Journal of Functional Foods 17 (2015) 422–433

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jff

Screening of the chemical composition and occurring antioxidants in jabuticaba (*Myrciaria jaboticaba*) and jussara (*Euterpe edulis*) fruits and their fractions

Kim Ohanna Pimenta Inada [a,b], *Andressa Alves Oliveira* [b], *Tamirys Barcellos Revorêdo* [a], *Ana Beatriz Neves Martins* [a], *Ellen Cristina Quirino Lacerda* [b], *Aline Soares Freire* [c], *Bernardo Ferreira Braz* [c], *Ricardo Erthal Santelli* [c], *Alexandre Guedes Torres* [b], *Daniel Perrone* [b], *Mariana C. Monteiro* [a,*]

[a] *Laboratório de Alimentos Funcionais, Instituto de Nutrição Josué de Castro, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho, 373, CCS, Bloco J, 2° andar, sala 16, 21941-902 Rio de Janeiro, Brazil*

[b] *Laboratório de Bioquímica Nutricional e de Alimentos, Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro, Av. Athos da Silveira Ramos 149, CT, Bloco A, sala 528A, 21941-909 Rio de Janeiro, Brazil*

[c] *Laboratório de Desenvolvimento Analítico, Departamento de Química Analítica, Instituto de Química, Universidade Federal do Rio de Janeiro, Av. Athos da Silveira Ramos 149, CT, Bloco A, Lab. A518, 21941-909 Rio de Janeiro, Brazil*

ARTICLE INFO

Article history:
Received 23 March 2015
Received in revised form 15 May 2015
Accepted 1 June 2015
Available online 17 June 2015

Keywords:
Anthocyanins
Antioxidant activity
Carotenoids
Mineral composition
Phenolic compounds
Tocopherols

ABSTRACT

We aimed at determining the bioactive compounds and chemical composition of jabuticaba (*Myrciaria jaboticaba* (Vell.) O. Berg) and jussara (*Euterpe edulis* Mart.) fruits and their fractions. With the exception of jabuticaba pulp, both fruits and their fractions might be exploited as dietary fibre sources. Jabuticaba fruit may be considered as source of vitamin A and its pulp source of Fe, Mn and Cu, while jussara pulp may be considered a source of Mn, Cu and vitamins A and E. The phenolic profile of jabuticaba fractions (pulp, peel, seeds and depulping residue) and jussara whole fruit and seed was investigated for the first time. Eleven phenolic compounds were determined in each fruit, of which soluble forms were predominant, anthocyanins being the most abundant phenolics. Jabuticaba and jussara presented higher antioxidant activity compared with berries. Our results indicate that jabuticaba and jussara have high commercial potential due to their nutritional and functional properties.

* *Corresponding author.* Laboratório de Alimentos Funcionais, Instituto de Nutrição Josué de Castro, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho, 373, CCS, Bloco J, 2° andar, sala 16, 21941-902 Rio de Janeiro, Brazil. Tel.: +55 21 3938 6449; fax: +55 21 3938 8213.
E-mail address: mariana@nutricao.ufrj.br (M.C. Monteiro).

Chemical compounds: Ascorbic acid (PubChem CID: 54670067); β-Carotene (PubChem CID: 5280489); Cyanidin-3-O-glucoside (PubChem CID: 441667); Cyanidin-3-O-rutinoside (PubChem CID: 441674); Delphinidin-3-O-glucoside (PubChem CID: 443650); Ellagic acid (PubChem CID: 5281855); Gallic acid (PubChem CID: 370); 4-Hydroxybenzoic acid (PubChem CID: 135); Rutin (PubChem CID: 5280805); γ-Tocopherol (PubChem CID: 92729).

http://dx.doi.org/10.1016/j.jff.2015.06.002

1. Introduction

The Brazilian fruits jabuticaba (*Myrciaria jaboticaba* (Vell.) O. Berg) and jussara (*Euterpe edulis* Mart.) are threatened plant species from the Brazilian Atlantic Forest. These fruits are highly appreciated for their taste and flavour but seldom used commercially, especially at an industrial scale. The commercial sustainable use of these fruits might help in the preservation of the Brazilian Atlantic Forest, which is one of the 35 biomes on the planet considered as biodiversity hotspots.

Jabuticaba is a fruit tree grown at a low-scale by small farmers mainly from the southeastern region of Brazil. The tree produces fruits once or, more rarely, twice a year, usually between the months of August and November. Jabuticaba fruit presents a dark purple to black peel, and a whitish pulp with sweet and slightly tangy taste and appreciable flavour. Because of these quality attributes, this fruit has good marketing potential, and it is used for producing high quality artisanal products, such as juice, jam, jelly, vinegar, liqueur and wine. Although jabuticaba is known and appreciated throughout Brazil, it does not have high economic value because of its perishability, which hampers commercialization (Wu, Long, & Kennelly, 2013).

Jussara palm is very similar to açaí palm (*Euterpe oleracea*); however, in contrast to açaí berry use, the main form of jussara commercialization is through extraction of the edible palm heart. Jussara palm is not commercially grown, being found in forests of the southern and southeastern regions of Brazil. Its rapid and illegal exploitation, without appropriate management, has contributed to jussara being included in the endangered species list. Jussara fruit is composed of a single seed covered with a thin shiny dark purple to black coloured skin. Jussara is harvested annually, from April to November, and the fruit is not eaten fresh. Jussara fruit is typically added to water to soften its skin and its pulp that may be used for producing juices and beverages, ice cream and jam, among other products, is extracted. Harvesting of jussara fruits is a sustainable activity potentially more profitable than extraction of the palm heart (Borges et al., 2011; da Silva et al., 2013).

In general, berries present a wide variety of phenolic compounds as well as carotenoids, vitamins and minerals (de Souza et al., 2014). These compounds are associated with berries' bioactivity and potential health benefits in humans (Nile & Park, 2014). Since jabuticaba and jussara are rich in bioactive compounds, such as anthocyanins and other phenolic compounds and show high antioxidant activity (AA) (Bicudo, Ribani, & Beta, 2014; Borges et al., 2011; Rufino et al., 2010; Wu, Dastmalchi, Long, & Kennelly, 2012), these Brazilian fruits might also present bioactivity.

In addition to providing novel compositional data, a more detailed nutritional and functional characterization of jabuticaba and jussara and their fractions (peel, seed and pulp) will hopefully allow assessing the technological potential of these fruits and value-added products, thus contributing to the sustainable use of Brazilian Atlantic Forest resources. Therefore, the aim of the present study was to determine the antioxidant activity and the chemical composition of jabuticaba and jussara fruits and their fractions (Supplementary Fig. S1), with emphasis on phenolic compounds, carotenoids, tocopherols and minerals.

2. Materials and methods

2.1. Standards and chemicals

Standard solutions of minerals were purchased from Quimlab Química & Metrologia® (São Paulo, Brazil). Folin–Ciocalteu reagent, 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ), 2,2′-azino-bis (2-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), potassium persulphate, (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), fluorescein, potassium phosphate, sucrose, glucose and tocopherol standards were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Sodium carbonate and aluminium chloride were purchased from Spectrum Chemical Manufacturing Corp. (Gardena, CA, USA). Iron (II) sulphate, fructose and L(+)-ascorbic acid were purchased from Merck KGaA (Darmstadt, Germany). Anthocyanin and non-anthocyanin standards were purchased respectively from Indofine Chemical Co. (Hillsborough, NJ, USA) and Sigma-Aldrich Chemical Co. β-Carotene standard was isolated from carrot by open column chromatography. All solvents were HPLC grade from Tedia (Fairfield, OH, USA). HPLC grade water (Milli-Q System, Millipore, Bedford, MA, USA) was used throughout the experiments.

