, and then to dark red; in some situations, the fruit becomes nearly black when lycopene is the major carotenoid present. The following carotenoids have been found in pitanga fruits (listed in decreasing quantitative order): lycopene, rubixanthin, *cis*-rubixanthin, β-cryptoxanthin, *cis*-lycopene, β-carotene, γ-carotene, zeaxanthin, lutein, violaxanthin, and β-carotene-5,6-epoxide [25].

Blackberries showed the lowest carotenoid levels, compared to the other fruits. The color of these fruits in the mature stage is primarily because of the presence of anthocyanins, whereas the carotenoid content decreases with maturation [21]. The composition of the carotenoids in the plant is affected by several factors such as variety, part of the plant, degree of maturity, climate, soil type, growing conditions and geographical area of production, harvest conditions, and processing and storage. This may explain the lower total carotenoid content of all fruits examined, compared to results reported in the literature for the same species and fruit varieties.

The content of ascorbic acid of the butiá was approximately 73 times greater than all other fruits studied (Table 1). Ascorbic acid, in addition to being the biologically active form of vitamin C, is the most commonly found and widely distributed in products of plant origin. It is primarily in citrus fruits and leafy vegetables. The extracts of pitanga and araçá showed low levels of ascorbic acid. They are characterized as poor sources of vitamin C. The vitamin content of fruit can vary, depending on the species, maturity stage at harvest time, genetic variants, postharvest handling, storage conditions, and processing. The content and stability of these nutrients in the fresh food can influence its nutritional quality [26]. The extracts of blackberries had very low ascorbic acid levels. Hassimotto et al [20] accordingly found only the oxidized form of vitamin C (L-dehydroascorbic acid) in the Tupy and Guarani cultivars grown in a tropical climate region of Brazil. This finding was attributed to the fast oxidation of vitamin C and absence of *de novo* synthesis of ascorbic acid during development or ripening.

We found a negative correlation between total phenolic content and ascorbic acid content ($r^2 = -0.674$; $p < 0.05$). This finding suggested that the lower the ascorbic acid content, the higher the total phenolic content in these fruits studied.

3.2. Profile of phenolic compounds

Phenolic compounds or polyphenols are a complex group of phytochemicals that possess several hydroxyl groups on aromatic rings. They are widely distributed throughout the plant kingdom and thus form an integral part of the human diet.

The HPLC-DAD chromatograns (at 280 nm and 360 nm) for the extracts of pitanga fruits are shown in Fig. 1. The main phenolic compounds identified are listed in Table 2. The chromatograms of three varieties of pitanga have many similarities in the profile of phenolic compounds. Gallic acid derivatives; quercetin derivatives; quercitrin, isoquercitrin, kaempferol derivatives; and cyanidin-3-glucoside were found in the three varieties of pitanga, whereas cyanidin derivatives and quercetin were found only in red- and purple-fleshed pitanga. Protocatechuic acid derivatives were found in red-fleshed pitanga and malvidin derivatives were found in purple-fleshed pitanga (Table 2). There are a range of phytochemicals already identified in pitanga leaves such as flavonoids (e.g., myricetin, quercetin, and quercetrin 3-l-ramnoside), steroids and triterpenoids, tannins, antraquinons and phenols, sineol, and essential oils [3,4]. However, few studies have evaluated these compounds in pitanga fruits. Celli et al [27] evaluated the flavonoids profile in red and purple pitanga fruits and identified several flavonoid derivatives of cyanidin, myricetin, and quercetin. Some anthocyanins such as cyanidin-3-glucoside and delfinidin-3-glucoside were also identified.

