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Characterization and Antioxidant Potential of Brazilian Fruits from the Myrtaceae Family

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ABSTRACT: The objective of this study is to evaluating the Brazilian biodiversity through physicochemical characterization and determination of antioxidant potential of three species from the Myrtaceae family, namely yellow guava (*Psidium cattleyanum* Sabine), guabiroba (*Campomanesia xanthocarpa* O. Berg), and uvaia (*Eugenia pyriformis* Cambess). Guabiroba had the greater quantity of phenolic compounds (9033 mg chlorogenic acid/100 g) and vitamin C (30.58 mg/g) and showed the best TSS/TTA (total soluble solid/total titratable acid) ratio (45.12). For the ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic) method, the guabiroba (507.49 μM Trolox/g) presented the highest antioxidant potential; however, in the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, uvaia (170.26 g/g DPPH) and guabiroba (161.29 g/g DPPH) were not statistically different. The uvaia outranked the other fruits with respect to its high carotenoid (909.33 μg/g) and vitamin A (37.83 μg/g) contents, and the yellow guava, although showing a lower bioactive compound content and antioxidant activity, nevertheless presented much higher values than many traditionally consumed fruits.

KEYWORDS: *Brazilian fruit, Myrtaceae, chemical composition, antioxidant activity, carotenoids*

INTRODUCTION

Brazil is recognized for the immense biological diversity of its flora and is considered to be one of the main centers of genetic diversity for fruit species in the world. Nevertheless, the greater part of this richness remains underused and its potential unknown. However, according to the Brazilian Institute of Fruits (IBRAF),[1] Brazil is one of the three largest fruit producers in the world, only losing to China and India, producing more than 43 million tons in 2008, which represents 5% of the world production. Although a greater variety of native Brazilian fruit species are found in the Amazon and the savanna, the southern region also shows great richness in wild fruits, among which the botanical family of Myrtaceae stands out for presenting the greatest number of species with food potential, which could be commercialized in natura for use in the manufacture of ice creams, juices, yogurts, liqueurs, desserts, cereal bars, sweetmeats, and jams. Of these fruits, the yellow guava (*Psidium cattleyanum* Sabine), guabiroba (*Campomanesia xanthocarpa* O. Berg), and uvaia (*Eugenia pyriformis* Cambess) are examples usually used in folk medicine and grown in home gardens that have great potential for economic exploration, since they show high productivity with low deployment and maintenance costs.

In a market hungry for novelty and consumers more conscious of the benefits of eating healthy food, such fruits provide greater variety to the diet, providing nutritious foods rich in functional compounds that could act as natural antioxidants, protecting the organism from chronic diseases and from premature aging. According to Ratnam et al.,[2] the human antioxidant defense system is incomplete without the dietetic antioxidants, confirming the importance of ingesting these compounds. Thus the consumption of antioxidants presents various benefits, providing an improvement in the quality of life of the population.

In a review by Steinmetz and Potter[3] involving the data from 206 epidemiological studies, it was shown that an elevated consumption of fruits and vegetables was related to a reduced incidence of certain types of cancer and beneficial effects on cardiovascular diseases, diabetes, obesity, and cataract.

The protective effect exerted by these foods has been attributed to the presence of antioxidant compounds, with emphasis on the vitamins and some special metabolites, such as phenolic compounds and carotenoids. Vitamin C can act by scavenging the oxygen radicals present in the medium by way of chemical reactions, consequently making them unavailable to act as a propagator of auto-oxidation. In addition, it has a high vitamin E regenerating capacity.[4]

Phenolic compounds act by neutralizing and sequestering free radicals and also chelatimg transition metals. The intermediate compounds formed by the action of the phenolic antioxidants are relatively stable due to charge distribution throughout the aromatic ring system. The antioxidant capacity of these compounds is attributed to the reducing power of the aromatic hydroxyl group, which reduces the reactive free radicals.[5]

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The mechanism by which carotenoids protect biological systems from free radicals depends on the transfer of energy from the excited oxygen to the carotenoid molecule. They react mainly with the peroxide radical and molecular oxygen. Carotenoids such as β-carotene and lycopene exert antioxidant functions in lipid phases, blocking the free radicals that damage the lipoprotein membranes.[6] Over and above the many functions of carotenoids, their provitamin A value is the most important. The lack of vitamin A in a diet can cause xerophtalmy, ceratomalace, blindness, and death.[7]

With the objective of spreading information that stimulates valorization of the Brazilian biodiversity and also of determining the potential of alternative fruits that provide health benefits, this work determined the chemical composition, antioxidant potential, total phenolic compounds, vitamin C content, and the carotenoid content and profile of three species of the Myrtaceae family native to the south of Brazil.

MATERIAL AND METHODS

Raw Material. The fruits used were the yellow guava (*P. cattleyanum* Sabine), guabiroba (*C. xanthocarpa* O. Berg), and uvaia (*E. pyriformis* Cambess), all obtained from the active germplasm bank of native fruit trees of Embrapa Temperate Climate (Pelotas/RS). All the samples were collected when fully mature, in two batches each containing about 3 kg of fruits. The the fruits were harvested in 2010, with yellow guava harvested between the months of February and April, guabiroba between November and December, and uvaia between February and March. The fruits were preselected considering the absence of visible injury and infections and also color and size uniformity and were stored frozen (−20 °C) until analyzed. For all the analyses, only the normally edible parts were used, that is, for the yellow guava and guabiroba, the skin, pulp, and seeds were used, and for uvaia, only the pulp and skin. All the fruits were analyzed fresh for the determination of antioxidant activity, but for the remaining analyses, samples stored in the frozen state were used (−20 °C). At the time of analysis, the fruits were thawed at room temperature and homogenized in an Ultra-Turrax homogenizer (Ika, Artur Nogueira, São Paulo, Brazil) to determine the contents of total soluble solids, total titratable acid, protein, sugars, ash, moisture, vitamin C, phenolic compounds, and antioxidant activity. To analyze the lipid and fiber contents, after homogenization the samples were freeze-dried and ground. At least 10 fruits were combined for each of the three replicated samples.