2.2. Jabuticaba and jussara samples

Jabuticaba fruits (*M. jaboticaba*, cv. Sabará) from Minas Gerais state, Brazil, from the 2012 crop, were purchased at Rio de Janeiro's agricultural trading centre. Jussara palm (*E. edulis*) fruits from the 2012 crop were obtained from Juçaí® processing company, situated in Resende (Rio de Janeiro state, Brazil). Fruits were selected, washed and sanitized in 100 ppm solution of sodium hypochlorite for 15 min.

Jabuticaba pulp was separated in a horizontal depulper, yielding a residue composed of peel and seeds, which were manually separated. Jussara fruits were immersed in water at 40 °C for 30 min for peel softening and processed in a vertical depulper with water addition (1 L of water per 2.5 kg of fruit), yielding jussara pulp and seeds. All samples were freeze-dried (Labconco, Kansas City, MO), with the exception of jussara seeds that were oven-dried, and stored at –20 °C.

2.3. Proximate composition, pH and total soluble solids

Moisture, protein, lipid, dietary fibre and ash contents of fruits and their fractions were determined in triplicate, according to official methods (Association of Official Analytical Chemists, 2000). Carbohydrate content was determined by difference. Total soluble solids (TSS) of jabuticaba and jussara pulps and pH values of fruits and their fractions were determined by official methods (Association of Official Analytical Chemists, 2000). Energy values were calculated from the contents of lipids, proteins and carbohydrates multiplied by the Atwater factors (9 kcal/g, 4 kcal/g, and 4 kcal/g, respectively).

2.4. Sugar contents by HPLC-RI

Glucose, fructose and sucrose in jabuticaba and jussara pulps were analysed in duplicate directly after centrifugation

(11,300× *g*, 10 min) and filtration through a 0.45 μm PTFE filter unit (Millipore, Barueri, Brazil). Sugars were determined according to Farah, Monteiro, Calado, Franca, and Trugo (2006), using a liquid chromatography system composed of a quaternary pump LC-20AT (Shimadzu®, Kyoto, Japan), a refractive index (RI) detector M-410 (Waters, Milford, MA, USA) and a signal integrator C-R6A (Shimadzu®). Chromatographic separation of sugars was achieved using NH_2 column (5 μm, 250 mm × 4.6 mm, Zorbax®) and isocratic elution with 82% acetonitrile in water at a flow rate of 1.0 mL/min. Identification of sugars was performed by comparison with retention time of the respective standard and quantification was performed by external calibration.

2.5. Mineral composition by ICP-OES and ICP-MS

Sample digestion was performed in triplicate according to official methods (Association of Official Analytical Chemists, 2000).

Calcium, sulphur, phosphorus, iron, magnesium, manganese, potassium and sodium contents were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES) with double vision configuration (axial and radial) (iCAP 6300 Thermo Scientific®, Cambridge, England), equipped with cyclonic spray chamber, MiraMist nebulizer (Mira Mist CE, Burgener Research Inc., Mississauga, ON, Canada), charge coupled-device detector (CCD) and iTEVA 2.0 operating software for data acquisition.

Fe (λ = 249.940), K (λ = 769.896), Mn (λ = 257.610), Na (λ = 589.592), P (λ = 213.618) and S (λ = 182.034) contents were determined on axial view and Ca (λ = 393.366) and Mg (λ = 280.270) contents on radial view. Quantification was performed by external calibration. The operating conditions of ICP-OES were 1200 W of incident power, 12 L/min of plasma gas flow, 1.0 L/min of auxiliary gas flow, 0.19 bar of nebulizer pressure, 50 rpm of rotation speed of the peristaltic pump during data acquisition and 1 s of integration.

Cadmium, cobalt, copper, nickel, zinc and selenium contents were determined using an inductively coupled plasma mass spectrometer (ICP-MS) (iCAP Qc, Thermo Fisher Scientific, Bremen, Germany) equipped with collision and reaction cells, sample cones, nickel skimmer, quartz double pass cyclonic spray chamber (baffled), Teflon® concentric nebulizer (FPA-ST), Peltier, quartz injector of 2.5 mm diameter, autosampler (ASX 520, CETAC Technologies, Omaha, NE, USA) and Qtegra operating software (version 1.5.1189.1) for data acquisition.

The elements were determined in the form of isotopes ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{82}Se and ^{111}Cd in standard mode. To correct the transport interferences in sample introduction and ionization, internal standardization was performed with the addition and monitoring of isotopes ^{45}Sc, ^{73}Ge, ^{103}Rh and ^{205}Tl at a final concentration of 5 μg/L. Quantification was performed by external calibration. The operating conditions of ICP-MS were 1500 W of incident power, 14 L/min of plasma gas flow, 0.8 L/min of auxiliary gas flow, 10 ms of dwell time and 1 channel per mass unit.

2.6. Ascorbic acid content by HPLC-DAD

Ascorbic acid in jabuticaba and jussara pulps was analysed in duplicate directly after centrifugation (11,300× *g*, 10 min) and filtration through a 0.45 μm PTFE filter unit. The liquid chromatography system (Shimadzu®) included a quaternary pump LC-20AT, automatic injector SIL-20AHT, diode-array detector (DAD) SPD-M20A, system controller CBM-20A and degasser DGU-20A5. Chromatographic separation of ascorbic acid was achieved using a reverse phase column (C18, 5 μm, 250 mm × 4.6 mm, Kromasil®) and isocratic elution with 2% KH_2PO_4 buffer at a flow rate of 0.4 mL/min, according to Scartezzini, Antognoni, Raggi, Poli, and Sabbioni (2006), with adaptations. Ascorbic acid was monitored at 243 nm.

Identification of ascorbic acid was performed by comparison with retention time and UV absorption spectrum of the standard. Quantification was performed by external calibration. Data were acquired by LC solution software (Shimadzu Corporation®, version 1.25, 2009).

2.7. Carotenoid and tocopherol contents by HPLC-DAD-FL

Carotenoids and tocopherols were analysed using the methods described by Rahmani and Csallany (1991) and Tan and Brzuskiewicz (1989), with adaptations. Whole fruits were extracted in triplicate with 5 mL of methanol and 5 mL of hexane in an ultrasound bath for 5 min and vortexed for 1 min. After centrifugation (1690× *g*, 10 min, 10 °C), the upper hexane phase was collected and the lower aqueous phase was re-extracted three times following the same procedure. Hexane extracts were combined, the solvent removed and the dry residue reconstituted in hexane. Saponification of extract was tested and found to be unnecessary.

The liquid chromatography system (Shimadzu®) included a quaternary pump LC-20AT, DAD SPD-M20A and fluorescence (FL) RF-10AXL detector, system controller CBM-20A and degasser DGU-20A5. Chromatographic separation of carotenoids and tocopherols was achieved using a normal phase silica column (5 μm, 250 mm × 4.6 mm, Zorbax®) and isocratic elution with hexane:isopropanol (99:1, *v*/*v*) at a flow rate of 1.0 mL/min. Carotenoids were monitored by DAD at 450 nm and tocopherols by FL at 330 nm for emission and 290 nm for excitation.

Identification of analytes was performed by comparison with retention time and absorption spectrum of the respective standard. Quantification was performed by external calibration. Data were acquired by LC solution software (Shimadzu Corporation®, version 1.25, 2009).