As observed for pitanga fruits, the two cultivars of blackberries also showed chromatograms with very similar profiles (Fig. 2). The phenolic compounds identified in the two cultivars studied were ellagic acid derivatives, quercetin derivatives, isoquercitrin, cyanidin-3-glucoside, and delphinidin derivatives. Protocatechuic acid derivatives, cyanidin derivatives, quercetin and quercitrin were found in the Xavante cultivar, whereas kaempferol derivatives were found in the Cherokee cultivar (Table 2). There are many studies on the phenolic compounds of blackberries, but few studies have been performed on blackberries grown and adapted to the southern region of Brazil. Mertz et al [28] analyzed the phenolic compounds in two blackberry species and identified gallic acid and galloyl esters, caffeic acid, *p*-coumaric acid, ferulic acid, epicatechin, ellagic acid, quercetin derivatives, kaempferol derivatives, cyanidin-3-glucoside, among others. Hassimotto et al [20] found cyanidin, flavan-3-ol epicatechin, quercetin, and kaempferol in blackberry cultivars from the southern region of Brazil. Some recent studies suggest that blackberries have among the highest antioxidant capacity of any fruit or vegetable—primarily because of its high content of cyanidin-3-glucoside [29].

Fig. 3 shows the chromatograms (at 280 nm and 360 nm) of the araçá and butiá extracts. These two fruits have yellow flesh, although the profile of these phenolic compounds showed significant differences because of variations in the botanical family: the araçá belongs to the Myrtaceae family and butiá belongs to the Arecaceae family. The major phenolic compounds identified in araçá were gallic acid derivatives, quercetin derivatives, apigenin derivatives, and isoquercitrin, although most chromatographic peaks were not identified
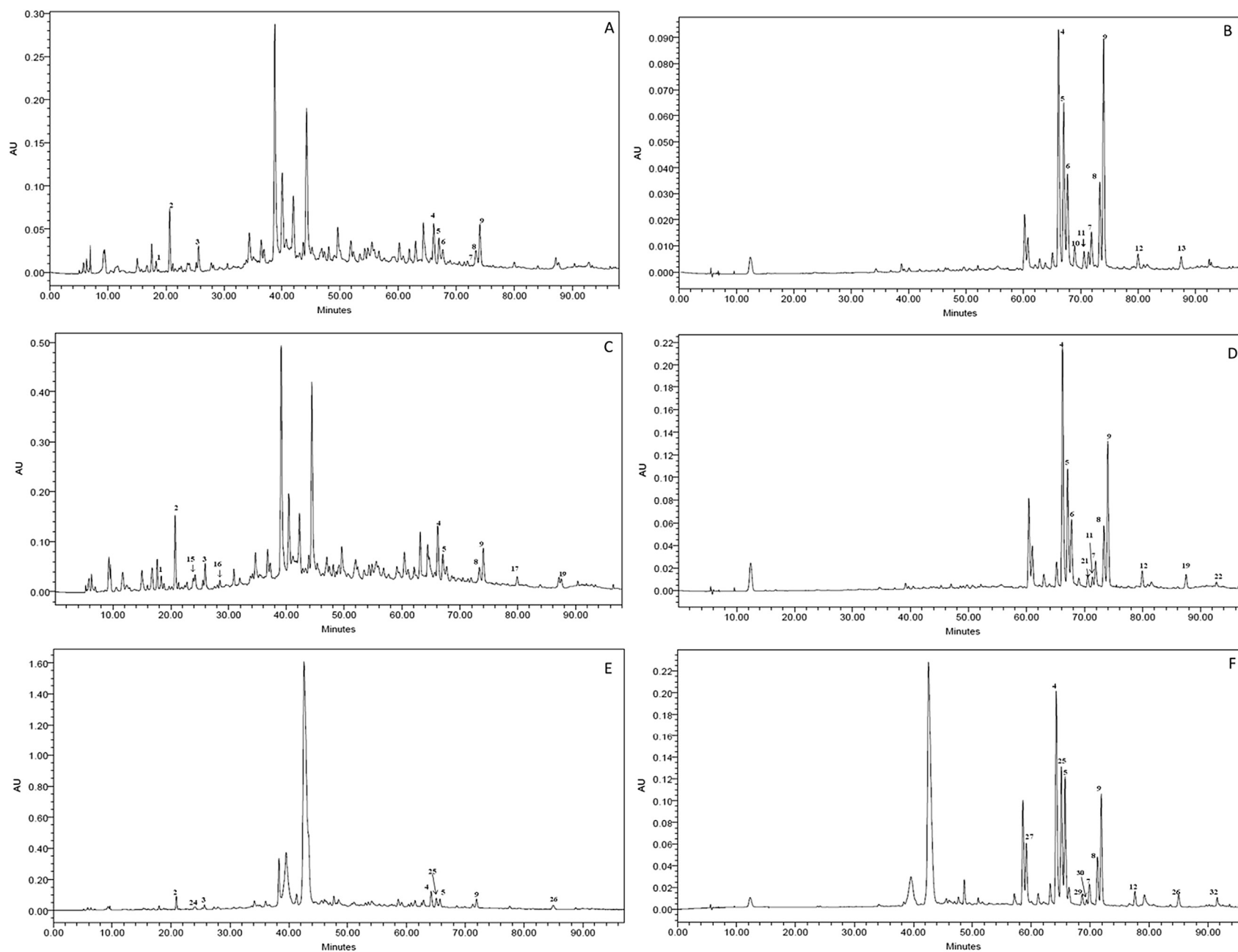
Fig. 1 – The high-performance liquid chromatography (HPLC) chromatograms of pitanga extracts from three different varieties: orange, 280 nm (A) and 360 nm (B); red, 280 nm (C) and 360 nm (D); and purple, 280 nm (E) and 360 nm (F). For the peak numbers, see Table 2.