Chemical Composition. All the analyses were carried out according to AOAC procedures.[8] The protein concentration was determined by the Kjeldahl method using a conversion factor of 5.75. The lipid concentration was determined by the Soxhlet extraction method, food fiber (total and insoluble) using the enzymatic-gravimetric method, ash in a muffle furnace controlled at 550 °C, moisture content by gravimetry, the total carbohydrate content by difference, and the reducing and nonreducing sugars by the Eynon−Lane method.

Total titratable acidity (TTA) was determined by titration and the total soluble solids (TSS) using a digital PAL-3 refractometer (Atago Co., Taiwan, China) according to AOAC methods.[8]

Total Phenolic Compounds. To extract these substances, 5 g of sample were homogenized in an Ultra-Turrax homogenizer with 20 mL of methanol, and the homogenate wascentrifuged for 20 min at 25 400*g* in a refrigerated centrifuge at 4 °C. A 250 μL aliquot of the supernatant was diluted in 4 mL of ultrafiltered water and a control also prepared containing 250 μL of methanol. The samples and the control were each combined with 250 μL of 0.25 N Folin−Ciocalteau reagent.[9] After 3 min of reaction, 500 μL of 1 N Na_2CO_3 was added, the mixtures were incubated for 2 h at room temperature, and the absorbance was read at 725 nm in an UV−vis spectrophotometer (Amersham, Modelo UV Vis Ultrospec-3100 Pro Amersham Bioscience)

A standard curve was constructed to quantitate the phenolic compounds, using chlorogenic acid in the concentration range from 0.05 to 0.50 μg/mL.

Antioxidant Activity. Methodologies based on sequestering the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical[10] and the 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic (ABTS)[11] radical were used to determine the antioxidant activity. The extract was obtained from 5 g of sample ground in methanol (50%) and acetone (70%), using three different dilutions (1:5, 1:10, 1:15). The fresh samples were weighed in centrifuge tubes and extracted sequentially with 40 mL of methanol/water (50:50, v/v) at room temperature for 1 h. The tubes were centrifuged at 25 400*g* for 15 min, and the supernatant was recovered. An aliquot of 40 mL of acetone/water (70:30, v/v) was then added to the residue at room temperature, extracted for 60 min, and centrifuged. The methanol and acetone extracts were combined, made up to 100 mL with distilled water, and used to determine the antioxidant capacity.

For the DPPH method, a 0.1 mL aliquot of each dilution of the extract was reacted with 3.9 mL of DPPH radical. The readings were made in a spectrophotometer at 515 nm after 30 min. The results are expressed as the concentration of antioxidant required to reduce the original amount of free radicals by 50% (EC_{50}), and the values are expressed as grams of fruit/gram of DPPH. For the ABTS method, a 30 μL aliquot of each extract dilution was reacted with 3.0 mL of ABTS radical and the reading taken at 734 nm after 6 min. The results are expressed as micromolar Trolox concentration/gram of fruit.

Determination of Vitamin C. The determination of vitamin C was based on the methodology proposed by Rosa et al.[12] with some modifications. Each 5 g sample was ground in an Ultra-Turrax with 20 mL of 0.05 M suprapure 96% sulfuric acid (MERCK, Darmstadt, Germany) for 1 min, centrifuged at 25 400*g* for 15 min and then filtered through a Teflon hydrophilic filter unit.

The analyses were carried out in a high-performance liquid chromatography unit (Agillent, Waldbronn, Germany), equipped with a degasser, quaternary solvent pump, and a UV/vis detector. The column used was a 250 mm × 4.6 mm i.d., 5 μm, C_{18} polymeric column (Vydac, Southborough, MA). The mobile phase was 0.05 M suprapure sulfuric acid at 1.0 mL/min, with an injection volume of 10 μL and wavelength of 254 nm. The vitamin C was quantitated using a standard curve constructed using ascorbic acid in a concentration range from 1 to 0.001 mg/mL.

Carotenoid Profile and Vitamin A Content. The carotenoid extract was prepared according to the method of Mercadante and Rodriguez-Amaya.[13] The main steps were the extraction of the pigments with acetone and saponification with 10% KOH in methanol overnight at room temperature. After removal of the alkali, the extract was concentrated in a rotary evaporator (Fisatom, Uberlândia, Minas Gerais, Brazil) (T < 35 °C), dried in a nitrogen flow, and stored in the freezer for subsequent quantitation by high-performance liquid chromatography.