2.8. Phenolic compounds profile by HPLC-DAD

2.8.1. Anthocyanins

Samples extraction was according to Rufino et al. (2010) with modifications, except for jabuticaba pulp. Samples were extracted with methanol 50% in an Ultraturrax extractor T18 BASIC (IKA®, Staufen, Germany) at 14,000 rpm for 1 min. After centrifugation (1700× *g*, 10 min, 20 °C), the residue was re-extracted once with methanol 50% and then with acetone:water:acetic acid (70:29.5:0.5, *v*/*v*/*v*) as many times as necessary to obtain a colourless supernatant. Supernatants were combined and filtered with paper filter (Whatman no. 1). Since jabuticaba pulp showed a very light pink colour, it was analysed directly after centrifugation (11,300× *g*, 10 min). All extractions were performed in triplicate.

The liquid chromatography system described in section 2.6 was employed. Chromatographic separation of anthocyanins was achieved using a reverse phase column (C18, 5 μm, 250 mm × 4.6 mm, Kromasil®), adapted from de Brito et al. (2007). The mobile phase consisted of a gradient of 1% aqueous formic acid (eluent A), 1% formic acid in methanol (eluent B) and acetonitrile (eluent C), at a flow rate of 2.0 mL/min. Eluent C concentration was kept constant at 2% during analysis. Prior to injection, the column was equilibrated with 18% B. After sample injection, solvent composition was kept constant until 2 min, increased to 32% B in 6 min and to 52% B in 8 min, and then decreased to 18% B in 18 min. Between injections, 10 min intervals were allowed to re-equilibrate the column with 18% B. Anthocyanins were monitored at 530 nm.

2.8.2. *Non-anthocyanin phenolic compounds*

Extraction of soluble and insoluble non-anthocyanin phenolic compounds from jabuticaba and jussara fruits was performed in triplicate, according to the adapted methodology of Mattila and Kumpulainen (2002). For soluble phenolic compounds, samples were extracted for 10 min with 20 mL of cold ethanol 80% and centrifuged (2500× *g*, 5 min, 10 °C). The supernatant was collected and the residue re-extracted. Supernatants were combined, the solvent removed and the dry residue was re-constituted in water.

For alkaline hydrolysis, the soluble phenolic compound extraction residue was incubated with 12 mL of water and 5 mL of NaOH (10 M) at room temperature in the dark for 16 hours with agitation. After this period, the pH was adjusted to 2 and the mixture was extracted for 30 s with 15 mL of ethyl acetate. After centrifugation (2500× *g*, 5 min, 10 °C), the supernatant was collected and the extraction procedure repeated twice. Supernatants were combined, the solvent removed and the dry residue was reconstituted in methanol 80%.

For acid hydrolysis, the alkaline hydrolysis extraction residue was incubated with 2.5 mL of concentrated HCl at 85 °C for 30 min. Then, the same extraction with ethyl acetate described for alkaline hydrolysis was performed. All extracts were filtered through a 0.45 μm cellulose ester membrane (Millipore®, São Paulo, Brazil) prior to HPLC analysis.

Chromatographic separation of non-anthocyanin phenolic compounds was achieved using a reverse phase column (C18, 5 μm, 250 mm × 4.6 mm, Phenomenex®), adapted from Alves and Perrone (2015). The mobile phase consisted of a gradient of 0.3% aqueous formic acid (eluent A), methanol (eluent B) and acetonitrile (eluent C), at a flow rate of 1.0 mL/min. Eluent C concentration was kept constant at 1% during analysis. Prior to injection, the column was equilibrated with 18% B. After sample injection, solvent composition changed to 20% B in 1 min, 43% B in 18 min, 85% in 23 min and kept constant until 30 min. Between injections, 10 min intervals were allowed to re-equilibrate the column with 18% B. Non-anthocyanin phenolic compounds were monitored from 190 to 370 nm.

Identification of all phenolic compounds was performed by comparison with retention time and absorption spectrum of the respective standard. Quantification was performed by external calibration. Data were acquired by LC solution software (Shimadzu Corporation®, version 1.25, 2009).

2.9. *Antioxidant activity*

The AA was determined in the same extracts used for anthocyanin analysis using Folin–Ciocalteu, FRAP (ferric reducing ability of plasma), TEAC (Trolox equivalent antioxidant capacity) and ORAC (oxygen radical antioxidant capacity) assays.

The Folin–Ciocalteu assay was performed as described by Singleton, Orthofer, and Lamuela-Raventós (1999). The results were expressed as g of gallic acid equivalents (GAE) per 100 g on a dry weight basis (dwb). The FRAP assay was performed according to Benzie and Strain (1996) with slight modifications. Results were expressed as mmol of Fe^{2+} equivalents per 100 g dwb. The TEAC assay was performed according to Re et al. (1999) with slight modifications. Results were expressed as mmol of Trolox equivalents per 100 g dwb. The ORAC assay was performed according to Zulueta, Esteve, and Frígola (2009) with slight modifications. Results were expressed as mmol of Trolox equivalents per 100 g dwb. Each extract was analysed in triplicate.

2.10. *Statistical analysis*

Data were expressed as mean ± standard deviation. Analysis of variance (one-way ANOVA) followed by Tukey's multiple comparison post-test was performed for comparing fruits fractions, using GraphPad Prism software for Windows, version 5.04 (GraphPad Software, San Diego, CA, USA). Pearson correlation analysis was performed to evaluate associations between variables, using Statistica software, version 7.0 (StatSoft Inc., Tulsa, OK). Results were considered significant when $p < 0.05$.

3. Results and discussion

3.1. *Proximate composition, total soluble solids, pH values and sugar contents*

Jabuticaba and jussara pulps exhibited high moisture contents, 87.3% on average (Table 1). Brazilian legislation classification of açaí pulp according to moisture content could be adopted for jussara pulp because of their similarities. In that case, the jussara pulp sample would be classified as "thick or special" (moisture <86 g/100 g). Jabuticaba fractions presented lower pH values (3.3 to 4.1) than jussara (4.8 to 6.2), which were similar to those previously reported (4.8) (Borges et al., 2011). Low pH values favour anthocyanin stability and inhibit microbial growth. Jabuticaba fruit and its fractions showed low lipid content (<1.8%). In contrast, jussara pulp exhibited high lipid content (46.6%), similar to those found in other studies regarding jussara (18% to 44%) and açaí pulps (33% to 49%) (Borges et al., 2011). Jabuticaba depulping residue and peel, and jussara pulp presented the highest ash contents among fractions (>3.4%) and all samples exhibited low protein contents (<8.5%). In general, our results are in accordance with previous published proximate composition data (Alezandro, Dubé, Desjardins, Lajolo, & Genovese, 2013; Borges et al., 2011; da Silva, Rodrigues, Mercadante, & de Rosso, 2014; Rufino et al., 2010).

In general, all fractions presented high contents of carbohydrates (>85.7%), with the exception of jussara pulp (42.5%)

Table 1 – Proximate composition and pH values of jabuticaba and jussara fruits and their fractions.

