Research Article

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Potential dietary sources of ellagic acid and other antioxidants among fruits consumed in Brazil: Jabuticaba (*Myrciaria jaboticaba* (Vell.) Berg)

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Abstract

BACKGROUND: The objectives of this study were (1) to evaluate the content of ellagic acid in fruits consumed by the Brazilian population, including native ones; (2) to further characterize rich sources in relation to ascorbic acid, phenolics contents and *in vitro* antioxidant capacity; and (3) to study the distribution and effect of ripening stage on ellagitannins content of jabuticaba (*Myrciaria jaboticaba*). The content of free ellagic acid and ellagic acid derivatives such as ellagitannins was analyzed using high-performance liquid chromatography (HPLC).

RESULTS: Ellagic acid was detected in 10 out of a total of 35 fruits analyzed. The content of free ellagic acid in fruits varied from 0.0028 to 0.085 g kg^{-1} (FW) and total ellagic acid varied from 0.215 to 3.11 g kg^{-1} (FW). All the seven fruits belonging to the Myrtaceae family evaluated in this study presented high contents of ellagitannins in their composition, with jabuticaba, grumixama and cambuci (all native from Brazil) showing the highest total ellagic acid contents. Jabuticaba, the most consumed in Brazil among those and already adapted to commercial plantations, contained concentrated phenolics compounds, including ellagitannins, in the peel. Anthocyanins (cyanidin derivatives) increased significantly through ripening of jabuticaba and were not present in the pulp or seeds. Samples collected from three different locations during summer, winter and spring had total ellagic contents varying from 1.88 to 3.31 g kg^{-1} (FW). The decrease in ellagic acid content with ripening was more accentuated for pulp (eight times) compared to seeds (2.3 times) and peel (2.0 times).

CONCLUSION: These results showed the potential of jabuticaba as dietary source of ellagic acid and reinforced consumption of the whole fruit by the population.

Keywords: ellagic acid; ellagitannins; fruits; jabuticaba; ripening stage

INTRODUCTION

There has been an increasing scientific interest in ellagic acid and ellagitannins due to their powerful antioxidant properties[1] and other beneficial biological activities such as cardioprotective effects *in vitro* and *in vivo*;[2] selective inhibition of the growth of human pathogenic bacteria;[3] inhibition of α-amylase and angiotensin I-converting enzymes (ACE) and antiproliferative activities against several different cancer cell lines.[4]

Ellagic acid can exist as either a free form, a glycoside or linked as ellagitannins esterified with glucose. In foods, ellagic acid is mainly found as polymeric ellagitannins and, for their detection and quantification, it is normally necessary to subject the sample to a basic or acid hydrolysis. When exposed to acids or bases, ester bonds are hydrolyzed and the hexahydroxydiphenic acid spontaneously rearranges into the water-insoluble ellagic acid. This reaction forms the basis for detection and quantification of ellagic acid in foods.[5]

The occurrence of ellagitannins is limited to a few berries and nuts, and the main sources of ellagic acid are blackberries, raspberries, pomegranate and walnuts.[6,7] Pinto *et al*.[8,9] reported the levels of ellagic acid in seven strawberry varieties cultivated in Brazil and in strawberry jams. The authors also evaluated the potential health benefits of purified ellagitannins from strawberries in relation to the antiproliferative, and *in vitro* inhibition of α-amylase, α-glucosidase and angiotensin I-converting enzyme (ACE) relevant for potential management of hyperglycemia and hypertension.[4] Recently, Abe *et al*.[10] evaluated the ellagic acid content of different nuts. There are no other reports about the ellagic acid content in foods consumed by the Brazilian population, and the richest known dietetic sources such as mulberries and raspberries are not commonly consumed in Brazil, which makes it necessary to find new potential sources of these compounds.

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According to this, the objectives of this study were (1) to evaluate the content of ellagic acid in fruits consumed by the Brazilian population, including native ones; (2) to further characterize rich sources in relation to ascorbic acid, phenolics contents and *in vitro* antioxidant capacity; and (3) to study the distribution and effect of ripening stage on ellagitannins content of jabuticaba (*Myrciaria jaboticaba*).

MATERIALS AND METHODS

Materials

Two kilograms of each fully ripened fruit, as ready for consumption, were obtained from the Central Market of São Paulo, Brazil (CEAGESP). The edible portions of the samples were cut into pieces, immediately frozen in liquid nitrogen, lyophilized and stored at −18 °C until analyses. At the time of analysis, samples were thoroughly homogenized. All chemicals and solvents were reagent or high-performance liquid chromatography (HPLC) grade. Ellagic acid, quercetin, chlorogenic acid and kaempferol were purchased from Sigma Chemical Co. (St Louis, MO, USA). The anthocyanidins cyanidin and pelargonidin and the respective 3-glucosides were obtained from Extrasynthèse (Genay, France). The samples evaluated in this study (common names, scientific and family names) are listed in Table 1.

