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Original

# Physicochemical characterization and antioxidant capacity of pitanga fruits (*Eugenia uniflora* L.)

*Caracterização físico-química e capacidade antioxidante de pitangas (Eugenia uniflora L.)*

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**Abstract**

This study was carried out to obtain more information about the physicochemical properties, composition, and antioxidant activity of pitanga fruits (*Eugenia uniflora* L.), particularly fruits from the State of Rio Grande do Sul, Brazil. Pitanga with different flesh colors (purple, red, and orange) from tree selections cultivated at Embrapa Clima Temperado (RS-Brazil) were analyzed. Only slight differences were observed in the quality parameters and in the proximate and fatty acid compositions among the fruits studied. The extracts from purple-fleshed pitanga had the highest total phenolic and anthocyanin contents along with the highest antioxidant capacity. The antioxidant capacity (DPPH and FRAP assays) of methanolic pitanga extracts was highly correlated with the total phenolic content, but in ethanolic extracts, the anthocyanin content was correlated only with the FRAP antioxidant capacity. Orange fleshed pitanga had higher β-cryptoxanthin and β-carotene levels than those of the red fruit, which had higher lycopene content. The results indicate that the purple-fleshed pitanga, cultivated in Rio Grande do Sul, is a rich source of phenolic compounds and has high antioxidant capacity. The red and orange-fleshed pitanga, on the other hand, are rich sources of carotenoids.

***Keywords:*** *sugars; insaturated fatty acids; β-cryptoxanthin; lycopene; β-carotene.*

**Resumo**

Este estudo foi realizado para obter mais informações sobre as propriedades físico-químicas, composição e atividade antioxidante de frutos de pitanga (*Eugenia uniflora* L.), especialmente os do Rio Grande do Sul (Brasil). Foram comparadas pitangas com diferentes colorações de polpa (roxa, vermelha e laranja) de seleções cultivadas na Embrapa Clima Temperado (RS-Brasil). Foram observadas pequenas diferenças nos parâmetros de qualidade e na composição centesimal e de ácidos graxos entre as frutas com diferentes colorações de polpa. Os extratos de pitanga roxa apresentaram maiores conteúdos totais de fenólicos e de antocianinas, bem como, a maior capacidade antioxidante. A capacidade antioxidante (valores de DPPH e FRAP) dos extratos metanólicos de pitanga apresentou alta correlação com o conteúdo de fenólicos totais, mas nos extratos etanólicos, o conteúdo de antocianinas correlacionou-se apenas com a capacidade antioxidante avaliada pelo método de FRAP. A pitanga de cor laranja apresentou maiores teores de β-criptoxantina e β-caroteno, enquanto que a de cor vermelha continha alto teor de licopeno. Os resultados indicam que a pitanga de cor roxa, cultivada no Rio Grande do Sul, é uma fonte rica de compostos fenólicos e possui alta capacidade antioxidante. As de cor vermelha e laranja, por outro lado, são fontes ricas de carotenoides.

***Palavras-chave:*** *açúcares; ácidos graxos insaturados; β-criptoxantina; licopeno; β-caroteno.*

## 1 Introduction

It is widely known, from epidemiological studies, that the consumption of fruits and vegetables imparts many health benefits, especially reduced risk of chronic diseases, such as cancer, cardiovascular disease, and stroke (BLOCK; PATTERSON; SUBAR, 1992; DILLARD; GERMAN, 2000; PRIOR; CAO, 2000; KAUR; KAPOOR, 2001). Fruits and vegetables contain different antioxidant compounds, such as vitamin C, vitamin E, and carotenoids. These phytochemicals may protect the human body against reactive oxygen and nitrogen species (DIPLOCK et al., 1998). Reactive oxygen species (ROS) are produced naturally in mammalian systems as a result of oxidative metabolism. However, excessive ROS production may damage cell membranes and DNA causing cancerous mutations. Moreover, the oxidation of low-density lipoprotein is a major factor in the pathogenesis of heart disease (RAHMAN; ADCOCK, 2006).