	Jabuticaba					*Jussara*		
	Whole fruit	Pulp	Peel	Seed	Depulping residue	Whole fruit	Pulp	Seed
Moisture[1] (%)	87.4 ± 0.2[b]	90.7 ± 6.2[a]	80.9 ± 0.2[c]	58.0 ± 0.2[e]	76.6 ± 1.9[d]	51.9 ± 0.3[b]	83.8 ± 0.5[a]	48.9 ± 0.2[c]
Ash[1] (%)	3.1 ± 0.0[b]	3.2 ± 0.2[b]	4.0 ± 0.0[a]	2.4 ± 0.0[c]	4.2 ± 0.1[a]	2.5 ± 0.0[b]	3.4 ± 0.0[a]	1.7 ± 0.0[c]
Lipid[1] (%)	1.8 ± 0.0[a]	0.2 ± 0.0[d]	0.6 ± 0.0[b]	0.6 ± 0.0[b]	0.5 ± 0.0[c]	6.9 ± 0.3[b]	46.6 ± 0.0[a]	0.7 ± 0.0[c]
Protein[1] (%)	5.0 ± 0.1[c]	3.5 ± 0.0[d]	8.5 ± 0.0[a]	7.1 ± 0.2[b]	7.5 ± 0.2[b]	5.0 ± 0.3[b]	7.5 ± 0.1[a]	4.3 ± 0.1[b]
Carbohydrate[1] (%)	90.1 ± 0.1[b]	93.1 ± 0.2[a]	86.9 ± 0.3[d]	89.8 ± 0.3[b]	87.9 ± 0.0[c]	85.7 ± 0.4[b]	42.5 ± 0.1[c]	93.3 ± 0.1[a]
Dietary fibre[1] (%)	38.2 ± 0.6[a]	ND[3]	38.4 ± 1.2[a]	31.8 ± 0.7[b]	36.1 ± 0.7[a]	71.8 ± 0.6[b]	27.1 ± 0.0[c]	79.0 ± 0.4[a]
Energy value (kcal/100g fwb[2])	31	36	44	112	56	66	83	41
pH	3.6 ± 0.0[b]	3.3 ± 0.0[d]	3.3 ± 0.0[d]	4.1 ± 0.0[a]	3.4 ± 0.0[c]	5.5 ± 0.0[b]	4.8 ± 0.0[c]	6.2 ± 0.0[a]

[1] Results expressed on dwb as mean ± SD for triplicates. Different letters in the line indicate significant difference between fractions of the same fruit (One-way ANOVA followed by Tukey *post hoc*, $p < 0.05$).
[2] Fresh weight basis.
[3] Not detected.

(Table 1). Dietary fibre comprised more than 64% of carbohydrates in jussara fruit and its fractions. In fact, low contents of fructose (0.5 ± 0.05 g/100 mL fwb) and glucose (0.3 ± 0.01 g/100 mL fwb) were found in jussara pulp, as well as low TSS (3.0 °Brix). In contrast, dietary fibre comprised less than 44% of carbohydrates in jabuticaba fruit and its fractions. Moreover, jabuticaba pulp carbohydrates were not composed of dietary fibre, but simple sugars, from which fructose (6.9 ± 0.3 g/100 mL fwb) was the most abundant, followed by glucose (4.2 ± 0.1 g/100 mL fwb) and sucrose (0.5 ± 0.02 g/100 mL fwb). These results were consistent with the high TSS (12.0 °Brix) and are in accordance with published data (Lima, Corrêa, Dantas-Barros, Nelson, & Amorim, 2011). The high amounts of sugars found in jabuticaba pulp are responsible for the fruit's sweet taste and high potential for juice production.

The consumption of 100 g of fresh jabuticaba fruit and jussara pulp would provide approximately 17% of the recommended daily intake of total fibre for adults (Otten, Hellwig, & Meyers, 2006). Even though jussara seeds and jabuticaba peel, seeds and depulping residue are non-edible fractions, they might be exploited as dietary fibre sources for food enrichment.

A small cup (200 mL) of fresh jabuticaba pulp contains 66 kcal, which represents only 3.3% of daily energy intake based on a 2000 kcal diet. This energy density is similar to that of blackberry juice (76 kcal) and lower than reported for highly consumed fruit juices like orange (90 kcal), apple (92 kcal), cranberry (92 kcal), pineapple (106 kcal) and grape (120 kcal) (United States Departament of Agriculture, Agricultural Research Service, 2014). On the other hand, jussara pulp presents higher energy density (0.8 kcal/mL), mainly for its high lipid content and therefore may be used as nutritional supplement in weight gain diets.

3.2. *Micronutrients*

3.2.1. *Minerals*

Fourteen minerals were quantified in jabuticaba and jussara fruits and their fractions (Table 2). Pulps, the main edible part of these fruits, presented the highest contents of most minerals. In general, jabuticaba mineral composition was in accordance with data reported by Alezandro et al. (2013) and Lima, Corrêa, Dantas-Barros et al. (2011), with the exception of iron, which content was 8.8-fold higher in the whole fruit than that reported by these same authors. This difference may be related to variations in cultivar, soil, weather, agricultural practices and ripeness stage (de Souza et al., 2014). To the best of our knowledge, cobalt, nickel and cadmium were analysed for the first time in jabuticaba fruit. These two latter heavy metals were found in contents that do not pose any risk of human intoxication through intake (European Food Safety Authority, 2006). Moreover, selenium, which has only been reported to occur as traces in jabuticaba (Alezandro et al., 2013; Lima, Corrêa, Dantas-Barros et al., 2011), was quantified in this fruit and its fractions in the present study due to the high sensitivity of the ICP-MS technique.

Although iron bioavailability from vegetable foods is generally lower than from animal sources, the iron content found in jabuticaba pulp (6.0 mg/100 g fwb) was much higher than that of well-known iron-rich vegetable foods, such as baked beans (1.5 mg/100 g fwb) and cabbage (0.5 mg/100 g fwb). Therefore, consumption of a small cup (200 mL) of fresh jabuticaba pulp would account for 70% and 31% of the RDA for male and female adults, respectively (Otten et al., 2006) (Supplementary Table S1). Considering the high prevalence of iron-deficiency in Brazil, jabuticaba fruit products might contribute to increase dietary iron intake. Jabuticaba may also be considered as a nutritional source of copper, contributing to 34% of the RDA for adults per 200 mL of pulp, and manganese, contributing to 17% and 22% of the RDA for male and female adults, respectively (Otten et al., 2006). Potassium was the most abundant mineral in jabuticaba pulp and its contribution to RDA (7.2% per 200 mL cup of pulp) (Otten et al., 2006) is similar to that of other juices recognized to be sources of potassium such as grapefruit (6.8%), tangerine (7.6%), orange (8.4%) and passion fruit (11.8%) (United States Departament of Agriculture, Agricultural Research Service, 2014).

The mineral contents in jussara pulp were similar, yet consistently lower than those reported by da Silva et al. (2013), with the exception of potassium, which concentration was 3.6-fold higher than that reported by these authors. The manganese

Table 2 – Mineral contents in jabuticaba and jussara fruits and their fractions.[1]