Table 2 – The tentative identification of phenolic compounds in the ethanolic extract of some Brazilian native fruits.

Peak no.[c]	tR (min)	DAD characteristics absorption maxima (nm)	Fruit	Tentative characterization[a]
Benzoic acid derivatives, hydroxycinnamic acid derivatives, and ellagic acid derivatives (280 nm)				
3; 24;	18.7; 25.6;	225, 272	Purple pitanga	Gallic acid derivative
2; 58	20.6;	225, 277	Purple pitanga	Gallic acid derivative
Flavonols (360 nm)				
4	64.2	225, 258, 348	Purple pitanga	Quercetin derivative
7; 25;	71.9; 66.9	225, 253, 354	Purple pitanga	Quercetin derivative
8; 10; 27; 50	73.3; 73.3; 71.3; 68.9; 59.2; 67.2	225, 258, 354	Orange, red, and purple pitanga; Cher.	Quercetin derivative
29; 30; 65	68.6; 69.4; 62.1	225, 263, 354	Purple pitanga; araçá	Quercetin derivative
32; 22	91.; 92.2; 92.7	225, 253, 371	Red and purple pitanga; Xav.	Quercetin
5	67.0; 67.1; 65.7; 66.6; 66.5; 67.5; 64.6	225, 253, 354	Orange, red and purple pitanga; Xav.; Cher.; araçá; butiá	Quercetin-3-b-D-glucoside
9	74.0; 74.0; 71.9; 72.4	225, 253, 349	Orange, red and purple pitanga; Xav.	Quercetin-3-rhamnoside
12; 17; 26; 52; 54	79.9; 79.9; 77.6; 79.9; 85.0; 69.2; 72.7	225, 263, 349	Orange, red and purple pitanga; Cher.	Kaempferol derivative
Anthocyanins (520 nm)[b]				
14	43.8; 43.9; 42.6; 43.0; 43.6	230, 277, 517	Orange, red and purple pitanga; Xav.; Cher.	Cyanidin-3-glucoside
23; 46	40.7; 39.6; 52.1	225, 517, 527	Red and purple pitanga; Xav.	Cyanidin derivative
33	47.6	225, 287, 537	Purple pitanga	Malvidin derivative

[a] The characterization is based on the standard retention time and standard UV visible spectra.
[b] The chromatograms are not shown.
[c] The peak numbers correspond to Figs. 1–3. The peaks are numbered according to their tR and DAD characteristics absorption maxima (nm). The same peaks received the same number in all samples, but different peaks with the same DAD characteristics absorption maxima (nm) received different numbers.
araçá = Psidium cattleianum; blackberry = Rubus sp.; butiá = Butia eriospatha; Cher. = blackberry Cherokee; DAD = diode array detection; pitanga = Eugenia uniflora; Xav. = blackberry Xavante.

(Table 2). In addition, the following compounds were identified in butiá: gallic acid derivatives, protocatechuic acid derivatives, caffeic acid derivatives, chlorogenic acid derivatives, isoquercitrin, quercetin derivatives, hyperoside, and rutin (Table 2). Few reports in the literature assess the profile of phenolic compounds in these fruits. However, hyperoside was the main phenolic compound in araçá, followed by cyanidin [22].