Table 1. Family, scientific and common names of fruits screened for the presence of ellagic acid

Family	Scientific name	Common name
Actinidiaceae	Actinidia chinensis	Kiwi
Anacardiaceae	*Anacardium occidentale*	Cashew
	Mangifera indica	Mango
Annonaceae	*Annona muricata*	Graviola
Arecaceae	*Mauritia flexuosa*	Buriti
	Astrocaryum aculeatum	Tucuma
Bromeliaceae	*Ananas comosus*	Pineapple
Leguminosae	*Tamarindus indica*	Tamarindo
Clusiaceae	*Platonia insignis*	Bacuri
Cucurbitaceae	*Cucumis metuliferus*	Kino
Ebenaceae	*Diospyros kaki*	Persimmon
Humiriaceae	*Endopleura uchi*	Uxi
Myrtaceae	*Campomanesia phaea*	Cambuci
	Psidium guajava	Red guava
	Psidium guajava	White guava
	Myrciaria jaboticaba	Jabuticaba
	Myrciaria dubia	Camu-camu
	Eugenia uniflora	Surinam cherry
	Eugenia brasiliensis	Grumixama
Moraceae	*Ficus carica*	Fig
	Morus nigra	Wild mulberry
Oxalidaceae	*Averrhoa carambola*	Star fruit
Passifloraceae	*Passiflora alata*	Sweet passion fruit
	Passiflora ligularis	Granadilha
Punicaceae	*Punica granatum*	Pomegranate
Rosaceae	*Prunus domestica*	Plum
	Prunus persica	Peach
	Prunus avium	Cherry
	Rubus fruticosus	Blackberry
	Fragaria ananassa	Strawberry
Sapindaceae	*Litchi chinensis*	Litchi
Solanaceae	*Solanum sessiflorum*	Mana cubiu
Sterculiaceae	*Theobroma grandiflorum*	Cupuaçu
Vitaceae	*Vitis vinifera*	Vinifera grape
	Vitis labrusca	American grape

Vitamin C content

Ascorbic acid was extracted from fresh fruits with *meta*-phosphoric acid (3% w/v) and analyzed by reversed-phase HPLC in a Hewlett-Packard 1100 system (Hewlett-Packard, Palo Alto, CA, USA) with autosampler and quaternary pump coupled to a diode array detector as previously reported.[11] The column used was a 150 mm × 3.6 mm i.d., 5 µm, HP®, NucleoSil 100 C18 and elution (flow rate of 0.8 mL min^{-1}) was performed in isocratic condition with 2 mmol L^{-1} potassium chloride buffer (pH 2.5), monitored at 245 nm. Total ascorbic acid was estimated after reduction of dehydroascorbic acid (DHA) with 10 mmol L^{-1} dithiotreitol. Results were expressed as g kg^{-1} sample in fresh weight (FW).

Sample extraction for total phenolics and antioxidant capacity assays

Lyophilized fruits (1 g) were extracted three times in a solvent mixture (100 mL the first time, 50 mL the next two times) comprising methanol/water (70 : 30, v/v) or methanol/water/acetic acid (70 : 30:5, v/v/v) (for samples containing anthocyanins), using a Brinkmann homogenizer (Polytron-Kinematica GmbH, Kriens-Luzern, Sweden), at moderate speed for 1 min, while cooled in ice. The homogenate was filtered under reduced pressure through filter paper (Whatman N° 1) and it was stored at −18 °C until analysis. All extractions were done in duplicate, and the subsequent assays were run in triplicate.

Folin–Ciocalteu reducing capacity and total phenolics

The analysis was performed according to Singleton *et al.*,[12] with some modifications. A 0.25 mL aliquot of the extract obtained above was mixed with 0.25 mL of the Folin–Ciocalteu reagent and 2 mL of distilled water. After 3 min at room temperature, 0.25 mL of a saturated sodium carbonate (Na_2CO_3) solution was added and the mixture placed at 37 °C in a water bath for 30 min. The absorbance was measured at 750 nm using a model Ultrospec 2000 UV–visible spectrophotometer (Amersham Biosciences, Cambridge, UK). Gallic acid was used as the reference standard, and the results were expressed as g gallic acid equivalents (GAE) kg^{-1} sample in fresh weight (FW). Total phenolics (GAE kg^{-1}) were calculated subtracting the value of Folin–Ciocalteu reducing capacity due to ascorbic acid, using a standard curve.

2,2-Diphenyl-1-picrylhydrazyl radical scavenging capacity

The antioxidant capacity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging method according to Brand-Williams *et al.*[13] with some modifications.[14] A 50 µL aliquot of the extract obtained above, previously diluted with methanol, and 250 µL of DPPH (0.5 mmol L^{-1}) were mixed and after 20 min the absorbance was measured at 517 nm using a Microplate Spectrophotometer (Benchmark Plus; Biorad, Hercules, CA, USA), using a methanolic solution of Trolox, at different concentrations (20, 40, 60, 80 and 100 µmol L^{-1}), as control. The antioxidant capacity was expressed as mmol Trolox equivalents kg^{-1} sample in fresh weight (FW).

Flavonoids and free ellagic acid content

The extraction was performed according to the method of Arabbi *et al*.[15] Briefly, lyophilized samples were extracted three times in methanol/water (70 : 30, v/v), or methanol/water/acetic acid (70 : 30:5, v/v/v) for anthocyanin-containing samples, using a Brinkmann homogenizer (Polytron-Kinematica), in an ice bath. The homogenate was filtered under reduced pressure through filter paper (Whatman N° 1). The extracts obtained were concentrated until methanol elimination on a rotatory evaporator (Rotavapor RE 120; Büchi, Flavil, Sweden) at 40 °C for posterior application to solid-phase extraction (SPE) columns. Aliquots of the extracts obtained were passed through polyamide SC 6 (Macherey-Nagel Gmbh and Co., Duren, Germany) columns (1 g/6 mL) and eluted with methanol followed by methanol/ammonia (99.5 : 0.5, v/v). Each eluate was evaporated to dryness under reduced pressure at 40 °C, redissolved in methanol or methanol/acetic acid (95 : 5, v/v) and filtered through a 0.22 µm polytetrafluoroethylene filter (Millipore Ltd., Bedford, MA, USA) prior to HPLC analysis. In these conditions, ellagitannins are retained in SPE columns and the sum of eluted free ellagic acid and ellagic acid glycosides was considered as 'free ellagic acid'.