Element	*Jabuticaba*				*Jussara*			
	Whole fruit	Pulp	Peel	Seed	Depulping residue	Whole fruit	Pulp	Seed
Ca (mg/100 g)	27.1 ± 2.6^{c}	67.4 ± 1.5^{a}	51.0 ± 0.8^{b}	17.1 ± 2.7^{d}	46.3 ± 2.9^{b}	63.8 ± 3.3^{b}	76.4 ± 2.9^{a}	50.7 ± 2.7^{c}
Fe (mg/100 g)	23.7 ± 1.3^{b}	32.8 ± 0.8^{a}	3.6 ± 0.3^{d}	1.3 ± 0.2^{e}	9.8 ± 0.3^{c}	1.67 ± 0.4^{b}	4.3 ± 0.6^{a}	2.3 ± 0.1^{b}
P (mg/100 g)	75.7 ± 8.8^{c}	176.6 ± 6.8^{a}	89.0 ± 8.7^{c}	95.1 ± 5.8^{bc}	111.2 ± 8.6^{b}	69.2 ± 12.2^{b}	41.2 ± 1.4^{c}	90.7 ± 2.7^{a}
K (mg/100 g)	700.7 ± 81.2^{d}	1978.5 ± 174.0^{a}	1006.0 ± 29.5^{b}	401.2 ± 70.4^{e}	806.9 ± 12.9^{cd}	361.0 ± 42.0^{a}	419.1 ± 26.9^{a}	333.3 ± 38.9^{a}
Na (mg/100 g)	23.3 ± 0.9^{d}	153.6 ± 11.0^{a}	60.7 ± 2.0^{b}	38.3 ± 5.6^{cd}	46.0 ± 6.2^{bc}	21.8 ± 2.5^{a}	17.3 ± 0.1^{a}	17.6 ± 0.3^{a}
Mn (mg/100 g)	1.1 ± 0.1^{b}	2.3 ± 0.2^{a}	1.0 ± 0.1^{b}	0.4 ± 0.1^{c}	1.2 ± 0.1^{b}	2.8 ± 0.9^{a}	3.0 ± 0.0^{a}	3.6 ± 0.2^{a}
Mg (mg/100 g)	72.3 ± 7.6^{bc}	187.9 ± 7.2^{a}	65.4 ± 1.2^{cd}	51.3 ± 8.0^{d}	85.3 ± 4.0^{b}	32.1 ± 4.2^{b}	47.4 ± 1.4^{a}	30.2 ± 0.8^{b}
S (mg/100 g)	31.0 ± 3.4^{c}	57.1 ± 8.5^{ab}	66.9 ± 8.8^{a}	35.1 ± 3.3^{c}	45.7 ± 2.9^{bc}	26.9 ± 2.9^{a}	35.4 ± 4.9^{a}	28.4 ± 0.5^{a}
Zn (mg/100 g)	1.1 ± 0.2^{bc}	3.1 ± 0.1^{a}	1.3 ± 0.1^{b}	0.8 ± 0.2^{c}	1.3 ± 0.2^{b}	0.6 ± 0.1^{b}	0.9 ± 0.0^{ab}	1.2 ± 0.3^{a}
Cu (mg/100 g)	0.8 ± 0.2^{b}	1.8 ± 0.4^{a}	0.6 ± 0.1^{b}	0.5 ± 0.1^{b}	0.5 ± 0.0^{b}	0.3 ± 0.0^{b}	0.5 ± 0.0^{a}	0.3 ± 0.0^{b}
Ni (mg/100 g)	2.8 ± 0.2^{b}	3.9 ± 0.0^{a}	0.2 ± 0.0^{d}	0.1 ± 0.0^{d}	0.9 ± 0.0^{c}	0.5 ± 0.0^{b}	1.0 ± 0.1^{a}	0.4 ± 0.0^{b}
Co (µg/100 g)	30.0 ± 3.4^{b}	37.2 ± 1.5^{a}	2.7 ± 0.2^{d}	0.9 ± 0.2^{d}	8.9 ± 0.5^{c}	13.6 ± 1.9^{b}	7.1 ± 0.2^{b}	49.5 ± 10.3^{a}
Se (µg/100 g)	1.9 ± 0.2^{a}	3.4 ± 1.5^{a}	1.2 ± 0.3^{a}	1.2 ± 0.1^{a}	3.1 ± 0.3^{a}	1.0 ± 0.1^{a}	0.5 ± 0.1^{b}	0.6 ± 0.1^{b}
Cd (µg/100 g)	1.0 ± 0.0^{b}	2.5 ± 0.5^{a}	1.2 ± 0.2^{b}	0.9 ± 0.1^{b}	1.0 ± 0.0^{b}	1.1 ± 0.2^{a}	1.2 ± 0.0^{a}	1.4 ± 0.1^{a}

[1] Results expressed on dwb as mean ± SD for triplicates. Different letters in each column indicate significant difference between fractions of the same fruit (One-way ANOVA followed by Tukey *post hoc* test, $p < 0.05$).

content of jussara pulp (0.5 mg/100 g fwb) is similar to other commonly consumed berries (0.3 mg/100 g to 0.7 mg/100 g fwb) (United States Departament of Agriculture, Agricultural Research Service, 2014). In fact, jussara may be considered as a nutritional source of manganese, contributing to 40% and 51% of the RDA for male and female adults, respectively, per 200 mL cup of pulp, and copper, contributing to 17% of the RDA for adults (Otten et al., 2006) (Supplementary Table S1).

3.2.2. *Vitamins*

The content of ascorbic acid in jabuticaba pulp (8.6 ± 0.2 mg/100 mL) was lower than that reported by Abe, Lajolo, and Genovese (2011) and Lima, Corrêa, Saczk, Martins, and Castilho (2011) (25 mg/100 mL) and much lower than that reported by Rufino et al. (2010) (238 mg/100 mL). These last authors also reported a much higher content for jussara pulp (186 mg/100 mL) than that observed in the present study (5.2 ± 0.1 mg/100 mL). It is noteworthy that the HPLC method employed in the present work is much more specific than the 2,6-dichlorophenol indophenol method used by Rufino et al. (2010), especially considering that the red colour of the titrimetric endpoint is very similar to the colour of these berries. Moreover, the variability of ascorbic acid contents could also be related to soil, weather, agricultural practices and ripeness stage, as well as losses during harvest, transport, storage and depulping process. Nevertheless, ascorbic acid contents in jabuticaba and jussara pulps are similar to other non-citrus fruit juices, such as blackberry, cranberry and prune (4 mg/100 mL to 11 mg/100 mL) (United States Departament of Agriculture, Agricultural Research Service, 2014), and a 200 mL cup of jabuticaba pulp would contribute to 19% and 23% of the RDA for male and female adults, respectively.

β-Carotene was the only carotenoid identified and quantified in jabuticaba (873 ± 27 µg/100 g dwb; 73 µg retinol activity equivalent/100 g dwb) and jussara whole fruits (86 ± 12 µg/100 g dwb; 7 µg retinol activity equivalent/100 g dwb) (Supplementary Fig. S2). If one considers that the pulp corresponds to approximately only 10% of the whole fruit weight, jussara pulp β-carotene content would be around 2500 µg/100 g dwb, similar to that reported by da Silva et al. (2014) (2354 µg/100 g dwb). These authors also reported 11 other carotenoids using HPLC-DAD-MS, including lutein, α-carotene, 13-*cis*-β-carotene, 9-*cis*-neoxanthin, *cis*-lutein, 15-*cis*-β-carotene, totalling 6522 µg/100 g dwb. To the best of our knowledge, our work is the first report on the carotenoid profile of jabuticaba.

According to the classification sources of carotenoids proposed by Britton and Khachik (2009), both jabuticaba whole fruit (110 µg/100 g fwb) and jussara pulp (414 µg/100 g fwb) would be considered as moderate sources, similarly to blackberry (128 µg/100 g fwb), papaya (274 µg/100 g fwb), watermelon (303 µg/100 g fwb) and guava (374 µg/100 g fwb). Commonly consumed berries, such as strawberry, raspberry and cherry (7 µg/100 g to 38 µg/100 g fwb) are classified as low sources of carotenoids (United States Departament of Agriculture, Agricultural Research Service, 2014).