The phenolic composition of fruits is determined by genetic and environmental factors, but may be modified by oxidative reactions during processing and storage [30]. The phenolic compounds are metabolized as a defense response against intense solar radiation and other adverse factors. Variations in the fruits result from the route of formation of these compounds since their presence differs in each fruit. Thus, the different contributions of individual phenolics in the extracts are expected to yield different antioxidant effects by the extracts.

3.3. Antioxidant capacity

Polyphenols have powerful antioxidant activity *in vitro* and are capable of scavenging a wide range of reactive oxygen, nitrogen, and chlorine species such as superoxide anion, hydroxyl radical, peroxyl radicals, hypochlorous acid, and peroxynitrous acid. They also chelate metal ions, and thus decrease their pro-oxidant activity. Because considerable evidence indicates that increased oxidative damage is associated with the development of most major age-related degenerative diseases, it has been speculated that polyphenols may have protective effects against such conditions [31].

Because of multiple reaction characteristics and mechanisms, a single antioxidant assay will not accurately reflect all antioxidant in a mixed or complex system. Thus, the use of different antioxidant assays help to identify variations in the response of the compounds extracted from fruits [31,32]. For this reason, three different antioxidant assays were conducted to clarify different aspects of the antioxidant capacity of extracts from fruits produced in the southern region of Brazil. The DPPH and FRAP assays were chosen because they are simple and rapid methods for assessing the antioxidant capacity of fruits and vegetables [32]. These two assays are based on different principles, and thus may be used to screen compounds that have different antioxidant mechanisms. The FRAP assay measures the ferric-reducing capacity of antioxidants [33], whereas the DPPH assay measures the ability of antioxidants to scavenge the DPPH radical [18].