Total ellagic acid content

Total ellagic acid was determined after extraction and acid hydrolysis according to Pinto *et al*.[9] An aliquot of 2 mL of the raw sample extract in 80% acetone was dried under nitrogen, 2 mL of 2 mol L^{-1} trifluoroacetic acid were added, and the hydrolysis was performed at 120 °C for 90 min. The hydrolyzed samples were evaporated to dryness under nitrogen, redissolved in methanol and filtered through a 0.22 µm polytetrafluoroethylene filter (Millipore) for HPLC analysis.

High-performance liquid chromatography quantification

Identification and quantification of flavonoids and phenolic acids were achieved using analytical reversed-phase HPLC in a Hewlett-Packard 1100 system with autosampler and quaternary pump coupled to a diode array detector controlled by the Chemstation software. The column used was 250 × 4.6 mm, i.d., 5 µm, Prodigy ODS3 reversed-phase silica (Phenomenex, Torrance, CA, USA) and elution solvents were: A, water/tetrahydrofuran/trifluoroacetic acid (98 : 2:0.1) and B, acetonitrile. Solvent gradient was the same as that used by Arabbi *et al*.[15] Eluates were monitored at 270, 328, 370 and 525 nm and samples were injected in duplicate. Calibration was performed by injecting the standards three times at five different concentrations, from 0.5 to 10 µg per injection ($R^2 = 0.999$). Peak identification was performed by comparison of retention times and diode array spectral characteristics with the standards and the library spectra. In the case of quercetin and kaempferol derivatives, results were expressed as milligrams of aglycone, anthocyanins were expressed as milligrams of cyanidin, catechin, epicatechin and phenolic acids such as ellagic acid and chlorogenic acid, were expressed as mg of the respective standard. All flavonoid and phenolic acid analyses were done in triplicate and results were expressed per kilogram of sample in fresh weight (FW).

Total tannin content

Total tannins were determined spectrophotometrically by using the method of Hagerman and Butler,[16] based on precipitation with bovine serum albumin (BSA). Briefly, adequately diluted methanolic extract (1 mL) was mixed with 2 mL of BSA (1 mg mL^{-1}) and allowed to stand at room temperature for 15 min. After centrifugation at 5000×*g* for 10 min, the supernatant was discarded, and the precipitate was dissolved in 4 mL of sodium dodecyl sulfate solution. One milliliter of the ferric chloride reagent was added, and after 20 min the absorbance at 510 nm was read on an Ultrospec 2000 (Amersham Biosciences). Results were given in g of tannic acid kg^{-1} FW.

Color measurement

The color (L^*, a^*, and b^* values) of jabuticaba at different ripening stages was measured using a reflectance spectrophotometer (Color Quest XE; HunterLab, Reston, VA, USA), calibrated by using a standard white tile (top of the scale)/light trap (bottom of the scale) included with the instrument. The color readings of the individual fruits were taken in the equatorial area of the intact fruits, measuring each fruit twice at two different sites. A total of five fruits from each ripening stage were measured. C^* (chroma) and h^* (hue angle) were calculated by using values of a^* and b^* coordinates according to the equations $C^* = (a^{*2} + b^{*2})^{\frac{1}{2}}$ and $h^* = \tan^{-1}(b^*/a^*)$.

Statistical analysis

All analyses were run in triplicate and were expressed as mean ± standard deviation (SD). Statistical analysis was done by using the Statistic software package version 5.0 (StatSoft, Inc., Tulsa, OK, USA). Differences between means were first analyzed by the ANOVA test and then the least significant difference (LSD) test ($P < 0.05$). Data were subjected to Pearson correlations.

RESULTS AND DISCUSSION

Ellagic acid was found in 10 of a total of 35 fruit samples analyzed (34 of them commercially available) (Table 2). The content of free ellagic acid in fruits varied from 0.0028 to 0.085 g kg^{-1} fresh weight (FW) and total ellagic acid, determined after acid hydrolysis, varied from 0.215 to 3.11 g kg^{-1} (FW). The proportion of free ellagic acid ranged from 0.08 to 2.7% of the total showing that ellagic acid is mostly present as ellagitannins. The presence of ellagic acid among the twenty botanical families of fruits evaluated in the screening was restricted to the Myrtaceae, Punicaceae and Rosaceae families. All the seven different fruits from the Myrtaceae family presented ellagic acid and the highest contents were found in jabuticaba, grumixama and cambuci, native from Brazil. Among fruits of the Rosaceae family, ellagic acid was present only in berries such as strawberry and blackberry while in peach, plum and cherry it was not detected.

Similar to our results, Williner *et al*.[17] did not detect ellagic acid in kiwi, oranges and red apples, and detected very low amounts in pineapple, banana, tangerine and green apples (less than 0.1 g kg^{-1} DW). Daniel *et al*.[6] also reported very low levels in peach, plum, cherry and kiwi (less than 0.1 g kg^{-1} DW). There are contradictions about the presence of ellagic acid in cherries. While Shahrzad and Bitsch[18] detected 0.25 mg L^{-1} of ellagic acid in cherry juice, Olsson *et al*.[19] did not detect this compound in the fruit.