To the best of our knowledge, this is the first study to report tocopherol contents in both jabuticaba and jussara fruits (Supplementary Fig. S3). Jabuticaba whole fruit contained α- (0.72 ± 0.04 mg/100 g dwb), β- (0.13 ± 0.01 mg/100 g dwb) and γ-tocopherols (0.01 ± mg/100 g dwb). Although β-tocopherol

content in jabuticaba fruit was similar to that of berries, such as strawberry, blueberry, raspberry and blackberry (0.11 mg/100 g to 0.42 mg/100 g dwb), total tocopherol content was much lower than that of these berries (4.2 mg/100 g to 21.5 mg/100 g dwb) (United States Departament of Agriculture, Agricultural Research Service, 2014). Jussara whole fruit also presented α- (0.59 ± 0.06 mg/100 g dwb), β- (0.03 ± 0.00 mg/100 g dwb) and γ-tocopherols (0.06 ± 0.00 mg/100 g dwb). Jussara pulp may be considered as a source of vitamin E, as 200 g of fresh pulp would contribute to 39% of the RDA for adults (Otten et al., 2006). Similarly to jussara, Darnet, Serra, Rodrigues, and da Silva (2011) reported α-, β- and γ-tocopherols in açaí pulp, but their total tocopherol content was twice higher than that estimated in the present study for jussara pulp.

3.3. *Phenolic compounds*

Phenolic compounds occur in plants as soluble (aglycones and conjugated glycosides) and insoluble forms. Phenolics in the insoluble forms are covalently bound to cell wall structural components such as cellulose, hemicellulose, lignin, pectin and structural proteins. This fraction is not extractable by organic solvents, being released after alkaline and acid treatments. While alkaline hydrolysis breaks ester bonds, acid hydrolysis breaks glycosidic bonds, but generally leaves ester bonds intact (Acosta-Estrada, Gutiérrez-Uribe, & Serna-Saldívar, 2014). To the best of our knowledge, the profile of soluble and insoluble forms of phenolic compounds of jabuticaba and jussara were investigated for the first time in the present study.

Eleven phenolic compounds were identified and quantified in jabuticaba whole fruit, being three hydroxybenzoic acid derivatives (gallic, 3,4-dihydroxybenzoic and ellagic acids), two hydroxycinnamic acid derivatives (*m*-coumaric and *trans*-cinnamic acids), four flavonols (rutin, myricetin, myricitrin and quercetin) and two anthocyanins (cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside) (Supplementary Fig. S4). All of these compounds have already been reported in jabuticaba whole fruit by other authors (Wu et al., 2013), with the exception of *m*-coumaric acid, which was found in jabuticaba for the first time, to the best of our knowledge, in the present study. Other thirty-six phenolic compounds (four anthocyanins, three flavonols, eleven gallotannins, twelve ellagitannins, two depsides, one hydroxybenzoic acid, one hydroxycinnamic acid and two hydroxycinnamic alcohols) have been reported to occur in jabuticaba fruit (Alezandro et al., 2013; Wu et al., 2013).

Total phenolic content in jabuticaba whole fruit was 815 mg/100 g dwb, being 56% of soluble phenolics and 44% of insoluble phenolics (Table 3). Of those insoluble forms of phenolics, half were linked to cell wall constituents by ester bonds and half by glycosidic bonds. As observed in the present study, soluble phenolics are those reported to be predominant in fruits and vegetables (Acosta-Estrada et al., 2014). Total soluble phenolic content in jabuticaba whole fruit (452 mg/100g dwb) was lower than that reported by Reynertson, Yang, Jiang, Basile, and Kennelly (2008) (604 mg/100 g dwb) and by Wu et al. (2012) (868 mg/100 g dwb). These differences may be related to variations in cultivar, soil, weather, agricultural practices and ripeness stage, but also to a larger number of phenolics identified by Wu et al. (2012) using LC-TOF-MS.

Cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, rutin, quercetin, myricitrin and *m*-coumaric acid were found exclusively as soluble forms in jabuticaba whole fruit. Myricetin was

Table 3 – Phenolic compound contents (mg/100 g dwb) in jabuticaba fruit and its fractions.[1]

Compound	Extract	Whole fruit	Pulp	Peel	Seed	Depulping residue
Gallic acid	Soluble phenolics	7.9 ± 0.1^{d}	3.2 ± 0.0^{d}	21 ± 1^{c}	30 ± 1^{b}	40 ± 2^{a}
Cyanidin-3-*O*-glucoside		280 ± 18^{c}	0.4 ± 0.0^{e}	1.261 ± 18^{a}	58 ± 4^{d}	707 ± 32^{b}
Delphinidin-3-*O*-glucoside		48 ± 1^{c}	ND[2]	269 ± 11^{a}	11.8 ± 0.4^{d}	157 ± 5^{b}
Rutin		77 ± 2^{c}	7.1 ± 0.6^{d}	247 ± 5^{a}	241 ± 12^{a}	195 ± 5^{b}
Myricetin		0.4 ± 0.0^{c}	0.2 ± 0.0^{c}	4.3 ± 0.2^{a}	2.0 ± 0.1^{b}	2.1 ± 0.0^{b}
Quercetin		0.4 ± 0.0^{c}	ND	3.5 ± 0.0^{a}	0.4 ± 0.0^{c}	1.6 ± 0.0^{b}
m-coumaric acid		0.3 ± 0.0^{c}	0.6 ± 0.0^{a}	0.2 ± 0.0^{d}	0.6 ± 0.1^{a}	0.4 ± 0.0^{c}
Myricitrin		3.5 ± 0.1^{b}	0.7 ± 0.0^{d}	20 ± 0^{a}	1.8 ± 0.0^{c}	20 ± 1^{a}
Ellagic acid		34 ± 1^{d}	5.3 ± 0.3^{e}	178 ± 5^{a}	83 ± 8^{c}	99 ± 4^{b}
Subtotal		**452 ± 22^{c}**	**18 ± 1^{d}**	**2004 ± 40^{a}**	**429 ± 26^{c}**	**1222 ± 49^{b}**
Gallic acid	Alkaline hydrolysis	135 ± 1^{c}	ND	123 ± 16^{c}	345 ± 5^{a}	253 ± 21^{b}
3,4-dihydroxybenzoic acid		4.4 ± 0.5^{c}	ND	16 ± 1^{a}	ND	8.4 ± 0.2^{b}
Myricetin		0.4 ± 0.0^{b}	0.7 ± 0.3^{b}	0.2 ± 0.0^{b}	2.1 ± 0.2^{a}	0.4 ± 0.0^{b}
Trans-cinnamic acid		1.4 ± 0.2^{a}	0.4 ± 0.0^{b}	0.5 ± 0.1^{b}	ND	0.6 ± 0.0^{b}
Ellagic acid		40 ± 1^{b}	ND	61 ± 7^{a}	41 ± 2^{b}	53 ± 4.0^{ab}
Subtotal		**181 ± 3^{c}**	**1.1 ± 0.3^{d}**	**201 ± 24^{c}**	**388 ± 7^{a}**	**315 ± 25^{b}**
Gallic acid	Acid hydrolysis	63 ± 3^{a}	1.3 ± 0.1^{e}	9.2 ± 0.5^{d}	47 ± 1^{b}	14 ± 1^{c}
Myricetin		ND	ND	0.6 ± 0.0^{a}	0.4 ± 0.1^{b}	0.4 ± 0.0^{ab}
Ellagic acid		119 ± 24^{a}	ND	37 ± 4^{b}	120 ± 13^{a}	106 ± 10^{a}
Myricitrin		ND	ND	ND	1.7 ± 0.1^{a}	ND
Subtotal		**182 ± 27^{a}**	**1.3 ± 0.1^{d}**	**47 ± 5^{c}**	**169 ± 14^{a}**	**120 ± 11^{b}**
Total		**815 ± 52^{d}**	**20 ± 1^{e}**	**2252 ± 69^{a}**	**986 ± 47^{c}**	**1658 ± 85^{b}**

[1] Results expressed as mean ± SD for triplicates. Different letters in the line indicate significant difference between fractions (One-way ANOVA followed by Tukey *post hoc* test, $p < 0.05$).