The column used was a 250 mm × 4.6 mm i.d., 3 μm, C_{30} reversed-phase polymeric column (YMC). The mobile phase was water/methanol/*tert*-methyl butyl ether (MTBE) (J. T. Baker−Mallinckrodt) starting at 5:90:5, reaching 0:95:5 in 12 min, 0:89:11 in 25 min, 0:75:25 in 40 min, and finally 0:50:50 after a total of 60 min, with a flow rate of 1 mL/min at 33 °C.[14]

The carotenoid standards were acquired from Sigma-Aldrich. For quantitation, a standard curve was constructed with β-carotene (5−50 μg/mL), α-carotene (2−25 μg/mL), lutein (1−65 μg/mL), cryptoxanthin (4−100 μg/mL), and zeaxanthin (1−40 μg/mL). The limits of quantitation (LOQ) and detection (LOD) were, respectively, for β-carotene and 9-*cis*-β-carotene, 10.89×10^{-2} mg/kg, 6.53×10^{-2} mg/kg; for lutein, 1.15×10^{-2} mg/kg, 6.9×10^{-3} mg/kg; for cryptoxanthin, 3.51×10^{-2} mg/kg, 2.11×10^{-2} mg/kg; for zeaxanthin, 1.59×10^{-2} mg/kg, 9.56×10^{-2} mg/kg; for α-carotene, 3.28×10^{-2} mg/kg, 1.97×10^{-2} mg/kg; for β-carotene 5,6-epoxide, 7.43×10^{-2} mg/kg, 4.46×10^{-2} mg/kg; and for 13-*cis*-β-carotene, 7.43×10^{-2} mg/kg, 4.46×10^{-2} mg/kg.

The vitamin A activity was calculated by assuming the factor of bioconversion proposed by Guilland et al.[15] This information corresponds

to a factor equivalence of 13 mg of β-carotene:1 mg of retinol. With regard to the structural configurations identified in β-carotene, the equivalence factor was estimated as 53, 38, and 21% of the bioactivity of the provitamin A β-carotene, for 13-*cis*-β-carotene, 9-*cis*-β-carotene, and 5.6-epoxy-β-carotene, respectively.[16]

Statistical Analysis. The results were analyzed by ANOVA and the Tukey means comparison test at a level of 5% of significance, using the software Statistica 10.0.

RESULTS AND DISCUSSION

Proximate Composition. According to the proximate composition (Table 1), the three samples presented significant differences for all the variables analyzed, except for the protein and carbohydrate contents, where the yellow guava and the guabiroba were considered the same. With respect to the carbohydrate composition, these two fruits presented about 3 times the amount found in the uvaia, whereas the inverse occurred for the protein content, with the yellow guava and guabiroba presenting almost 3 times less than that presented by the uvaia.

Table 1. Chemical Composition of Fruits from the Myrtaceae Family Native to the South of Brazil[a]

proximate composition	yellow guava	guabiroba	uvaia
moisture (g/100 g fresh matter)	83.31 ± 0.01 b	82.21 ± 0.19 c	94.50 ± 0.10 a
ash (g/100 g fresh matter)	0.63 ± 0.01 a	0.47 ± 0.01 b	0.23 ± 0.01 c
protein (g/100 g dry matter)	4.24 ± 0.13 b	5.53 ± 0.18 b	15.82 ± 0.54 a
lipid (g/100 g dry matter)	1.53 ± 0.01 b	3.7 ± 0.05 a	0.52 ± 0.03 c
TDF[b] (g/100 g dry matter)	11.95 ± 0.17 a	9.75 ± 0.02 b	3.09 ± 0.08 c
IDF[c] (g/100 g dry matter)	11.55 ± 0.06 a	9.47 ± 0.23 b	3.09 ± 0.08 c
carbohydrate (g/100 g dry matter)	15.08 ± 0.04 a	15.68 ± 0.23 a	4.37 ± 0.12 b
total sugar (g/100 g dry matter)	22.74 ± 0.39 c	34.45 ± 0.50 b	36.72 ± 0.72 a
reducing sugar (g/100 g dry matter)	18.6 ± 0.03 c	34.06 ± 0.78 b	36.54 ± 0.18 a

[a]Values are expressed as the mean ± standard deviation. The same letters in the same row indicate no significant difference at a level of 5%. [b]TDF: total dietary fiber. [c]IDF: insoluble dietary fiber.

In addition to the highest protein content, the uvaia also showed the highest moisture content. Kinupp and Barros[17] found high protein contents in native fruits of the same genera as the uvaia, including araçá-pitanga (*Eugenia multicostata*) and pessegueiro-do-mato (*Eugenia myrcianthes*), with 10.9% and 8%, respectively. Some traditionally consumed fruits, such as passion fruit (*Passiflora edulis f. flavicarpa*) (11.7% protein)[18] also show this characteristic.

With respect to the yellow guava, in addition to the carbohydrate content mentioned above, the ash and fiber contents also stood out, with higher values than those presented by the other two fruits examined, showing almost 3 times the amount detected in the uvaia for both these components. With respect to fiber content, that of the yellow guava can be compared to the value found in mangoes (*Mangifera indica* L.) (9.6%).[18]

The guabiroba stood out for its elevated lipid content as compared with the yellow guava and uvaia, showing 2 and 7 times the amounts found in these fruits, respectively. The result found for the guabiroba was higher than that found in banana (0.3%), papaya (0.8%), and orange (mean of 1.2%) and similar to that found in strawberries (3.5%).[18]

Physicochemical Analyses. According to the results obtained for total soluble solids (TSS) (Table 2), there was a statistically significant difference between the three fruits analyzed, the guabiroba showing the greatest content, representing twice the amount found in the uvaia.