Pomegranate juice is known for presenting high contents of ellagic acid, which are strongly influenced by processing conditions. Gil *et al*.[1] reported ten-fold higher ellagic acid concentration and 50-fold higher punicalagin (ellagitannin) content in commercial juices compared to those obtained by hand pressing of the arils. Besides factors such as added

Table 2. Free and total ellagic acid contents (g kg^{-1} sample FW) of fruits

Family	Fruit	Free ellagic acid	Total ellagic acid
Actinidiacea	Kiwi	ND	ND
Anacardiaceae	Cashew	ND	ND
	Mango	ND	ND
Annonaceae	Graviola	ND	ND
Arecaceae	Buriti	ND	ND
	Tucuma	ND	ND
Bromeliaceae	Pineapple	ND	ND
Cesalpinaceae	Tamarindo	ND	ND
Clusiaceae	Bacuri	ND	ND
Curcubitaceae	Kino	ND	ND
Ebenaceae	Persimmon	ND	ND
Humiriaceae	Uxi	ND	ND
Myrtaceae	Cambuci	0.0033 ± 0.0001^{h}	2.67 ± 0.07^{b}
	Red guava	0.0056 ± 0.0005^{g}	0.25 ± 0.02^{g}
	White guava	0.0028 ± 0.0001^{i}	0.215 ± 0.005^{h}
	Jabuticaba	0.06 ± 0.003^{b}	3.11 ± 0.19^{a}
	Camu-camu	0.016 ± 0.001^{c}	0.59 ± 0.03^{e}
	Surinam cherry	0.009 ± 0.001^{f}	0.96 ± 0.06^{d}
	Grumixama	0.085 ± 0.005^{a}	2.70 ± 0.08^{b}
Moraceae	Fig	ND	ND
	Wild mulberry	ND	ND
Oxalidaceae	Star fruit	ND	ND
Passifloriaceae	Sweet passion fruit	ND	ND
	Granadilha	ND	ND
Punicaceae	Pomegranate	0.0096 ± 0.0002^{f}	0.62 ± 0.04^{e}
Rosaceae	Plum	ND	ND
	Peach	ND	ND
	Cherry	ND	ND
	Blackberry	0.0138 ± 0.0008^{e}	1.40 ± 0.03^{c}
	Strawberry	0.022 ± 0.001^{d}	0.42 ± 0.02^{f}
Sapindaceae	Litchi	ND	ND
Solanaceae	Mana cubiu	ND	ND
Sterculiaceae	Cupuaçu	ND	ND
Vitaceae	Vinifera grape	ND	ND
	American grape	ND	ND

Results are expressed as means ± SD for triplicates.
[a,b,c,d,e,f,g,h,i] Means in the same column with common superscript letters are not significantly different ($P < 0.05$).
ND, not detected.

enzymes, thermal treatment and concentration process, the industrial process includes the hydrostatic pressure to crush the whole fruit, extracting the water soluble ellagitannins from the rind proportionally to the force used. The edible portion of pomegranate evaluated here presented 0.62 g kg^{-1} of total ellagic acid, higher than guava, strawberry and camu-camu. However, we found a much higher content in pomegranate rind (5.34 ± 0.20 g kg^{-1} FW), which is in accordance with the high content of ellagic acid present in commercial juices.

Berries of Rosaceae family are well-known sources of ellagic acid with contents ranging from 1.03 to 3.30 g kg^{-1} (FW).[20,21] Blackberry evaluated in the present study had 1.40 g kg^{-1} (FW) and strawberry (cv Camarosa) 0.42 g kg^{-1} (FW). Häkkinen and Torronen[22] reported values from 0.40 to 0.52 g kg^{-1} (FW) and Pinto *et al.*[9] from 0.19 to 0.47 g kg^{-1} (FW) for different strawberry cultivars.

All fruits belonging to the Myrtaceae family presented important amounts of ellagic acid. White and red guavas presented the lowest amounts, 0.20–0.25 g kg^{-1} FW. Camu-camu (Amazonian fruit) and Surinam cherry (Brazilian cherry) showed intermediate ellagic acid levels. Jabuticaba, grumixama and cambuci, in this descending order, were the three fruits with the highest contents of ellagic acid. Similar to our results, Genovese *et al.*[11] also found a high content of ellagic acid for cambuci. Jabuticaba cultivation occurs mainly in Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo and Paraná States and is already commercially available, with about 2000 tones of jabuticaba fruits sold in 2008 by the Central Market in São Paulo (CEAGESP). The fruit has an appearance and texture similar to grapes but with a thicker, tougher purple-colored skin.

Grumixama is common in domestic orchards and is not commercialized; cambuci originates from the Atlantic Forest, and both are only consumed locally but present potential for agro-industrial exploitation, mainly cambuci, already used in jams, ice creams, and liquors due to the excellent flavor. These results show that native Brazilian fruits can represent important sources of ellagic acid, contrary to what occurs in other countries, such as in Finland, where dietary sources of ellagitannins are almost exclusively represented by certain berries of the Rosaceae family.[21]

The ten ellagic acid-rich fruits identified here were further characterized for total phenolic content, *in vitro* antioxidant capacity and ascorbic acid content. Besides this, the identification and quantification of phenolic compounds were carried out by HPLC.

Vitamin C content

Vitamin C plays an important role in human health mainly as an antioxidant and anti-inflammatory agent.[23] Among the fruits rich in ellagic acid, camu-camu had the highest total ascorbic acid content, of 4 g kg^{-1} (FW) (Table 3), but lower than the values previously reported for the pulps, from 13 to 30 g kg^{-1}, depending on the region of origin.[24] High incidence of solar radiation and excess of potassium in the soil can increase ascorbic acid content, while excess of nitrogen and phosphorus can decrease it.[25]

Guava fruit also showed high content of vitamin C. The white variety had 1.42 g kg^{-1} (FW), two-fold higher than the level found in the red variety and in strawberry. Lima *et al.*[26] found 0.52 to 2.09 g kg^{-1} of ascorbic acid in 10 guava cultivars and also observed higher contents for white varieties. These results are important since white guava is destined for fresh consumption while red guava is mostly used to prepare jams, jellies and juices.