Vitamins and carotenoids are not the sole compounds that contribute to the antioxidant activity of fruits and vegetables.

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Polyphenolic compounds such as flavonoids also contribute to the beneficial effects of this group of foods (BORS et al., 1990). Polyphenolic compounds have shown antiallergenic, antiviral, antibacterial, antifungal, antitumor, and antihemorragic activities (PIETTA, 2000).

*Eugenia uniflora* L. is a widely distributed tree in South American countries, mainly in Brazil, Argentina, Uruguay, and Paraguay (CONSOLINI; SARUBBIO, 2002). Its leaves are used in popular medicine as infusion in the treatment of fever, rheumatism, stomach diseases, disorders of the digestive tract, hypertension, yellow fever, and gout. It may also reduce weight, blood pressure, and serve as a diuretic (ADEBAJO; OLOKI; ALADESANMI, 1989). Pitanga fruits, also known as Brazilian cherry or Suriname cherry, contain various volatile compounds that are also found in the essential oil of pitanga leaves (WEYERSTAHL et al., 1988; OLIVEIRA et al., 2006). Like the leaves, pitanga fruits may also have health benefits. In the Brazilian food industry, pitanga fruits have mostly been used to produce juice and frozen pulp. Pulp production has high economic potential because the product has consumer appeal and high concentrations of antioxidant compounds, such as anthocyanins, flavonols, and carotenoids (LIMA; MÉLO; LIMA, 2002).

Carotenoids are known to have various biological functions, such as vitamin A activity and prevention of cataract, age-related macular degeneration, cancer, and cardiovascular diseases (KRIS-ETHERTON et al., 2002; TAPIERO; TOWNSEND; TEW, 2004; KRINSKY; JOHNSON, 2005; STAHL; SIES, 2005). However, the carotenoid composition of pitanga fruits is variable. Lycopene is the major carotenoid in pitanga fruits cultivated in the following states: São Paulo, Pernambuco, and Paraná (Brazil), but other carotenoids have been found in different proportions depending on the geographic origin of the fruits (CAVALCANTE; RODRIGUEZ-AMAYA, 1992; AZEVEDO-MELEIRO; RODRIGUEZ-AMAYA, 2004; PORCU; RODRIGUEZ-AMAYA, 2008). Climate was found to influence the carotenoid composition of this fruit. The mean lycopene content of ripe pitanga fruits from Pernambuco (73 $\mu g.g^{-1}$) was slightly higher than that of Campinas, São Paulo (71 $\mu g.g^{-1}$), which was much higher than that of Medianeira, Paraná (14 $\mu g.g^{-1}$). As for β-crytoxanthin and rubixanthin, the levels were much higher in fruits from Pernambuco (47 and 23 $\mu g.g^{-1}$, respectively) than those from São Paulo (12 and 9 $\mu g.g^{-1}$, respectively) and Paraná (13 and 12 $\mu g.g^{-1}$, respectively). In addition to carotenoids, environmental factors can influence other components of fruits such as the phenolics (ROBARDS; ANTOLOVICH, 1997; AHERNE; O'BRIEN, 2002). However, these data are lacking for pitanga fruits, and no data have been found for pitanga from the Southern region of Brazil.

Knowledge of the proximate composition and the contents of bioactive compounds in different fruit varieties may be useful for genetic improvement programs to select the varieties with higher nutritional value. Thus, the objective of this work was to evaluate the physicochemical characteristics of pitanga fruits produced in the State of Rio Grande do Sul and to determine the antioxidant capacity of flesh extracts. We compared pitanga fruits with different flesh colors (purple, red, and orange). The fruits evaluated were from tree selections cultivated at Embrapa Clima Temperado (RS-Brazil) and have been studied to yield cultivars adapted to the Southern region of Brazil.