Table 2. Total Soluble Solids (TSS) and Total Titratable Acidity (TTA) in Fruits of the Myrtaceae Family from the South of Brazil[a]

analyses	yellow guava	guabiroba	uvaia
TSS (°Brix)	13.8 ± 0.0 [b]	15.34 ± 0.0 [a]	7.70 ± 0.0 [c]
TTA (% citric acid)	0.88 ± 0.02 [b]	0.34 ± 0.002 [c]	1.17 ± 0.01 [a]
TSS/TTA	15.68 [b]	45.12 [a]	6.58 [b]

[a]Values expressed as the mean ± standard deviation. The same letters in the same row indicate no significant difference at a level of 5%.

The TSS content identified in guabiroba was in the range recommended for fruits destined for processing, certifying a better, more natural flavor for the product, since elevated contents of these constituents in the raw material imply in a reduced addition of sugars, less time to evaporate off the water, less energy expenditure, and a greater product yield, resulting in a more economical process. The TSS content of the yellow guava was also considered high when compared to that of the uvaia, which presented approximately half of the value of the yellow guava.

It can be seen from the results for TTA (Table 2) that all the samples were statistically different from each other. The uvaia showed the most accentuated values for TTA among the fruits tested, with about 3 times the value found in the guabiroba. The yellow guava was classified as second in acidity among the three fruits analyzed.

The TSS/TTA ratio is one of the best ways of evaluating the flavor, being more representative than the isolated measurement of sugars or acidity, providing a good perception of the balance between these two components. Therefore, the ratio found for guabiroba indicated that it was a very sweet, tasty fruit, the opposite of uvaia, which would probably have limited use for consumption in natura. Nevertheless, uvaia could have an immediate marketing potential for the production of frozen, concentrated pulp, due to its abundant, juicy, and aromatic pulp.

Total Phenolic Compounds. With respect to the total phenolic compound contents (Table 3), it was observed that all the fruits were statistically different from each other, guabiroba standing out by presenting the highest levels, more than twice the amounts detected in the yellow guava and uvaia. However, all the fruits showed considerably higher levels than 13 plum cultivars (678 mg equiv chlorogenic acid/100 g dry matter),[19] a value about 13 times lower than that found in the guabiroba.

Antioxidant Activity. According to the ABTS method, the guabiroba stood out for presenting an antioxidant potential 1.5 and 2 times higher than that of the uvaia and yellow guava, respectively. However, using the DPPH method, the uvaia and guabiroba were not statistically different, showing more than twice the antioxidant activity found in yellow guava (Table 3).

The values found in the guabiroba and uvaia fruits also surpassed the values found in açaí (95 μM Trolox/g fruit, 678 g fruit/g DPPH), puçá-coroa-defrade (103 μM Trolox/g fruit,

Table 3. Total Phenolic Compounds (TPC), Antioxidant Activity (DPPH and ABTS methods), and Vitamin C (Vit C) Contents in Fruits of the Myrtaceae Family Native to the South of Brazil[a]

analyses	yellow guava	guabiroba	uvaia
TPC[b]	3713.24 ± 335.98 b	9033.19 ± 1428.3 a	3482.04 ± 74.1 b
ABTS[c]	242.30 ± 4.08 c	507.49 ± 29.17 a	336.29 ± 38.19 b
DPPH (EC_{50})[d]	389.74 ± 7.26 b	161.29 ± 12.09 a	170.26 ± 13.21 a
Vit C[e]	0.3 ± 0.01 b	30.58 ± 3.91 a	0.7 ± 0.37 b

[a]The values are expressed as the mean ± standard deviation. The same letters in the same row indicate no significant difference at the 5% level. [b]Expressed as mg equiv chlorogenic acid/100 g dry matter. [c]Expressed in μM equiv Trolox/g dry matter. [d]Expressed as g dry matter/g DPPH. [e]Expressed as mg ascorbic acid/g dry matter.

Table 4. Carotenoid Compositions of Fruits of the Myrtaceae Family Native to the South of Brazil[a]

peak no.	carotenoids[c]	range of t_R[b] (min)	yellow guava	guabiroba	uvaia
1	lutein	18.03−18.10	26.380 ± 1.41 b	81.91 ± 18.33 b	307.49 ± 68.07 a
2	zeaxanthin	21.07−21.18	3.29 ± 1.29 b	32.45 ± 3.68 a	40.38 ± 6.36 a
3	β-carotene 5,6-epoxide	29−30	1.20 ± 0.10 c	8.49 ± 3.68 b	16.39 ± 2.09 a
4	cryptoxanthin	31.89−32.04	0.95 ± 0.62 b	121.08 ± 10.92 a	159.09 ± 68.27 a
5	13-*cis*-β-carotene	34−35	1.18 ± 0.06 b	5.86 ± 0.09 b	38.30 ± 10.28 a
6	α-carotene	38.40−38.53	4.01 ± 0.2 b	16.64 ± 2.13 b	124.39 ± 36.26 a
7	β-carotene	42.95−43.43	2.95 ± 0.45 b	34.33 ± 2.35 b	191 ± 71.29 a
8	9-*cis*-β-carotene	44	1.25 ± 0.04 b	4.75 ± 2.28 b	32.27 ± 8.27 a
	total		41.22 ± 2.46 b	305.53 ± 22.98 b	909.33 ± 270.89 a

[a]Values expressed as the mean ± standard deviation. The same letters in the same row indicate no significant difference at the 5% level. [b]t_R: retention time. [c]Expressed in g/g dry matter.