The main sources of ellagic acid, jabuticaba, grumixama and cambuci, presented lower ascorbic acid contents (less than 0.25 g kg^{-1} FW).

Total phenolic content

The content of total phenolics varied significantly, from 0.42 (white guava) to 14.76 (camu-camu) g kg^{-1} (FW) (Table 3). Although Surinam cherry and red guava showed low total phenolic content among the ellagic acid-rich fruits, these levels are still higher than those of other fruits evaluated here for comparison, such as mango, kiwi, pineapple and persimmon, which showed total phenolic contents ranging from 0.31 to 0.74 g kg^{-1} (FW).

Variation in the content of total phenolics among fruits can be due to intrinsic and extrinsic factors. Values ranging from 0.0145 to 5.27 g kg^{-1} (FW) have been reported, with the highest values for

Table 3. Folin–Ciocalteu reducing capacity (g GAE kg^{-1} FW), total phenolic content (g GAE kg^{-1} FW), DPPH radical scavenging capacity (mmol Trolox eq. kg^{-1} FW) and vitamin C content (g kg^{-1} FW) of ellagic acid-rich fruits

Fruits	Folin–Ciocalteu reducing capacity	DPPH radical scavenging capacity	Vitamin C	Total phenolics
Cambuci	7.00 ± 0.28^{c}	19.5 ± 1^{c}	ND	7.00 ± 0.28^{c}
Red guava	1.88 ± 0.02^{f}	13.5 ± 0.5^{d}	0.70 ± 0.04^{c}	1.37 ± 0.02^{f}
White guava	1.52 ± 0.03^{g}	10.7 ± 0.5^{e}	1.42 ± 0.08^{b}	0.42 ± 0.03^{g}
Jabuticaba	7.56 ± 0.32^{c}	62 ± 6^{b}	0.25 ± 0.01^{d}	7.44 ± 0.32^{c}
Camu-camu	17.97 ± 0.49^{a}	141 ± 7^{a}	3.97 ± 0.21^{a}	14.76 ± 0.49^{a}
Surinam cherry	1.07 ± 0.01^{h}	5.6 ± 0.5^{f}	0.22 ± 0.02^{d}	0.95 ± 0.01^{h}
Grumixama	10.52 ± 0.19^{b}	64 ± 4^{b}	0.20 ± 0.01^{d}	10.40 ± 0.19^{b}
Pomegranate	2.62 ± 0.04^{e}	13 ± 1^{d}	ND	2.62 ± 0.04^{e}
Blackberry	2.96 ± 0.06^{d}	15 ± 1^{d}	0.051 ± 0.004^{e}	2.96 ± 0.06^{d}
Strawberry	2.62 ± 0.08^{e}	14 ± 2^{d}	0.65 ± 0.03^{c}	2.17 ± 0.08^{e}

Results are expressed as means ± SD for triplicates.
[a,b,c,d,e,f,g,h] Means in the same column with common letters are not significantly different ($P < 0.05$).
ND, not detected.

berries and grapes and the lowest for mango, banana, persimmon and litchi.[27]

Folin–Ciocalteu reducing capacity is related to the content of phenolics and ascorbic acid.[28] However, for cambuci, pomegranate, and blackberry, due to the absence/very low content of vitamin C, it results exclusively from the presence of phenolic compounds. For jabuticaba and grumixama the contribution of vitamin C is also low, of less than 2%. The extremely high value presented by camu-camu is 82% due to phenolics, similar to strawberry (83%) and Surinam cherry (89%), although it has the highest vitamin C content. The highest contribution of vitamin C to Folin-reducing capacity was observed for red guava (27%) and white guava (72%).

When the Pearson correlation was evaluated excluding camu-camu, it was observed high correlation ($r = 0.88$) between Folin–Ciocalteu reducing capacity and ellagic acid content, showing that ellagic acid can be the main phenolic compound in these fruits. However, when camu-camu was included, the correlation coefficient decreased to 0.27, probably due to the fact that camu-camu is rich in other phenolics besides ellagic acid.

2,2-Diphenyl-1-picrylhydrazyl radical scavenging capacity

Ellagic acid-rich fruits were evaluated for antioxidant capacity by DPPH free radical scavenging method, which is based on the measurement of the reducing ability of antioxidants toward DPPH. The DPPH radical scavenging capacity of ellagic acid rich-fruits varied greatly, from 5.6 to 141 mmol Trolox kg^{-1} (FW) (Table 3). Camu-camu, jabuticaba and grumixama presented the highest values among the 10 selected fruits. The strong antioxidant capacity of camu-camu can be primarily attributed to the high content of phenolics in general and vitamin C, while for grumixama and jabuticaba can be due to the anthocyanins responsible for their strong purple color. Cambuci, which has no anthocyanins, but also a high content of ellagic acid, presented a much lower antioxidant capacity compared to grumixama and jabuticaba, suggesting that compounds such as ellagic acid and anthocyanins can act by synergism.

As expected, the Folin–Ciocalteu reducing capacity and the DPPH radical scavenging capacity showed high correlation ($r = 0.87$). It was observed a strong correlation ($r = 0.83$) between DPPH radical scavenging capacity and ellagic acid content when camu-camu was not included. However, the inclusion of camu-camu decreased the coefficient to 0.16, explained by the fact that the high antioxidant activity of camu-camu is also due to other phenolics and vitamin C. The intensely high antioxidant activity of camu-camu and its relation with the vitamin C content had already been reported.[11,29]

Flavonoids and phenolic acids

The composition and concentration of flavonoids varied significantly among ellagic acid-rich fruits (Table 4). Flavanols were detected only in four of the 10 fruits analyzed. The catechin content of pomegranate, red and white guava ranged from 0.013 to 0.031 g kg^{-1} (FW), while epicatechin was only detected in blackberry (0.10 g kg^{-1} FW). High content of flavanols are not common in fruits as demonstrated by Harnly *et al.*,[30] who reported values ranging from 0.001 to 0.095 g kg^{-1} (FW) in 14 varieties of fruits.