[2] Not detected.

equally distributed as soluble and insoluble forms. Gallic and ellagic acids were found predominantly as insoluble forms (89% on average) and 3,4-dihydroxybenzoic and *trans*-cinnamic acids were found exclusively as insoluble forms, being linked by ester bonds. In plants, phenolic acids occur mostly in the insoluble forms whereas flavonoids are usually found as soluble forms (Acosta-Estrada et al., 2014), which is in accordance with the phenolic profile observed in the present study.

Cyanidin-3-*O*-glucoside was the most abundant phenolic compound in jabuticaba whole fruit (34%), in accordance with data from other authors (Reynertson et al., 2008; Wu et al., 2012). However, differently from these authors, gallic (25%) and ellagic acids (24%) were also major phenolics. These compounds could only be found in large amounts because insoluble forms were considered in the present study, highlighting the relevance of alkaline and acid hydrolyses for properly quantifying phenolic compounds in fruits. In fact, Abe et al. (2011) and Alezandro et al. (2013) reported even higher levels of ellagic acid than that found in the present study, probably due to the employment of an extraction and hydrolysis procedure specific for the analysis of ellagitannins.

We also investigated for the first time the phenolic profile in different jabuticaba fractions (pulp, peel and seed) and depulping residue. Jabuticaba peel showed the highest total phenolic compounds content (2252 mg/100 g dwb), followed by the residue (1658 mg/100 g dwb), seed (986 mg/100 g dwb) and pulp (20 mg/100 g dwb) (Table 3). The highest content of phenolic compounds in jabuticaba peel was expected since the synthesis of these compounds is increased under stress conditions, such as UV radiation (Winkel-Shirley, 2002).

Cyanidin-3-*O*-glucoside was also the major phenolic compound in both peel (56%) and residue (43%), while rutin and gallic acid were the major phenolic compounds in pulp (36%) and seed (43%), respectively. Phenolic compounds were found predominately in the soluble forms in jabuticaba pulp (90%), peel (89%) and residue (74%), whereas in the seed phenolic compounds were mainly found as insoluble forms (56%).

Eleven phenolic compounds were identified and quantified in jussara pulp, being three hydroxybenzoic acid derivatives (gallic, 4-hydroxybenzoic and 3,4-dihydroxybenzoic acids), four hydroxycinnamic acid derivatives (*m*-coumaric, *p*-coumaric, ferulic and *trans*-cinnamic acids), two hydroxyphenylacetic acid derivatives (4-hydroxyphenylacetic and 3,4-dihydroxyphenylacetic acids) and two anthocyanins (cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside) (Supplementary Fig. S5). From those, *m*-coumaric, *trans*-cinnamic, 4-hydroxyphenylacetic and 3,4-dihydroxyphenylacetic acids were observed for the first time in jussara pulp. Other twenty-eight phenolic compounds (eight anthocyanins, three flavanols, four flavonols, seven flavones, three hydroxybenzoic acids and three hydroxycinnamic acids) were observed in jussara pulp by other authors (Bicudo et al., 2014; Borges et al., 2011; da Silva et al., 2014; de Brito et al., 2007). In jussara whole fruit, 3,4-dihydroxybenzoic, 3,4-dihydroxyphenylacetic and *trans*-cinnamic acids were not found probably due to their low concentration in the seed (which comprises about 90% of the whole fruit weight) and in the pulp.

Total phenolic content in jussara pulp was 1783 mg/100 g dwb, being 95% of soluble phenolics and 5% of insoluble phenolics (Table 4). The relative abundance of soluble phenolics was similar to that observed for jabuticaba pulp in the present study and might be explained by their lower fibre contents in relation to other fruit fractions. Total soluble phenolic content in jussara pulp (1695 mg/100 g dwb) was lower than that reported by da Silva et al. (2014) (4087 mg/100 g dwb) and by de Brito et al. (2007) (2956 mg/100 g dwb) and higher than that reported by Bicudo et al. (2014) for soluble and insoluble phenolics (244 mg/100 g dwb). It has already been reported that ripeness stage (Bicudo et al., 2014) and geographical origin (Borges et al., 2011) affect jussara phenolic compound contents.

Table 4 – Phenolic compounds contents (mg/100 g dwb) in jussara fruit and its fractions.[1]

Compound	Extract	Whole fruit	Pulp	Seed
Gallic acid	Soluble phenolics	3.8 ± 0.0^{a}	4.4 ± 0.3^{a}	1.0 ± 0.1^{b}
Cyanidin-3-*O*-glucoside		61 ± 1^{a}	425 ± 8^{b}	ND
Cyanidin-3-*O*-rutinoside		195 ± 20^{a}	1255 ± 17^{b}	ND
3,4-dihydroxybenzoic acid		ND[2]	4.5 ± 0.1^{a}	1.1 ± 0.0^{b}
4-hydroxybenzoic acid		ND	3.4 ± 0.1^{a}	2.3 ± 0.2^{b}
trans-cinnamic acid		ND	0.03 ± 0.01^{a}	ND
m-coumaric acid		0.03 ± 0.01^{b}	0.4 ± 0.0^{a}	ND
4-hydroxyphenylacetic acid		2.9 ± 0.3^{a}	2.6 ± 0.4^{a}	ND
Subtotal		**263 ± 21^{b}**	**1695 ± 26^{a}**	**4.4 ± 0.3^{c}**
p-coumaric acid	Alkaline hydrolysis	5.5 ± 0.1^{a}	4.6 ± 0.2^{b}	2.3 ± 0.2^{c}
Ferulic acid		19 ± 1^{a}	17 ± 2^{b}	4.0 ± 0.3^{c}
4-hydroxybenzoic acid		24 ± 1^{a}	20 ± 1^{ab}	19 ± 1^{b}
3,4-dihydroxybenzoic acid		ND	1.1 ± 0.1^{a}	ND
Subtotal		**49 ± 2^{a}**	**43 ± 3^{b}**	**25 ± 2^{c}**
3,4-dihydroxyphenylacetic acid	Acid hydrolysis	ND	5.9 ± 0.7^{a}	ND
Ferulic acid		3.3 ± 0.4^{b}	4.6 ± 0.4^{a}	1.4 ± 0.1^{c}
4-hydroxybenzoic acid		19 ± 2^{b}	34 ± 0^{a}	16 ± 1^{b}
Subtotal		**22 ± 2^{b}**	**45 ± 1^{a}**	**17 ± 1^{c}**
Total		**334 ± 26^{b}**	**1783 ± 30^{a}**	**47 ± 3^{c}**

[1] Results expressed as mean ± SD for triplicates. Different letters indicate significant differences between fractions (One-way ANOVA followed by Tukey *post hoc* test, $p < 0.05$).

[2] Not detected.

Moreover, differences in analytical methodologies may also explain the observed variations.

Cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside and *trans*-cinnamic, *m*-coumaric, gallic and 4-hydroxyphenylacetic acids were found exclusively as soluble forms in jussara pulp. 3,4-dihydroxybenzoic acid was predominantly found in the soluble form (80%) and the insoluble form was exclusively linked by ester bonds (released after alkaline hydrolysis). Ferulic, *p*-coumaric, 4-hydroxybenzoic and 3,4-dihydoxyphenylacetic acids were exclusively or mostly found as insoluble forms (98% on average). Once again, the distribution of phenolic classes between soluble and insoluble forms is in accordance with literature data (Acosta-Estrada et al., 2014).