476 g fruit/g DPPH), gurguri (140 μM Trolox/g fruit, 350 g fruit/g DPPH), uvaia from Ceará/Brazil (168 μM Trolox/g fruit, 347 g fruit/g DPPH), murta (189 μM Trolox/g fruit, 242 g fruit/g DPPH), and jaboticaba (265 μM Trolox/g fruit, 207 g fruit/g DPPH).[20] However, they showed similar values to that found in puçá-preto (348 μM Trolox/g fruit, 149 g fruit/g DPPH) and lower values than those found in juçara (799 μM Trolox/g fruit, 168 g fruit/g DPPH), West Indian cherry (1073 μM Trolox/g fruit, 60 g fruit/g DPPH), and camu−camu (1500 μM Trolox/g fruit, 49 g fruit/g DPPH).[20]

Among the native species analyzed, the yellow guava came in last place with respect to antioxidant capacity but showed greater values than many other fruits traditionally consumed throughout the world.

The yellow guava fruit showed higher values than carnaúba (36 μMTrolox/g fruit, 1040 g fruit/g DPPH), umbu (52 μM Trolox/g fruit, 856 g fruit/g DPPH), yellow mombim (57 μM Trolox/g fruit, 1278 g fruit/g DPPH), cashew (85 μM Trolox/g fruit, 936 g fruit/g DPPH), mangaba (159 μM Trolox/g fruit, 311 g fruit/g DPPH), and jambolão (197 μM Trolox/g fruit, 457 g fruit/g DPPH).[20]

Vitamin C. The guabiroba had a significantly higher vitamin C content (Table 3), about 100 times the amount found in the yellow guava and 40 times that in the uvaia, whereas the latter two did not differ between themselves. Fruits such as mangaba (21 mg ascorbic acid/g), juçara (19 mg ascorbic acid/g), jaboticaba (17 mg ascorbic acid/g), cashew (14 mg ascorbic acid/g), jambolão (7 mg ascorbic acid/g), murta (7 mg ascorbic acid/g), and murici (4 mg ascorbic acid/g) also showed lower levels.[20]

The vitamin C contents obtained for guabiroba were lower than those found in acerola (151 mg ascorbic acid/g) and camu−camu (184 mg ascorbic acid/g).[20] The uvaia showed a low vitamin C content but nevertheless showed more than twice the amount detected in yellow guava and also surpassed the values found in the starfruit (0.6 mg/g).[21] Uvaia presented a value similar to that of mango (0.7 mg/g).[21]

For its part, despite being considered as a fruit with a high vitamin C content, generally 3−4 times higher than that of citric fruits, in the present study the yellow guava failed to show this potential, although it did show more than found in bacuri (0.09 mg/g).[20] However, there are many factors that can influence the vitamin contents of fruits, including the species, stage of maturity when harvested, genetic variations, post-harvest handling, storage conditions, and processing.

Carotenoid Profile and Vitamin A. Due to their involvement in human health, the carotenoids most studied are β-carotene, α-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin. Fruits show a much more complex and diversified carotenoid composition than leafy vegetables, with considerable variations even for the main carotenoids. Typically, fruits contain only a few carotenoids but in high concentrations, together with a series of minor components present in much smaller or trace amounts.

Table 4 shows that the uvaia stood out with the highest total carotenoids content, with 3 and 22 times the values found for guabiroba and yellow guava, respectively. Furthermore, uvaia showed the highest amounts of all the individual carotenoids identified, although not differing significantly from guabiroba concerning the amounts of zeaxanthin and cryptoxanthin. Guabiroba had the second highest total and individual carotenoid contents, although the results were not statistically different from those found for yellow guava, except for the β-carotene 5,6-epoxide contents.

Uvaia also had the highest vitamin A content (Table 5), representing more than 5 and 50 times the values found in guabiroba and yellow guava, respectively. Despite the fact that guabiroba showed a value almost 10 times greater than that found in guava, there was no statistical difference between the two.

Table 5. Estimated Vitamin A Activity in Fruits of the Myrtaceae Family Native to the South of Brazil Based on the Carotenoid Content[a]

carotenoids	yellow guava	guabiroba	uvaia
β-carotene	0.492 ± 0.074	5.722 ± 0.277	31.833 ± 8.402
13-*cis*-β-carotene	0.104 ± 0.005	0.517 ± 0.006	3.383 ± 0.642
9-*cis*-β-carotene	0.079 ± 0.003	0.301 ± 0.102	2.044 ± 0.370
5,6-epoxy-β-carotene	0.042 ± 0.003	0.297 ± 0.081	0.573 ± 0.052
vitamin A[b]	0.718 ± 0.080 b	6.838 ± 0.454 b	37.834 ± 9.466 a

[a]Values expressed as the mean ± standard deviation. The same letters in the same row indicate no significant difference at the 5% level. [b]Expressed in μg equiv of retinol/g dry matter.

Many vegetables and fruits contain significant amounts of β-carotene and other provitamin A carotenoids that can be absorbed and converted to vitamin A in the human body. Examples such as tomato (10 μg/g), sweet potato (25 μg/g), and malabar spinach (24 μg/g)[22] are vegetables showing vitamin A contents lower than that of uvaia. On the other hand, fruits such as cagaita (5394 μg/g)[23] greatly surpass this value. In many developing countries, the largest vitamin A intake contribution comes from the provitamin A carotenoids in plant foods, which may contribute up to 82% of the total vitamin A intake. Food-based strategies are one of the means used to combat vitamin A deficiency in developing countries.[24] In the uvaia (Figure 1A), 33.8% of the total carotenoid content was represented by lutein and 21% composed of β-carotene, the amount of β-carotene corresponded to 1.7 and 21.4 times the amounts of this compound found in guabiroba and yellow guava, respectively.