All samples, with the exception of pomegranate, presented quercetin derivatives. Their content ranged from 0.0035 (cambuci, white guava) to 0.20 (grumixama) g kg^{-1} (FW). Kaempferol was detected in low levels in grumixama, blackberry and strawberry, 0.0056 to 0.018 g kg^{-1} (FW). Hakkinen *et al.*[31] reported quercetin contents ranging from 0.06 to 1.58 g kg^{-1} (FW) in 25 varieties of berries. The lowest contents were observed for raspberries and strawberries, while the highest were present in berries belonging to the Ericaceae family. Kaempferol was less frequently detected, similar to the present study.

Anthocyanins are flavonoids widely distributed in nature and responsible for the red, purple, blue, and violet colors of flowers and fruits. The content of anthocyanins in ellagic acid-rich fruits varied from 0.021 to 1.69 g kg^{-1} (FW), whereas the highest content was observed in grumixama. Anthocyanins were absent in cambuci, white and red guava, and present in very low amounts in Surinam cherry and pomegranate. The coloration of red guava and Surinam cherry is related to licopene, the main carotenoid in these fruits.[32] The content of total flavonoids varied from 0.0035 (cambuci) to 1.91 (grumixama) g kg^{-1} (FW), and the highest amounts corresponded to samples rich in anthocyanins. There was no correlation between total flavonoids and antioxidant capacity (0.18), total phenolics (0.31), and total ellagic acid (0.44).

Hydroxycinnamic acids were detected only in Surinam cherry (0.0131 g kg^{-1} FW). Peach, plum, cherry and pear are the main sources of hydroxycinnamic acid among the fruits.[33] Peach and

Table 4. Flavonoids and phenolic acid content (g kg^{-1} FW) of ellagic acid-rich fruits

	Flavanols		Flavonols				
Fruit	Catechin	Epicatechin	Quercetin	Kaempferol	Anthocyanins	Hydroxycinnamic acids	Total flavonoids
Cambuci	ND	ND	0.0035 ± 0.0002^{g}	ND	ND	ND	0.0035 ± 0.0002^{i}
Red guava	0.024 ± 0.001^{b}	ND	0.0095 ± 0.0009^{e}	ND	ND	ND	0.0335 ± 0.002^{h}
White guava	0.013 ± 0.001^{c}	ND	0.0035 ± 0.0002^{g}	ND	ND	ND	0.0165 ± 0.0012^{h}
Jabuticaba	ND	ND	0.0056 ± 0.0003^{f}	ND	0.32 ± 0.02^{d}	ND	0.33 ± 0.02^{d}
Camu-camu	ND	ND	0.030 ± 0.001^{d}	ND	0.28 ± 0.01^{e}	ND	0.31 ± 0.01^{e}
Surinam cherry	ND	ND	0.15 ± 0.01^{b}	ND	0.021 ± 0.001^{f}	0.0131 ± 0.0007	0.17 ± 0.01^{f}
Grumixama	ND	ND	0.20 ± 0.01^{a}	0.018 ± 0.001^{a}	1.69 ± 0.05^{a}	ND	1.91 ± 0.06^{a}
Blackberry	ND	0.10 ± 0.01	0.091 ± 0.005^{c}	0.0056 ± 0.0003^{c}	0.67 ± 0.01^{b}	ND	0.87 ± 0.03^{b}
Pomegranate	0.031 ± 0.001^{a}	ND	ND	ND	0.041 ± 0.002^{f}	ND	0.072 ± 0.003^{g}
Strawberry	ND	ND	0.027 ± 0.002^{d}	0.0079 ± 0.0003^{b}	0.44 ± 0.02^{c}	ND	0.48 ± 0.02^{c}

Results are expressed as means ± SD for triplicates.
^{a,b,c,d,e,f,g,h,i} Means in the same column with common superscript letters are not significantly different ($P < 0.05$).
ND, not detected.
Anthocyanins were expressed as g cyanidin kg^{-1} FW, hydroxycinnamic acid as g chlorogenic acid kg^{-1} FW.

Table 5. Ratios among seed, pulp and peel (%), colour and anthocyanin contents (g cyanidin kg^{-1} FW) of jabuticaba in different ripening stages

	Colour						
Ripening stage	Parameter	Value	Anthocyanin (g kg^{-1} FW)	Part	% of fresh fruit	Moisture (%)	Ratio pulp : seed (freeze dried)
Stage 1	L^*	33.70	ND	Peel	74	87.4	–
	C^*	23.09	–	Pulp + seed	26	82.0	58 : 42
	h^*	275.94	–	–	–	–	–
Stage 2	L^*	26.65	0.01 ± 0.00	Peel	64	87.8	–
	C^*	16.91	–	Pulp + seed	36	84.6	67 : 33
	h^*	56.36	–	–	–	–	–
Stage 3	L^*	21.62	0.071 ± 0.1	Peel	56	87.5	–
	C^*	9.07	–	Pulp + seed	44	88.5	72 : 28
	h^*	21.65	–	–	–	–	–
Stage 4	L^*	18.71	0.55 ± 0.01	Peel	44	83.6	–
	C^*	4.50	–	Pulp + seed	56	83.5	85 : 15
	h^*	5.99	–	–	–	–	–
Stage 5	L^*	8.98	1.23 ± 0.03	Peel	33	84.3	–
	C^*	2.32	–	Pulp + seed	67	85.4	90 : 10
	h^*	344.22	–	–	–	–	–

Stage 1, green–unripe to Stage 5, deep purple–fully ripe.
L^*, lightness (+100 = white; −100 = black); C^*, chroma (an increase in value = colour intensity); h^*, hue(0° = red, 90° = yellow, 180° = green, 270° = blue).
ND, not detected.

cherry evaluated in this study had 0.28 and 0.50 g kg^{-1} (FW), respectively, of hydroxycinnamic acids, representing the main phenolic compounds in these fruits.