## 2 Materials and methods

### *2.1 Samples*

Samples of purple, red and orange-fleshed breeding lines of pitanga fruits (*Eugenia uniflora* L.) were harvested at Embrapa Clima Temperado (Rio Grande of Sul, Brazil) in the years 2007 and 2008. Each sample was a mixture of completely ripe fruits from various plant selections with the same flesh color. Three independent lots were collected, frozen at -18 °C, and transported to the Federal University of Santa Maria. Fruits were thawed and the flesh (edible portions) was manually separated from seeds and homogenized in a blender. The samples were immediately analyzed for the carotenoids composition or stored at -18 °C until required for other assays.

### *2.2 Determination of quality parameters*

The parameters of quality evaluated were pH, total soluble solids, and acidity. These parameters were evaluated according to AOAC (ASSOCIATION..., 1995).

### *2.3 Determination of proximate composition*

Except for fat, the analyses were carried out according to AOAC (ASSOCIATION..., 1995). Moisture was determined as the weight loss after 24 hours at 60 °C in a vacuum oven (method 925.09/17). Ash content was determined at 550 °C (method 923.03). Protein content (N × 6.25) was determined by the microkjeldahl procedure (method 960.2). Fat was extracted using chloroform and methanol as described by Bligh and Dyer (1959); the extract was used for the determination of the fat content and fatty acid profile. To prevent lipid oxidation during and after extraction, 0.02% butyl hydroxyl toluene was added to the chloroform solution used.

### *2.4 Determination of fatty acid composition*

Aliquots (2-3 mL) of the chloroform-lipid extract were evaporated at 50 °C using a vaccum pump. Fat was saponified and methylated with methanolic sulfuric acid solution, as described by Hartman and Lago (1973). The methylated samples were analyzed using an Agilent Technologies (HP 6890) gas chromatograph with flame ionization detector. The methylated fatty acids were separated in a capillary column DB-23 (50% cyanopropyl-methylpolysiloxane; 60 m × 0.25 mm × 0.25 μm; Agilent Technologies). The oven temperature was held at 140 °C for 5 minutes, increased to 240 °C at a rate of 4 °C/minute, and held at the latter temperature for 5 minutes. The injector port and detector temperature were adjusted at 250 °C. The samples (1 μL) were injected in a split mode (split ratio 1:50). Nitrogen was used as carrier gas at a flow rate of 0.6 mL/minute.

### *2.5 Determination of phenolic content*

The extraction of phenolic compounds was performed using the method of Escarpa and González (2001) with some modifications, as described by Pellegrini et al. (2007). This method allows a quantitative extraction of the main polyphenolic classes: hydroxybenzoic acids, hydroxycinnamic acids, and flavonoids. The homogenized sample (4 g) was extracted in an ultrasonic bath at room temperature in the absence of light with an aqueous solution consisting of 800 mL methanol and 50 mL formic acid per liter. The samples were sequentially extracted with 6 mL of solvent for 1 hour and 6 mL for 30 minutes and 3 mL for 30 minutes. After each extraction, the extracts were filtered under vacuum. The combined filtrate was brought to a final volume of 25 mL with the solvent and stored at -18 °C until required for analysis.

Total phenolic content was determined using the method of Singleton and Rossi (1965). An aliquot of 0.1 mL pulp extract was mixed with 2.5 mL 0.25 N Folin-Ciocalteu reagent. After 5 minutes, 2 mL 1 N $Na_2CO_3$ was added. The absorbance was determined at 740 nm after 1 hour in the dark. Gallic acid was used as a standard for the calibration curve. The amount of total phenolic compounds was calculated and expressed as mg gallic acid. 100 $g^{-1}$ sample.

### *2.6 Determination of anthocyanin content*

The extraction of anthocyanins was performed as described by Lees and Francis (1972). The pulp was homogenized in the extracting solvent containing 95% ethanol and 1.5 N HCl 85:15 v/v. The proportion sample/extracting solvent was 0.8 $g.mL^{-1}$. The sample was stored for 12 hours at 4 °C, filtered under vacuum, and the residue was exhaustingly washed with the extracting solvent for complete removal of pigments. The filtrates were collected in a volumetric flask, brought to 50 mL with the extracting solvent, and left to stand in the absence of light for 2 hours at room temperature; absorbance was measured at 535 nm.