Lutein is found in abundance in green leafy vegetables, mainly in the darker leaves such as kale (1052 μg/g), parsley (1312 μg/g),[25] lettuce (531 μg/g),[25] spinach (591 μg/g), chicory (358−497 μg/g), and rocket (407−564 μg/g).[26] However, uvaia showed higher lutein levels than the values found in vegetables such as the yellow pepper (166 μg/g),[27] guabiroba more than green pepper (64 μg/g),[27] and yellow guava more than cabbage (4 μg/g)[22] and broccoli (2 μg/g).[27] This carotenoid can be found in smaller amounts in fruits such as nectarine (97 μg/g), blackberry (38 μg/g), gooseberry (16 μg/g), blackcurrant (10 μg/g), plum cherry (11 μg/g), apricot (3 μg/g), and peach (3 μg/g),[25] where the values are all lower than those found in uvaia.

Lutein and zeaxanthin constitute the yellow pigments of the macula of the human retina and are also responsible for the protective ophthalmologic effect of the carotenoids, acting both as antioxidants and as filters of the high-energy blue light. Although not all studies show this relationship, the consumption of these carotenoids by ingesting foods shows an inverse correlation with the risk of macular degeneration, the main cause of loss of sight in the aged.[28]

β-Carotene is considered to be the carotenoid with the greatest vitamin A potential, since one molecule of β-carotene can be cleaved by a specific intestinal enzyme into two molecules of vitamin A. In addition, other health-promoting effects have been attributed to the carotenoids, such as immunomodulation and a reduction of the risk of contracting chronic degenerative diseases such as cancer and cardiovascular diseases. Such physiological activities have been attributed to their antioxidant properties, specifically, their ability to sequester singlet oxygen and interact with free radicals.[29]

Fruits such as starfruit (3 μg/g), papaya (12 μg/g),[21] nectarine (29 μg/g), morello cherry (32 μg/g),[25] watermelon (38 μg/g),[21] cagaita (46 μg/g),[23] apricot (57 μg/g), grapefruit (58 μg/g),[25] mango (58 μg/g), and muskmelon (67 μg/g)[21] show smaller β-carotene contents than the uvaia, despite β-carotene being their main carotenoid. However, when compared with the values found in vegetables such as the sweet potato (322 μg/g)[22] and carrot (320 μg/g),[27] foods considered as β-carotene sources, the uvaia showed a much lower value.

Nevertheless, considering that fruits, when produced in hot regions, show expressively higher carotenoid contents than those produced in regions with temperate climates, where peaches and nectarines are practically the only fruits showing appreciable amounts of β-carotene, the uvaia could be an interesting option.

Guabiroba (Figure 1B stands out for showing the highest amounts of zeaxanthin, β-carotene 5,6-epoxide, and cryptoxanthin, the latter compound representing about 39.6% of its total carotenoid content. This result corresponded to 134.6 and 2.5 times the values detected in the yellow guava and uvaia, respectively.

Cryptoxanthin is the main carotenoid in many fruits that have orange-colored pulp, but despite this, the guabiroba and uvaia stood out when compared to many other fruits such as papaya (5 μg/g), apricot (6 μg/g), nectarine (8 μg/g), clementine (38 μg/g), tomato (62 μg/g),[25] yellow tree tomato (108 μg/g), and red tree tomato (126 μg/g)[30] and vegetables like red pepper (34 μg/g) and yellow pepper (32 μg/g).[27]

The yellow guava (Figure 1C) showed the lowest amounts of all the carotenoid components evaluated and consequently showed the lowest total carotenoid content, almost 8 times less than that found in the guabiroba and uvaia, which did not vary from each other. As in the case of the uvaia, lutein was the principal carotenoid of the yellow guava, representing 63.9% of the total content.

Although fruit species generally show little variation with respect to their proximate compositions, frequently presenting low lipid and protein contents and high carbohydrate and moisture contents, they are important sources of vitamins and bioactive compounds. In this context, the guabiroba stood out for presenting excellent total phenolic compound contents and a considerable concentration of vitamin C, in amounts much higher than those normally found in the more traditionally consumed fruits. The guabiroba also showed high antioxidant potential by the ABTS method, surpassing the other fruits analyzed and also when compared with fruits recognized worldwide as important functional foods. However, using the DPPH method, the uvaia and guabiroba (161 g/g DPPH) were not statistically different. Although the evaluation methods used and results reported have not yet been sufficiently standardized, making comparisons difficult, the data still add valuable information to current knowledge on the bioactive properties of native fruits. The uvaia also stood out for presenting a relevant carotenoid and vitamin A content, and the yellow guava, although showing a lower phenolic compound content and antioxidant activity then the guabiroba, nevertheless showed much higher values than many traditionally consumed fruits.