The evaluation of antioxidant activity by the DPPH method showed a large variation among the different fruit extracts (Table 3), which may be associated with differences in the profile of phenolic compounds among these fruits. The lowest IC$_{50}$ value, which corresponds to the highest scavenging activity of DPPH radicals, was obtained for the extracts of purple-fleshed pitanga, Xavante blackberry, araçá, and Cherokee blackberry; by contrast, the orange- and red-fleshed pitanga had intermediate scavenging capacity and butiá had the lowest scavenging capacity (Table 3). Purple-fleshed pitanga, araçá, and the Xavante and Cherokee blackberries also showed the highest FRAP value, followed by orange- and red-
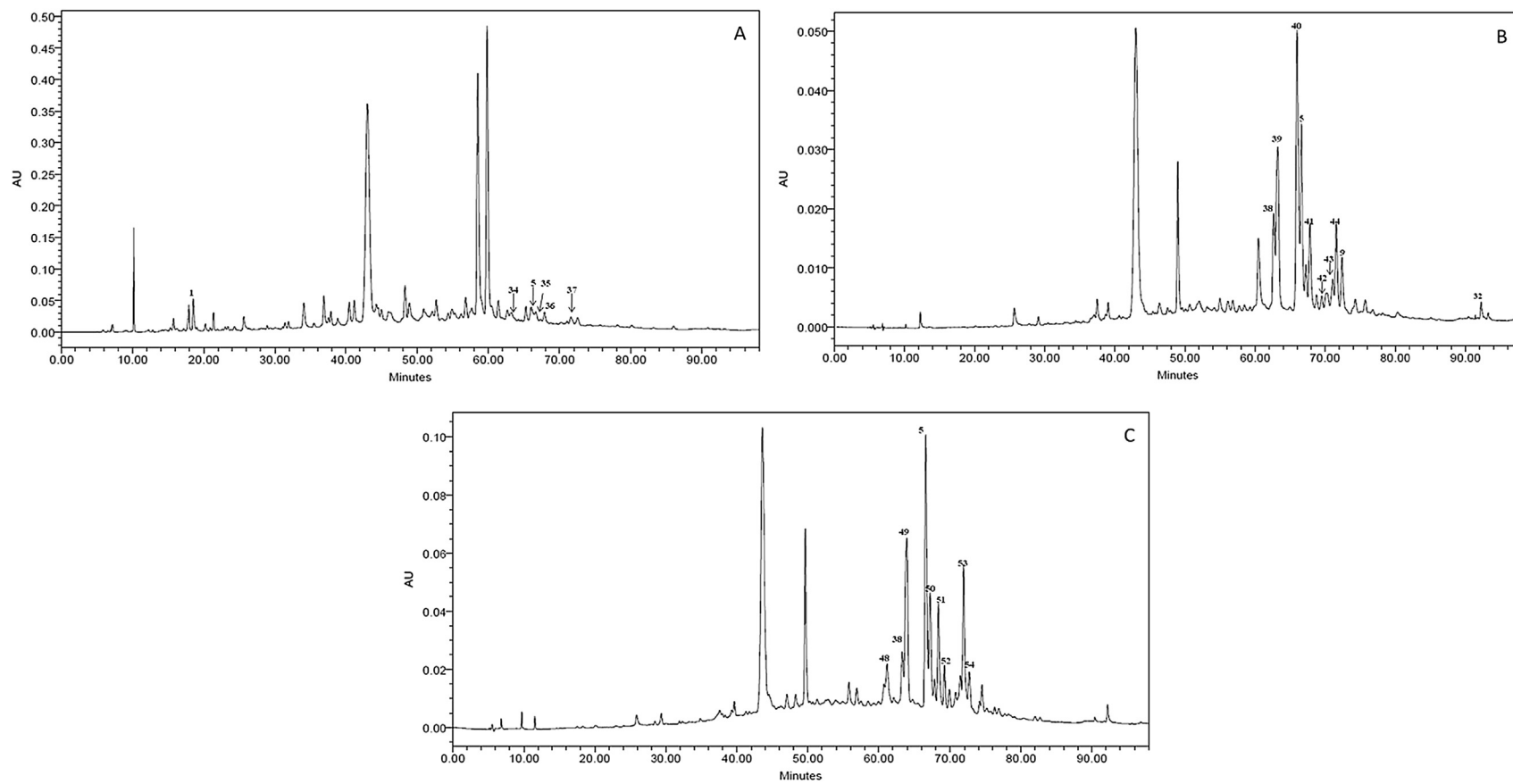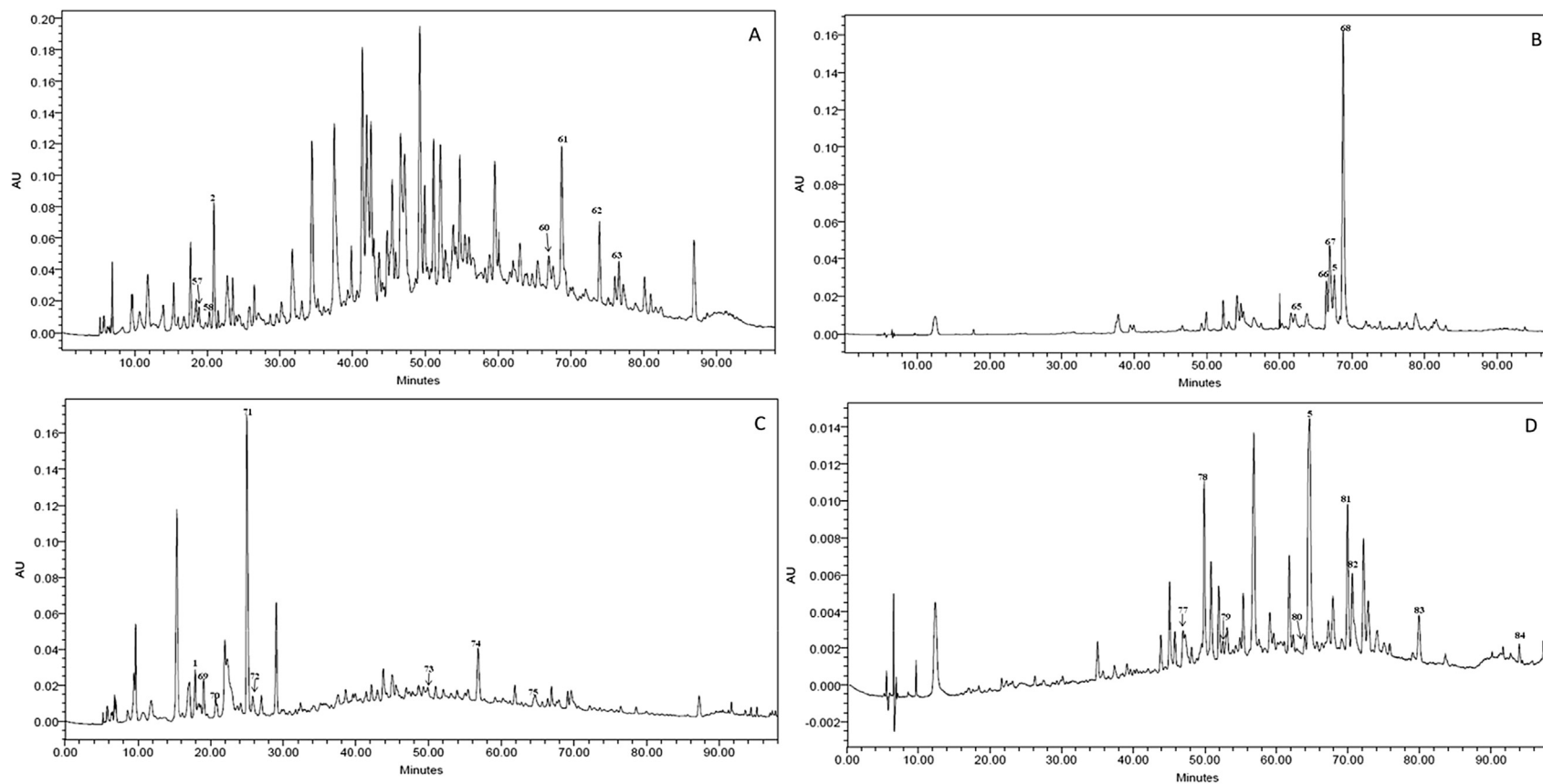
Fig. 2 — The high-performance liquid chromatography (HPLC) chromatograms of blackberry extracts from two different varieties: Xavante, 280 nm (A) and 360 nm (B); and Cherokee, 360 nm (C). For the peak numbers, see Table 2.