### *2.7 Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay*

A solution of DPPH was used for the determination of the antioxidant activity of extracts according to Brand-Williams, Cuvelier and Berset (1995). DPPH solution was previously diluted until 1.10 ± 0.02 absorbance at 515 nm was obtained. The extract (0.05 mL) was mixed with 1.9 mL diluted methanolic DPPH solution. The antiradical power of the different extracts was determined by measuring the decrease of DPPH absorbance after 24 hours in the dark against a blank. Trolox was used as a standard for the calibration curve and the results were expressed as mmol trolox equivalents. 100 $g^{-1}$ sample.

### *2.8 Ferric-reducing antioxidant power (FRAP) assay*

The method of Benzie and Strain (1996) was used for the FRAP assays. Ferric-2,4,6-trypyridyl-s-triazine (TPTZ) solution was prepared by mixing 2.5 mL 10 mM TPTZ solution in 40 mM HCl, 2.5 mL 20 mM $FeCl_3.6H_2O$ and 25 mL 0.3 M acetate buffer at pH 3.6. The sample (40 µL) was mixed with 1.2 mL of ferric-TPTZ reagent and incubated at 37 °C for 15 minutes. The absorbance of the colored complex formed with $Fe^{+2}$ and TPTZ was taken at 593 nm. Trolox was used as a standard for the calibration curve and the results were expressed as mmol trolox equivalents.100 $g^{-1}$ sample.

### *2.9 Carotenoid analysis*

Carotenoid analyses were performed at the Carotenoid Laboratory of the University of Campinas-UNICAMP. Carotenoids were extracted with cold acetone, partitioned to petroleum ether:ethyl ether (2:1), saponified overnight with 10% KOH in methanol with 0.1% butyl hydroxy toluene, washed with water, and concentrated in a rotary evaporator (RODRIGUEZ-AMAYA, 1999). Saponification was necessary to hydrolyze carotenoid esters. HPLC–DAD analyses were carried out on a Waters separation module (model 2690) equipped with a quaternary pump, a four-channel in-line vacuum degasser, and an autosampler injector, controlled by Millenium 2010 workstation. A monomeric C18 Spherisorb ODS 2, 3 µm, 4.6 i.d. × 150 mm column was used for all samples. After drying the extract with nitrogen gas, the carotenoids were dissolved in 2 mL acetone, filtered through a 0.22 µm PTFE syringe filter (Millipore), and 10 µL were injected. The mobile phase consisted of acetonitrile (containing 0.05% triethylamine), methanol, and ethyl acetate. A concave gradient was employed, from 95:5:0 to 60:20:20 in 20 minutes, maintaining the last proportion until the end of the run. Reequilibration took 15 minutes (time of set up), and the flow rate was 0.5 mL/minute. For quantification, calibration curves were constructed for β-cryptoxanthin, β-carotene and lycopene with five concentration levels, each in triplicate. The carotenoid quantification was performed by the comparison of the peak area of the sample with that of the standard, injected daily. The identification of carotenoids was performed according to Rodriguez-Amaya (1999) by the combined use of chromatographic behavior, UV-visible spectra obtained with a photodiode array detector, and co-chromatography with authentic carotenoid standards. The results were expressed as $\mu g.g^{-1}$ fresh sample.

### *2.10 Statistical analysis*

All measurements were carried out in triplicate. The results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey´s test when appropriate. Carotenoid data were analyzed by the Student´s *T* test. The results were considered significant when $p < 0.05$. Statistical analyses were carried out using Statistica 6.0 (Copyright Sta Soft, Inc 1984-2001).