These fruits constitute a native resource with technological and economic potential for the region, principally for

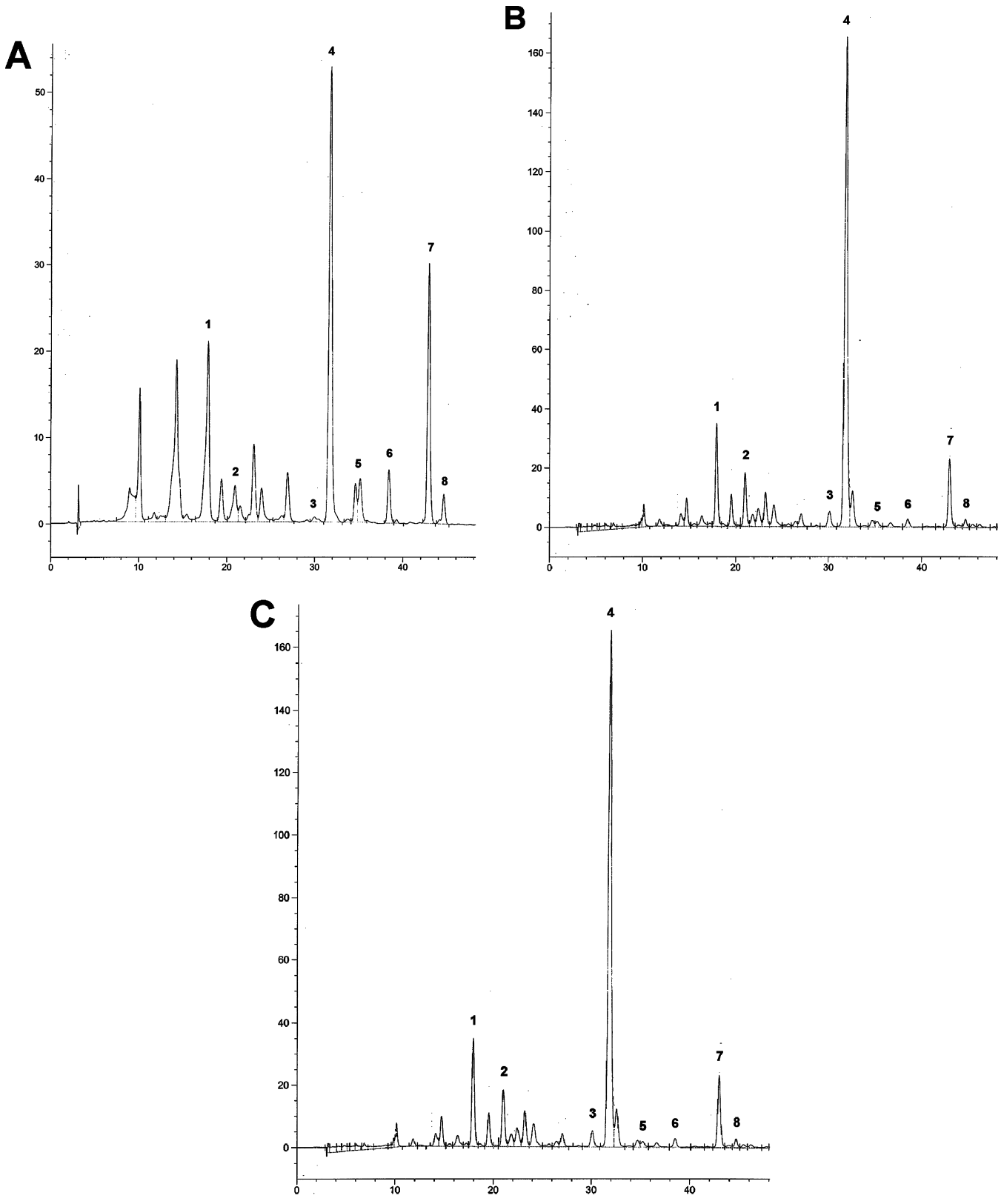

Figure 1. HPLC analysis of carotenoids in (A) uvaia, (B) guabiroba, and (C) yellow guava fruit native to the South of Brazil. Peaks 1−8 represent the following: 1, lutein; 2, zeaxanthin; 3, *β*-carotene-5,6-epoxide; 4, cryptoxanthin; 5, 13-*cis*-*β*-carotene; 6, *α*-carotene; 7, *β*-carotene; 8, 9-*cis*-*β*-carotene.

application in the pharmaceutical, cosmetic, and nutritional sectors. However, some actions are required for these fruits to be introduced into the production systems. The obtaining and diffusion of information to allow for the cultivation of these species on a commercial scale, and hence allow the product to be offered on the market, represents one of these actions.

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Additional studies will be needed for the steps of isolation, characterization of the phenolic compounds, and elucidation of the mechanism of action of these compounds and possible synergism between them. "In vivo" studies should be encouraged to evaluate the true bioavailability and effect of these compounds in the human body.

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Notes

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REFERENCES

(1) Instituto Brasileiro de Frutas (IBRAF), 2009, ano 4, ed. 12.

(2) Ratnam, D.; Ankola, D.; Bhardwaj, V.; Sahana, D.; Kumar, M. Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. *J. Controlled Release* **2006**, *113*, 189−207.

(3) Steinmetz, K. A.; Potter, J. D. Vegetables, fruits, and cancer prevention: A review. *J. Am. Diet. Assoc.* **1996**, *96*, 1027−1039.

(4) Hamilton, I. M.; Gilmore, W. S.; Benzie, I. F. F.; Mulholland, C. W.; Strain, J. J. Interactions between vitamins C and E in human subjects. *Br. J. Nutr.* **2000**, *84*, 261−267.

(5) Saito, K.; Kohno, M.; Yoshizaki, F.; Niwano, Y. Extensive screening for edible herbal extracts with potent scavenging activity against superoxide anions. *Plant Foods Hum. Nutr.* **2008**, *63*, 65−70.

(6) Tapiero, H.; Townsend, D. M.; Tew, K. D. The role of carotenoids in the prevention of human pathologies. *Biomed. Pharmacother.* **2004**, *58*, 100−110.

(7) Olson, J. A. Biological actions of carotenoids—Introduction. *J. Nutr.* **1989**, *119*, 94−5.