Ellagic acid in jabuticaba

Among the ellagic acid rich-fruits selected in this study, the most popular is by far jabuticaba, which has been successfully adapted to commercial plantations and presents a very pleasant aroma and sweet taste. To study the effect of environmental factors on the ellagic acid content, samples of jabuticaba were collected from three different locations in São Paulo State, during three different periods, summer, winter and spring. Results showed that for the same cultivar (Sabará) the total ellagic content varied almost 60%, from 1.88 to 3.31 g kg^{-1} FW, depending on the time of year and region of cultivation. In spite of this fluctuation, these contents allowed the classification of jabuticaba among the richest sources of ellagic acid among fruits.

To study the effect of ripening on phenolics contents, fruits were classified in five ripening stages according to peel color: stage 1, green color; stage 2, green with red parts; stage 3, red; stage 4, purple; stage 5, deep purple. Anthocyanin contents and color parameters (C, L, and h) in these stages are shown in Table 5. No anthocyanins were detected in pulp and seeds, independent of the ripening stage, and the main anthocyanins found in peel were cyanidin glycosides. As expected, anthocyanins concentration in peel increased significantly during ripening reaching a very high

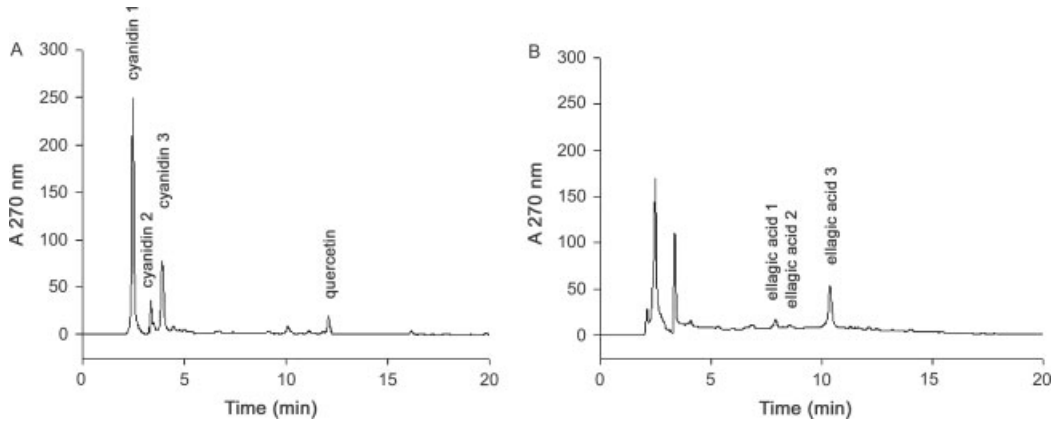

Figure 1. (A) HPLC 270 nm chromatogram of methanol fraction of jabuticaba extract. (B) HPLC chromatogram of methanol/ammonia fraction of jabuticaba extract.

Table 6. Effect of ripening stage on total ellagic acid distribution and concentration (g kg^{-1} DW) in jabuticaba

	Ellagic acid (g kg^{-1} DW)					
	Pulp		Peel		Seeds	
Ripening	Total	Free	Total	Free	Total	Free
Stage 1	36.8 ± 2.5	0.358 ± 0.001	43.95 ± 0.24	0.108 ± 0.004	91.73 ± 1.83	2.65 ± 0.04
Stage 2	28.9 ± 1.1	0.22 ± 0.02	43.36 ± 0.83	0.24 ± 0.01	68.47 ± 0.17	1.18 ± 0.01
Stage 3	22.0 ± 0.5	0.135 ± 0.001	37.85 ± 0.08	0.19 ± 0.01	58.29 ± 1.29	1.84 ± 0.16
Stage 4	8.2 ± 0.2	0.0303 ± 0.0001	38.7 ± 0.3	0.174 ± 0.003	37.61 ± 0.31	0.422 ± 0.002
Stage 5	4.6 ± 0.2	0.022 ± 0.001	22.5 ± 1.3	0.146 ± 0.005	40.18 ± 0.60	0.27 ± 0.03

Stage 1, green–unripe to Stage 5, deep purple–fully ripe.

value at stage 5 (fully ripe), of 1.2 g kg^{-1} FW. HPLC chromatograms at 270 nm of flavonoids identified in fully ripe jabuticaba are presented in Fig. 1, confirming cyanidin glycosides as the main compounds.

Separation of seeds from the pulp of fresh fruits was not possible due to adherence. In this way, the separation was performed after freeze drying. The proportion of the different parts, peel, pulp and seeds, and their moisture content are shown in Table 5. It was observed that there was an increase in pulp amount in relation to those of peel and seeds with ripening. In the stage 1, pulp and seeds were present in a 1 : 1 ratio, while in stage 5 the ratio was of 9 : 1 (w/w), respectively. The peel, which represented 74% of the fresh fruit at stage 1, corresponded to only 33% of the fully ripe fresh fruit. Water content did not change significantly, with an average value of around 85%.