## 3 Results and discussion

The quality parameters of purple, red, and orange-fleshed pitanga fruits are shown in Table 1. Purple-fleshed pitanga had higher pH, total soluble solids, and acidity than those of red and orange-fleshed fruits ($p < 0.05$). Orange and red-fleshed pitanga had similar pH and total soluble solids, but the red-fleshed fruit had higher acidity than that of the orange-fleshed pitanga ($p < 0.05$). The pH of red-fleshed pitanga is close to that

**Table 1.** Quality parameters of purple, red, and orange-fleshed pitanga (*Eugenia uniflora* L.).

| Samples | pH | TSS (°Brix ) | Acidity (% citric acid) | Yield (%) |
|---|---|---|---|---|
| Legal limits (Brazil) | 2.5 - 3.4 | > 6.0 | > 0.92 | - |
| Purple | 3.38 ± 0.02[a] | 13.8 ± 0.2[a] | 1.87 ± 0.09[a] | 74.4 |
| Red | 2.88 ± 0.06[b] | 11.5 ± 0.0[b] | 1.67 ± 0.01[b] | 76.8 |
| Orange | 3.01 ± 0.11[b] | 11.8 ± 0.2[b] | 1.63 ± 0.02[c] | 77.2 |

Results are means ± standard deviations (n = 3). Means with different letters within the same column are statistically different ($p < 0.05$). TSS: total soluble solids.

previously reported for commercial frozen pulp of pitanga (2.89) (SALGADO; GUERRA; MELO FILHO, 1999). However, the total soluble solids content obtained in the present study is higher than that found for frozen pulp (SALGADO; GUERRA; MELO FILHO, 1999). The quality characteristics of the purple, red, and orange pitanga fruits analyzed are within the legal limits established for frozen fruit pulp in Brazil (BRASIL, 2000) indicating that the maturation stage of the pitanga fruits evaluated was suitable for processing into frozen pulp.

The moisture content varied significantly among the pitanga samples with different flesh color: orange > red > purple (Table 2). Although the moisture content of all samples was lower than previously reported for pitanga (88%) (UNIVERSIDADE..., 2006) and for commercial frozen pitanga pulps (90.5%) (SALGADO; GUERRA; MELO FILHO, 1999), it is still considered high. This characteristic is common among fruits from the Myrtaceae family, which are classified as succulent (GEMTCHÜJNICOV, 1976).

Purple-fleshed pitanga had the highest ash content followed by orange and red- fleshed pitanga ($p < 0.05$). The ash content of all samples was higher than previously reported for pitanga (0.4%) (UNIVERSIDADE..., 2006), which demonstrates the good concentration of minerals in the samples analyzed. No significant difference was observed in the protein, fat, and carbohydrate contents, which were higher than previously reported for pitanga (0.9, 0.2 and 10.2%, respectively) (UNIVERSIDADE..., 2006). As observed for other fruits, these data show that carbohydrates were the major contributors to the caloric value of pitanga with minor contribution from protein and fat.

The predominant fatty acids in all pitanga samples were palmitic (C16:0), followed by oleic (C18:1n9c) and linoleic acids (C18:2n6) (Table 3). No studies were found on the fatty acid composition of pitanga or other fruits from the Myrtaceae family.

Pitanga fruits had a high proportion of unsaturated fatty acids (49-56%), 20-25% monounsaturated and 29-32% polyunsaturated fatty acids. No significant difference was found in palmitic and linolenic (C18:3n3) acids among the samples (Table 3). Oleic acid was found at a higher proportion in purple pitanga, followed by red and orange pitangas, whereas palmitoleic acid (C16:1n7c) was higher in orange and red fruits than in purple pitanga. Linoleic acid was found at a higher proportion in purple and red fruits than in orange pitanga.

The phenolic compounds influence the fruit quality contributing both to their sensory and health-promoting properties (SCALZO et al., 2005). The phenolic content and antioxidant capacity of the extracts of the three fruits are shown in Table 4. The methanolic extract from purple-fleshed pitanga had higher phenolic content than those of the red and orange-fleshed fruits ($p < 0.05$; Table 4), possibly due to the presence of anthocyanins. According to Reynerston et al. (2008), who analyzed and quantified several antioxidants and anti-inflammatory flavonols, phenolic acids, and anthocyanins from 14 underutilized tropical Myrtaceae fruits, the anthocyanins are the most abundant compounds among those quantified and are responsible for the bright color of those fruits.