Fig. 3 – The high-performance liquid chromatography (HPLC) chromatograms of araçá and butiá extracts: araçá, 280 nm (A) and 360 nm (B); and butiá, 280 nm (C) and 360 nm (D). For the peak numbers, see Table 2.

Table 3 – Antioxidant capacity and nonenzymatic potential of the extracts of some Brazilian native fruits.

	DPPH (IC$_{50}$ mg/L)*	FRAP (µmol FeSO$_4$·7H$_2$O/g fw)	TRAP (AUC)**	TAR (I$_0$/I)**
Butia	253.80 ± 25.4^a	9.32 ± 0.9^d	25.73 ± 0.23^{a,b}	72.05 ± 0.01^c
Araça	48.05 ± 12.1^{d,e}	89.09 ± 13.0^a	26.14 ± 0.34^a	71.62 ± 10.63^c
Orange pitanga	110.91 ± 18.9^{b,c}	33.17 ± 2.8^{c,d}	26.19 ± 0.17^a	81.80 ± 9.27^{a,b}
Red pitanga	121.87 ± 8.3^b	23.43 ± 4.4^d	25.90 ± 0.40^a	88.94 ± 4.89^a
Purple pitanga	36.78 ± 5.8^e	81.62 ± 10.1^a	25.27 ± 0.31^b	68.30 ± 8.97^c
Blackberry Xavante	44.70 ± 2.1^{d,e}	52.51 ± 3.3^{b,c}	26.21 ± 0.15^a	72.85 ± 7.01^{a,b}
Blackberry Cherokee	78.25 ± 8.1^{c,d}	66.60 ± 4.3^{a,b}	25.59 ± 0.17^{a,b}	68.78 ± 4.77^c