(8) Official Method. In *Official Methods of Analysis*, 16th ed.; AOAC International: Gaithersburg, MD, 1997

(9) Swain, T.; Hillis, W. E. The phenolic constituents of *Prunus domestica* I.— The quantitative analysis of phenolic constituents. *J. Sci. Food Agric.* **1959**, *10*, 63−68.

(10) Brand-Wiliams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Food Sci. Technol.* **1995**, *28*, 25−30.

(11) Kuskoski, E. M.; Asuero, A. G.; Troncoso, A. M.; Mancini-Filho, J.; Fett, R. Aplicatíon de diversos métodos químicos para determinar actividad antioxidante en pulpa de frutos. *Ciênc. Tecnol. Aliment.* **2005**, *25*, 726−732.

(12) Rosa, J. S.; Godoy, R. L. O.; Oiano Neto, J.; Campos, R. S.; Matta, V. M.; Freire, C. A.; Silva, A. S.; da; Souza, R. S. Desenvolvimento de um método de análise de vitamina C em alimentos por cromatografia liquida de alta eficiência e exclusão iônica. *Ciênc. Tecnol. Aliment.* **2007**, *27*, 837−846.

(13) Mercadante, A. Z.; Rodriguez-Amaya, D. B. Effects of ripening, cultivar differences, and processing on the carotenoid composition of mango. *J. Agric. Food Chem.* **1998**, *46*, 128−130.

(14) Zanatta, C. F.; Mercadante, A. Z. Carotenoid composition from the Brazilian tropical fruit camu-camu (*Myrciaria dubia*). *Food Chem.* **2007**, *101*, 1526−1532.

(15) Guilland, J. C.; Lequeu, B. *As Vitaminas: Do Nutriente ao Medicamento*; Santos: São Paulo, Brasil, 1995. (Original Les Vitamines Tec & Doc; Lavoisier: Paris.)

(16) Bauernfeind, J. C. Carotenoid vitamin A precursors and analogs in foods and feeds. *J. Agric. Food Chem.* **1972**, *20*, 456−473.

(17) Kinupp, V. F.; Barros, I. B. I. de. Teores de proteína e minerais de espécies nativas, potenciais hortaliças e frutas. *Ciênc. Tecnol. Aliment.* **2008**, *28*, 846−857.

(18) NEPA/UNICAMP. Tabela brasileira de composição de alimentos−TACO, 2006, versão 2. From http://www.unicamp.br/nepa/taco (Accessed 10/12/2011).

(19) Chun, O. K.; Kim, D. O. Consideration on equivalent chemicals in total phenolic assay of chlorogenic acid-rich plums. *Food Res. Int.* **2004**, *37*, 337−342.

(20) Rufino, M.; do, S. M.; Alves, R. E.; Brito, E. S.; de; Pérez-Jiménez, J.; Saura-Calixto, F.; Mancini-Filho, J. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chem.* **2010**, *121*, 996−1002.

(21) Shofian, N. M; Hamid, A. A.; Osman, A.; Saari, N.; Anwar, F.; Dek, M. S. P.; Hairuddin, M. R. Effect of freeze-drying on the antioxidant compounds and antioxidant activity of selected tropical fruits. *Int. J. Mol. Sci.* **2011**, *12*, 4678−4692.

(22) Kidmosea, U.; Yangb, R.-Y.; Thilstedc, S. H.; Christensena, L. P.; Brandtd, K. Content of carotenoids in commonly consumed Asian vegetables and stability and extractability during frying. *J. Food Compos. Anal.* **2006**, *19*, 562−571.

(23) Cardoso, L. de M.; Martino, H. S. D.; Moreira, A. V. B.; Ribeiro, S. M. R.; Pinheiro-Sant'Ana, H. M. Cagaita (*Eugenia dysenterica* DC.) of the Cerrado of Minas Gerais, Brazil: Physical and chemical characterization, carotenoids and vitamins. *Food Res. Int.* **2011**, *44*, 2151−2154.

(24) Gibson, R. S.; Hotz, C. Dietary diversification/modification strategies to enhance micronutrient content and bioavailability of diets in developing countries. *Br. J. Nutr.* **2001**, *85*, 159−166.

(25) Müller, H. Determination of the carotenoid content in selected vegetables and fruit by HPLC and photodiode array detection. *Z. Lebensm. Unters. Forsch A.* **1997**, *204*, 88−94.

(26) Žnidarčič, D. Z.; Ban, D.; Šircelj, H. Carotenoid and chlorophyll composition of commonly consumed leafy vegetables in Mediterranean countries. *Food Chem.* **2011**, *129*, 1164−1168.

(27) Burns, J.; Fraser, P. D.; Bramley, P. M. Identification and quantification of carotenoids, tocopherols and chlorophylls in commonly consumed fruits and vegetables. *Phytochemistry* **2003**, *62*, 939−947.

(28) Alves-Rodrigues, A.; Shao, A. The science behind lutein. *Toxicol. Lett.* **2004**, *150*, 57−83.

(29) Palozza, P.; Krinsky, N. I Antioxidant effects of carotenoids in vivo and in vitro: An overview. *Methods Enzymol.* **1992**, *213*, 403−420.

(30) Mertz, C.; Gancel, A.-L.; Gunata, X.; Alter, P.; Dhuique-Mayer, C.; Vaillant, F.; Perez, A. M.; Ruales, J.; Brat, P. Phenolic compounds, carotenoids and antioxidant activity of three tropical fruits. *J. Food Compos. Anal.* **2009**, 381−387.