The phenolic content of purple fleshed pitanga was higher than previously reported for purple (325 mg catechin.100 $g^{-1}$ f.w.) and red (257 mg catechin.100 $g^{-1}$ f.w.) mature pitanga from Pernambuco (LIMA; MÉLO; LIMA, 2002) and for cambuci (*Campomanesia phea Berg.*), which also belongs to the Myrtaceae family (246 mg gallic acid.100 $g^{-1}$ f.w.) (GENOVESE et al., 2008). Although red and orange-fleshed pitanga had lower phenolic content when compared to the purple samples, its phenolic content is higher than that of araçá (*Psidium guineensis* Sw.), which is also a Myrtaceae fruit (129 mg gallic acid.100 $g^{-1}$ f.w.) (GENOVESE et al., 2008). Moreover, the phenolic content of pitanga fruits was higher than that of mulberry, grape, and açaí pulps (119, 117 and 137 mg gallic acid.100 $g^{-1}$ f.w., respectively) (KUSKOSKI et al., 2006). However, the phenolic content of pitanga fruits was 5 to 10 fold lower than that of pitanga seeds (BAGETTI et al., 2009).

DPPH and FRAP assays are indicated as simple and rapid methods for assessing the antioxidant capacity of fruits and vegetables. The antioxidant capacity of pitanga fruits, determined by the FRAP and DPPH assays, was expressed as equivalents of the standard antioxidant trolox, which is a hydrosoluble analog of vitamin E. Both the ferric-reducing power and the DPPH radical scavenging capacity were higher for the methanolic extracts from purple fleshed color pitanga than for the red and orange fruits ($p < 0.05$) (Table 4). Several authors demonstrated a strong positive correlation between phenolic content and the antioxidant capacity of fruits (VISON et al., 1998; KAUR; KAPPOR, 2001; ABIDILLE et al., 2005; PINTO; LAJOLO; GENOVESE, 2007). We also found a highly positive correlation between the content of phenolics and DPPH ($r^2 = 0.987$; $p < 0.05$) and FRAP ($r^2 = 0.983$; $p < 0.05$) values. This suggests that phenolics are the major responsible

**Table 2.** Proximate composition (%) of purple, red, and orange-fleshed pitanga (*Eugenia uniflora* L.).

| Samples | Moisture | Ash | Protein | Fat | Carbohydrate* |
|---|---|---|---|---|---|
| Purple | 81.2 ± 0.0[c] | 2.4 ± 0.1[a] | 1.2 ± 0.5[a] | 0.4 ± 0.0[a] | 14.8 ± 0.4[a] |
| Red | 83.9 ± 0.0[b] | 1.1 ± 0.0[c] | 1.4 ± 0.0[a] | 0.4 ± 0.0[a] | 13.2 ± 0.0[a] |
| Orange | 84.7 ± 0.2[a] | 1.7 ± 0.8[b] | 1.1 ± 0.0[a] | 0.5 ± 0.0[a] | 12.9 ± 1.1[a] |

Results are means ± standard deviations (n = 3). Means with different letters within the same column are statistically different ($p < 0.05$). *Calculated by difference.

**Table 3.** Fatty acid composition (% of total fatty acids) of purple, red, and orange-fleshed pitanga (*Eugenia uniflora* L.).

| Fatty acids | Purple | Red | Orange |
|---|---|---|---|
| C16:0 | 34.6 ± 1.0[a] | 33.4 ± 0.2[a] | 34.7 ± 0.2[a] |
| C16:1n7c | 2.7 ±0.1[b] | 3.2 ± 0.1[a] | 3.0 ± 0.1[a] |
| C18:1n9c | 22.4 ± 0.1[a] | 21.1 ± 0.2[b] | 17.6 ± 0.2[c] |
| C18:2n6c | 18.9 ± 0.2[a] | 18.8 ± 0.4[a] | 17.0 ± 0.3[b] |
| C18:3n3 | 12.8 ± 0.6[a] | 13.1 ± 0.2[a] | 12.4 ± 0.3[a] |
| NI | 8.6 ± 0.6 | 7.8 ± 0.7 | 7.9 ± 0.1 |

Results are means ± standard deviations (n = 3). Means with different letters within the same row are statistically different ($p < 0.05$). C12:0, C14:0, C14:1n5, C18:0, C18:1n9t, C18:2n6t, C20:1n9, C20:4n6, C20:5n3, C22:0, C22:5n3 and C22:6n3 were not detected. NI: unidentified compounds.