In dry weight, total ellagic acid concentration was higher in jabuticaba seeds compared to pulp and peel (Table 6). The lowest concentrations were present in pulp, independent of the ripening stage, and the decrease in ellagic acid content with ripening was more accentuated for pulp (eight times) compared to seeds (2.3 times) and peel (two times).

Figure 2 shows that both total phenolics and ellagic acid contents decreased with ripening, and in all cases the main contribution to the contents of the whole fruit was from the peel. At the ripe stage, the contribution for total ellagic content was of 1 : 3:1 for pulp, peel and seeds, respectively. A similar tendency was observed for total tannin content (Fig. 3). However, total tannin concentrations of jabuticaba were higher and decreased more drastically during ripening than those of ellagic acid, mainly for the pulp (Table 7). In dry weight, the decrease was of 27 times for pulp and 4.3 and 3.1 times for peel and seeds, respectively. This result indicates that gallotannins and/or proanthocyanidins could be also present in jabuticaba, which are known for a much higher affinity for proteins than ellagitannins.[34] This fact is probably associated with the loss of astringency observed during jabuticaba ripening.

Table 7. Effect of ripening stage on total tannin distribution and concentration (g tannic acid kg^{-1} DW) in jabuticaba

	Total tannin (g kg^{-1} DW)		
Ripening	Pulp	Peel	Seeds
Stage 1	315 ± 2	211 ± 13	202 ± 5
Stage 2	208 ± 1	167 ± 1	170 ± 2
Stage 3	108 ± 2	145 ± 1	144 ± 2
Stage 4	29.1 ± 0.8	113 ± 1	92 ± 2
Stage 5	11.6 ± 0.3	48 ± 3	65 ± 5

Stage 1, green–unripe to Stage 5, deep purple–fully ripe.

Similar to the observed for jabuticaba, ripening of strawberries also causes a decrease in ellagic acid contents.[17,35] For five cultivars evaluated, the ellagic acid content was highest in green (0.09–0.18 g kg^{-1} FW), intermediate in mid-ripe (0.04–0.09 g kg^{-1} FW) and lowest in full-ripe strawberries

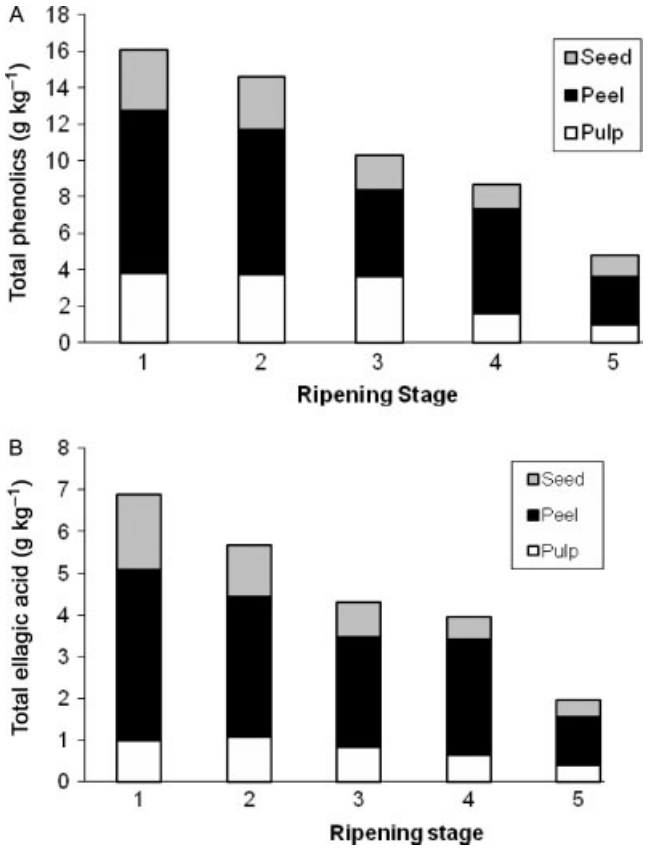

Figure 2. Total phenolic (A) and ellagic acid contents (B) (g kg^{-1} FW) during ripening stages of jabuticaba (stage 1, green–unripe to stage 5, deep purple–fully ripe).

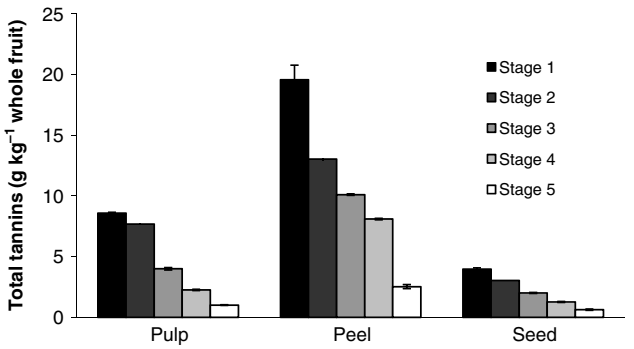

Figure 3. Total tannin contents (g tannic acid kg^{-1} whole fruit FW) in peel, pulp and seeds of jabuticaba as affected by ripening stages (stage 1, green–unripe to stage 5, deep purple–fully ripe).

(0.02–0.04 g kg^{-1} FW).[17] In contrast, in muscadine grapes an increase in ellagic acid content with ripening was observed, both in pulp and seeds.[36]

CONCLUSION

The main sources of ellagic acid among fruits consumed by the Brazilian population were those belonging to the Myrtaceae family, such as jabuticaba, grumixama and cambuci, all native fruits. Similar to strawberries, ellagitannin content decreased with ripening of jabuticaba meanwhile the anthocyanin increased significantly. Jabuticaba, due to the very pleasant flavor, is a promising source of ellagic acid derivatives in the diet.

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