The results are presented as mean ± standard deviation ($n = 3$).
$^{a-e}$ The values with the same letters in the same column are not significantly different (Tukey's test, $p < 0.05$).
* The results are expressed as milligrams of fresh fruit per liter of extract.
** For all fruits, the TRAP and TAR values are obtained by using an extract amount equivalent to 66 µg fruit.
araça = *Psidium cattleianum*; AUC = area under the curve; blackberry = *Rubus* sp.; butia = *Butia eriospatha*; DPPH = 2,2-diphenyl-2-picrylhydrazyl hydrate; FeSO$_4$.7H$_2$O = iron(II) sulfate heptahydrate; FRAP = ferric-reducing antioxidant power; fw = fresh weight; IC$_{50}$ = 50% inhibitory concentration; pitanga = *Eugenia uniflora*; TAR = total antioxidant reactivity; TRAP = total reactive antioxidant potential.

fleshed pitanga; however, butiá had the lowest FRAP value (Table 3).

The total phenolic content of fruit extracts was negatively correlated with the IC$_{50}$ value for the DPPH antioxidant assay ($r^2 = -0.758$; $p < 0.05$) and positively correlated with the FRAP value ($r^2 = 0.773$; $p < 0.05$). In addition, a significant positive correlation was found between the ascorbic acid content of fruits and the IC$_{50}$ value for the DPPH antioxidant assay ($r^2 = 0.918$; $p < 0.05$) and a negative correlation was found between ascorbic acid content and the FRAP value ($r^2 = -0.718$; $p < 0.05$). These correlations between bioactive compounds and the antioxidant activity of extracts suggests that phenolic compounds are primarily responsible for the antioxidant activity in the DPPH and FRAP assays. Ascorbic acid may not be important.

Several authors [9,34] demonstrated a strong positive correlation between the total phenolic content and the antioxidant capacity of fruits such as pitanga [35] and blackberries [29]. According to Bagetti et al [35] the ferric-reducing power and the DPPH radical scavenging capacity were higher for extracts from purple-fleshed pitanga than from the red- and orange-fleshed fruits. Furthermore, it was recently found that araçá has high antioxidant activity, which is possibly attributable to the synergism of their phenolic compounds, and that the antioxidant activity varies in a concentration-dependent manner in red and yellow fruits of same species [22].

The ability of species to scavenge reactive oxygen depends on the type of antioxidant. Fruits contain many different antioxidant components [36], and their relative quantities may also vary and thereby affect the total antioxidant capacity of fruits. The antioxidant activity of polyphenols occurs by different mechanisms. The most important is the sequestration of free radicals, which depends on the structure of the compound involved. The intensity of the antioxidant activity exhibited by these phytochemicals differs, primarily because of the number and position of hydroxyl groups in the molecule [37]. These facts may explain the higher antioxidant activity observed in fruits with red and purple flesh, which had higher phenolic content and a predominance of flavonoids and anthocyanins such as cyanidin and quercetin derivatives. However, araçá, a yellow-fleshed fruit, also showed strong antioxidant activity, which can be explained by the presence of several phenolic compounds that could not be identified in the HPLC chromatogram, and by the presence of flavonoid derivatives such as quercetin and Quercetin-3-glucoside.

The TRAP was determined using a method that is based on quenching Luminol-enhanced chemiluminescence derived from the thermolysis of a water-soluble azo compound, AAPH, used as a reliable and quantifiable source of alkyl peroxyl radicals [19]. This widely used assay has proven to be a simple, sensitive, and reproducible method that can be used to determine the antioxidant capacity in complex mixtures such as plant extracts [38]. The TRAP measurement is an index of the nonantioxidant capacity and indicates the quantity of antioxidants present in the plant extract, whereas TAR indicates the quality (given by the reactivity) in those extracts with antioxidant activity. In this study, we observed the highest nonenzymatic antioxidant potential, based on a decreased AUC in the TRAP assay, for the purple-fleshed pitanga, followed by the Cherokee blackberry and butiá. By contrast, the other fruits had the lowest antioxidant potential (Table 3). However, the TAR was higher for the red- and orange-fleshed pitanga, followed by Xavante blackberry, butiá, araçá, Cherokee blackberry, and purple-fleshed pitanga (Table 3). No significant correlation was found between the total phenolic content and the antioxidant activity assessed by the TRAP or TAR assays.