**Table 4.** Phenolic content and antioxidant capacity of methanolic extracts from purple, red, and orange-fleshed pitanga (*Eugenia uniflora* L.).

| Samples | Phenolic content (mg gallic acid.100 $g^{-1}$) | DPPH (mmol trolox.100 $g^{-1}$ ) | FRAP (mmol trolox.100 $g^{-1}$) |
|---|---|---|---|
| Purple | 463 ± 16[a] | 3.1 ± 0.7[a] | 3.1 ± 0.6[a] |
| Red | 210 ± 3[b] | 1.4 ± 0.1[b] | 1.4 ± 0.3[b] |
| Orange | 179 ± 5[b] | 1.4 ± 0.0[b] | 1.1 ± 0.1[b] |

Results are expressed as gallic acid or trolox equivalents per 100 g of fresh pulp used to prepare the extract and are the means ± standard deviations (n = 3); DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: ferric reducing antioxidant power. Different letters within the same column indicate significant differences ($p < 0.05$).

compounds for the antioxidant capacity of the methanolic extracts from pitanga samples.

Among phenolic compounds, the anthocyanin content has been suggested as an important criterion for predicting a high antioxidant activity of fruits since anthocyanin-rich samples usually show the highest antioxidant capacity (HASSIMOTTO; GENOVESE; LAJOLO, 2005). Therefore, we extracted anthocyanins from pitanga pulp using an ethanolic solution. The results of anthocyanin content and antioxidant capacity of these extracts are shown in Table 5. As with the phenolic content, the ethanolic extract from purple-fleshed pitanga had the highest anthocyanin content followed by the extracts from red and orange samples ($p < 0.05$). The anthocyanin contents of purple and red fleshed pitanga are higher than those of frozen pulps of blackberry (*Morus nigra*) (41.8 mg.100 $g^{-1}$), grape (*Vitis vinifera*) (30.9 mg.100 $g^{-1}$), and açaí fruit (*Euterpe oleracea)* (22.8 mg.100 $g^{-1}$) although lower than those of methanolic (578 mg.100 $g^{-1}$) and ethanolic extracts (596 mg.100 $g^{-1}$) from baguaçu (*Eugenia umbelliflora* Berg.) (KUSKOSKI et al., 2006), which is from the same genus as pitanga.

The DPPH radical scavenging capacity was not different among the ethanolic extracts from pitanga samples. However, the ferric-reducing power of the ethanolic extract from purple-fleshed pitanga was higher than those of the other samples. Moreover, the anthocyanin content had a positive correlation with FRAP values ($r^2 = 0.938$; $p < 0.05$), but showed no relationship with DPPH values (data not shown). The antioxidant capacity (DPPH and FRAP values) of anthocyanin extracts (Table 5) was (2.6 to 29 fold) higher than that of the phenolic extracts (Table 4).

Two possible explanations can be given for the discrepancy noted above. Firstly, the two assays are based on different principles. While the FRAP assay measures the ferric reducing capacity of antioxidants, the DPPH assay measures the ability of antioxidants to scavenge the DPPH radical. Secondly, the conditions used to obtain the anthocyanin extract like the successive washing until complete extraction of pigments or the polarity of the extracting solution might have led to the extraction of more compounds with greater antioxidant capacity. In agreement with this proposal, Beekwilder et al. (2005) found that anthocyanins, ellagitanins, and proanthocyanidins are the major compounds responsible for the antioxidant capacity of raspberry samples.

The results demonstrated that pitanga is a rich source of anthocyanins when compared with other fruits, and that purple pitanga, in general, had the highest antioxidant capacity when compared to the other fleshed color samples.

α-Carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin are the most studied and are considered

**Table 5.** Total anthocyanin content and antioxidant capacity of ethanolic extracts from purple, red, and orange-fleshed pitanga *(Eugenia uniflora* L.).

| Samples | Anthocyanin content (mg.100 $g^{-1}$) | DPPH (mmol trolox.100 $g^{-1}$ ) | FRAP (mmol trolox.100 $g^{-1}$) |
|---|---|---|---|
| Purple | $136 \pm 6^a$ | $37 \pm 2^a$ | $8.2 \pm 0.4^a$ |
| Red | $69 \pm 3^b$ | $41 \pm 0^a$ | $4.4 \pm 0.3^b$ |
| Orange | $25 \pm 1^c$ | $41 \pm 0^a$ | $4.2 \pm 0.4^b$ |

Results are expressed as anthocyanin content or trolox equivalents per 100 g of fresh pulp used to prepare the extract and are the means ± standard deviations (n = 3); DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: ferric reducing antioxidant power. Different letters within the same column indicate significant differences ($p < 0.05$).

**Table 6.** Carotenoid composition (μg.$g^{-1}$) of red and orange-fleshed pitanga (*Eugenia uniflora* L.).

| Samples | β-Cryptoxanthin | Lycopene | β-Carotene |
|---|---|---|---|
| Red | 16 ± 2 | 166 ± 7 | 2.9 ± 0.8 |
| Orange | 34 ± 7* | 151 ± 30 | 5.1 ± 0.8* |

Results are the mean ± standard deviation (n = 3). *Different from red samples (Student's *T* test, $p < 0.05$).

the most important carotenoids in terms of human health (RODRIGUEZ-AMAYA, 1999). Among them, lycopene has remarkably high antioxidant efficiency (DI MASCIO; KAISER; SIES, 1989) and has been suggested to protect humans against degenerative disorders (CLINTON, 1998). The carotenoids found in red and orange pitanga samples in the present study were lycopene, β-cryptoxanthin, and β-carotene (Table 6). Rubixanthin was also detected, but as a minor carotenoid.

The lycopene contents of the red and orange samples analyzed in this work were much higher than those of pitanga samples from São Paulo (71 μg.$g^{-1}$) and Paraná (14 μg.$g^{-1}$) (PORCU; RODRIGUEZ-AMAYA, 2008). Moreover, the pitanga samples from the state of Rio Grande do Sul had higher lycopene content than that of the fruits considered as important sources of lycopene such as watermelon (36 μg.$g^{-1}$) (NIIZU; RODRIGUEZ-AMAYA, 2003), guava (53 μg.$g^{-1}$) (PADULA; RODRIGUEZ-AMAYA, 1986), and papaya (cv. Formosa, 26 μg.$g^{-1}$) (KIMURA; RODRIGUEZ-AMAYA; YOKOYAMA, 1991). Orange-fleshed pitanga had higher β-carotene and β-cryptoxanthin content than red pitanga ($p < 0.05$). The β-carotene and β-cryptoxanthin contents of red pitanga obtained in the present study are similar to those previously reported for red pitanga from São Paulo and Paraná (PORCU; RODRIGUEZ-AMAYA, 2008).

## 4 Conclusions

Only slight differences were observed in the quality parameters and in the proximate and fatty acid compositions among the fruits with different flesh color. Although the red-fleshed pitanga had higher lycopene content, the orange-fleshed pitanga had higher β-cryptoxanthin and β-carotene concentrations than the red fruit. The extracts from purple-fleshed pitanga had the highest total phenolic and anthocyanin content along with the highest antioxidant capacity. The antioxidant capacity determined by the DPPH and FRAP assays of the methanolic pitanga extracts was highly correlated with the total phenolic content, but in ethanolic extracts, the anthocyanin content was correlated only to FRAP antioxidant capacity. The results showed that purple fleshed pitanga cultivated in the Rio Grande do Sul is a rich source of phenolics, whereas the orange and red-fleshed pitanga fruits are rich sources of carotenoids.

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