We found that the purple-fleshed pitanga had a lower quality (i.e., reactivity) of these antioxidants based on the TAR assay, compared to the other fruits studied. This is despite its higher content of phenolic compounds, higher antioxidant activity in the DPPH and FRAP assays, and the greater amount of antioxidants observed by TRAP method. Furthermore, the red- and orange-fleshed pitanga, which showed intermediate phenolic content and antioxidant activity in the DPPH and FRAP assays, showed a higher TAR value. This finding indicated the greater reactivity of phenolic compounds in these fruits in comparison to the other fruits, regardless of their quantity.

These differences can be the result of efficient and inefficient antioxidants in the extracts [37]. All extracts studied actively reduced Luminol-enhanced chemiluminescence,

which indicated the presence of compounds with peroxyl scavenging properties. However, the amount of total phenolic compounds was not directly associated with the peroxyl scavenging property, and the possible beneficial effects of these fruits was probably because different bioactive compounds can act in synergism or antagonism.

The antioxidant activity of a specific phenolic compound is associated with the number of available hydroxyl groups in the chemical structure and depends on their donor-proton capacity [37]. Therefore, the manner these compounds neutralize free radicals will depend on their relative concentrations in the sample matrix. In addition, phenolic compounds can act synergistically, additively, or antagonistically to inhibit reactive species.

The efficiency of flavonoids (e.g., flavonols, and isoflavones), which have a diphenylpropane skeleton, as free radical scavengers seems to depend primarily on the number of hydroxyl groups and the position of these groups on the molecule. The antioxidant potency is associated with the structure, based on electron delocalization of the aromatic nucleus. In addition, glycosylation of the molecule (e.g., rutin) may decrease its antioxidant activity. In general, flavonoid structural arrangements impart the greatest antioxidant activity [37]. Thus, quercetin satisfies all of the aforementioned determinants and is a more effective antioxidant.

The antioxidant activity of phenolic acids (e.g., hydroxybenzoic acid, hydroxyphenylacetic acid, and hydroxycinnamic acid) and their ester derivates depends on the number of hydroxyl groups in the molecule that are affected by steric hindrance from their carboxylate group [37]. The closeness of the carboxylate group and the hydroxyl groups on the phenolic ring in hydroxybenzoic acids negatively affects their donor–proton ability. As a result, higher antioxidant activities are usually observed on hydroxycinnamic acids (i.e., coumaric acid, caffeic acid, and ferulic acid), compared to their hydroxybenzoic counterparts [37].

Thus, the fruits with the highest content of phenolics (purple-fleshed pitanga, blackberries, and araçá) and fruits that had quercetin derivatives and cyanidin derivatives (which possess high antioxidant activity, as mentioned previously) also had the highest antioxidant activity in the DPPH and FRAP assays since these phenolic compounds may differentially contribute to the antioxidant capacity in these fruits.

4. Conclusion

The results of the current study revealed that the fruits with the highest content of phenolics (i.e., purple-fleshed pitanga, blackberries, and araçá) also had the highest antioxidant activity in the DPPH and FRAP assays. Furthermore, we observed that butiá had the highest content of ascorbic acid, although its antioxidant activity in the DPPH and FRAP assays was the lowest. In the TRAP assay, purple-fleshed pitanga, Cherokee blackberry, and butiá showed the highest capacity to scavenge the peroxyl radical. However, the amount of total phenolic compounds was not directly associated with the peroxyl scavenging property. This finding is probably because different bioactive compounds can provide increase efficiency

or inefficiency in the antioxidant response, as observed in TRAP and TAR assays. Several phenolic compounds were identified in fruits such as gallic acid derivatives, quercetin derivatives, quercitrin, isoquercitrin, and cyanidin derivatives, which may differentially contribute to the antioxidant capacity. These data reinforce the importance of a regular fruit intake to provide antioxidant polyphenols in the human diet and indicate that purple-fleshed pitanga, blackberries, and araçá have a great antioxidant potential. However, more studies are necessary to identify and quantify all phenolic compounds present in these fruits and determine the contribution of the major compounds to the antioxidant activity.

Conflicts of interest

None of the authors have any conflicts of interest to declare.

Acknowledgments

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