Cyanidin-3-*O*-rutinoside was the most abundant phenolic compound in jussara pulp (70%), followed by cyanidin-3-*O*-glucoside (24%), in accordance with previous studies (Bicudo et al., 2014; Borges et al., 2011; da Silva et al., 2014). Considering that these anthocyanins were exclusively found as soluble forms in jussara pulp, hydrolysis procedures are of less relevance for the investigation of phenolic compounds in this fruit.

Phenolic composition data of jussara available in the literature are limited to its pulp. In the present study, we investigated for the first time the phenolic profile of jussara whole fruit and seed. Jussara whole fruit and its seed contained total phenolic compound contents of 334 mg/100 g dwb and 47 mg/100 g dwb, respectively. While soluble phenolic compounds were the most abundant in the whole fruit (79%), the majority of phenolic compounds in the seed were insoluble linked to carbohydrates (89%). Differently from the whole fruit and its pulp, the major phenolic compound in jussara seed was 4-hydroxybenzoic acid, which represented 79% of the total phenolic compounds content.

3.4. *Antioxidant activity*

Folin–Ciocalteu, FRAP, TEAC and ORAC values for jabuticaba and jussara fruits and their fractions are shown in Table 5. Our results are similar to those reported in the literature for these fruits (Batista et al., 2014; Bicudo et al., 2014; Rufino et al., 2010; Wu et al., 2013) and higher than those of berries, such as açaí, blackberry, blueberry, jambolão, raspberry and strawberry (de Souza et al., 2014; Rufino et al., 2010). Jabuticaba peel, seed and depulping residue and jussara pulp stood out among fractions independently of the assay used. On the other hand, jabuticaba pulp and jussara seed showed the lowest AA among fractions.

A positive correlation ($r > 0.82$, $p < 0.01$) was observed between total phenolic compounds and AA values, independently of the assay, indicating that these bioactive compounds are the major contributors to AA of jabuticaba and jussara fruits and their fractions. It should be noted that other well-known fruit antioxidants, like flavan-3-ols monomers, proanthocyanidins, gallotannins and ellagitannins, which were not evaluated in the present study, could also contribute to the AA of these two fruits. Folin–Ciocalteu, FRAP, TEAC and ORAC values were strongly correlated, indicating that these assays were all suitable for measuring the AA of these samples ($r > 0.97$, $p < 0.0001$). However, if we plot the content of total phenolic compounds *vs*. AA values in jabuticaba, the slope of the linear regression for the FRAP assay (0.083 mmol Fe^{2+}/mg phenolics) was higher than that for TEAC (0.042 mmol Trolox/mg phenolics), ORAC (0.034 mmol Trolox/mg phenolics) and Folin–Ciocalteu assays (0.005 g GAE/mg phenolics), suggesting that FRAP was the most sensitive assay in measuring the AA of jabuticaba. For jussara, on the other hand, ORAC was the most sensitive assay as it presented the highest slope (0.086 mmol Trolox/mg phenolics) in comparison to FRAP (0.058 mmol Fe^{2+}/mg phenolics), TEAC (0.038 mmol Trolox/mg phenolics) and Folin–Ciocalteu assays (0.004 g GAE/mg phenolics).

The highest sensitivity of FRAP and ORAC assays in measuring the AA of jabuticaba and jussara fruits, respectively, may be explained by their anthocyanin profiles. FRAP assay was more sensitive in measuring the AA of cyanidin-3-*O*-glucoside (slope = 0.150 mmol Fe^{2+}/mg), the major anthocyanin in jabuticaba, than cyanidin-3-*O*-rutinoside (slope = 0.079 mmol Fe^{2+}/mg), the major anthocyanin in jussara. Conversely, ORAC assay was more sensitive in measuring the AA of cyanidin-3-*O*-rutinoside (slope = 0.119 mmol Trolox/mg) than cyanidin-3-*O*-glucoside (slope = 0.061 mmol Trolox/mg). It has already been reported that the sugar moiety affects the AA of flavonoids. Moreover, glycosylation with rutinose leads to higher scavenging of free radicals than with other sugars (Heim, Tagliaferro,

Table 5 – Antioxidant activity of jabuticaba and jussara fruits and their fractions measured by Folin–Ciocalteu, FRAP, TEAC and ORAC assays.[1]

	Folin–Ciocalteu (g GAE[2]/100 g)	FRAP (mmol Fe^{2+}/100 g)	ORAC (mmol Trolox/100 g)	TEAC (mmol Trolox/100 g)
Jabuticaba				
Whole fruit	6.0 ± 0.1^{d}	71.2 ± 13.0^{c}	36.3 ± 2.2^{c}	43.5 ± 6.2^{d}
Pulp	0.6 ± 0.0^{e}	5.1 ± 0.1^{d}	5.6 ± 1.6^{d}	4.6 ± 0.1^{c}
Peel	12.1 ± 0.2^{a}	192.1 ± 8.7^{a}	82.7 ± 0.9^{a}	97.6 ± 0.8^{a}
Seed	10.7 ± 0.1^{b}	186.6 ± 2.9^{a}	65.4 ± 1.8^{b}	97.9 ± 0.2^{a}
Depulping residue	9.0 ± 0.2^{c}	167.1 ± 7.7^{b}	71.6 ± 5.0^{b}	92.6 ± 1.0^{b}
Jussara				
Whole fruit	1.2 ± 0.1^{b}	15.9 ± 0.3^{b}	33.6 ± 0.9^{b}	14.3 ± 0.8^{b}
Pulp	7.5 ± 0.7^{a}	100.4 ± 5.4^{a}	154.4 ± 10.0^{a}	67.7 ± 2.9^{a}
Seed	0.2 ± 0.0^{c}	1.2 ± 0.0^{c}	2.2 ± 0.1^{c}	1.0 ± 0.1^{c}

[1] Results expressed on dwb as mean ± SD for triplicates. Different letters in each column indicate significant difference between fractions of the same fruit (One-way ANOVA followed by Tukey *post hoc* test, $p < 0.05$).
[2] Gallic acid equivalents.

& Bobilya, 2002), corroborating our findings regarding ORAC, as this assay follows this reaction mechanism.

3.5. *Potential application of jabuticaba and jussara fruits*

Jabuticaba pulp may be considered a nutritional source (contributes to more than 15% of the RDA per 200 mL) of iron, copper, manganese and vitamin C (Fig. 1A). However, this edible fraction of jabuticaba presented insignificant contents of phenolic compounds, as well as low AA. This rich nutritional value, associated with the desirable sensory characteristics of the pulp, is an incentive for the industrial production and commercialization of jabuticaba juice. On the other hand, jabuticaba depulping residue, the non-edible fraction of this fruit, presented high contents of both anthocyanin and non-anthocyanin phenolic compounds, which were associated with its high AA. Moreover, this residue is a dietary source of fibre and iron (Fig. 1A). The major phenolic compounds identified in jabuticaba depulping residue (anthocyanins and gallic and ellagic acids) have been associated with lower risks of development of certain cancers, cardiovascular diseases, diabetes and other chronic diseases (Liu et al., 2015; Nile & Park, 2014; Zanwar, Badole, Shende, Hegde, & Bodhankar, 2014; Zhang et al., 2014). The potential functional properties of jabuticaba depulping residue underline the importance of the recovery of this by-product, which could be used for the production of functional ingredients and dietary supplements by the food and pharmaceutical industries.

Jussara pulp presented both nutritional value (dietary source of fibre, copper and manganese) and functional potential (high contents of anthocyanins) (Fig. 1B) and therefore has good marketing potential. In fact, jussara pulp composition is very similar

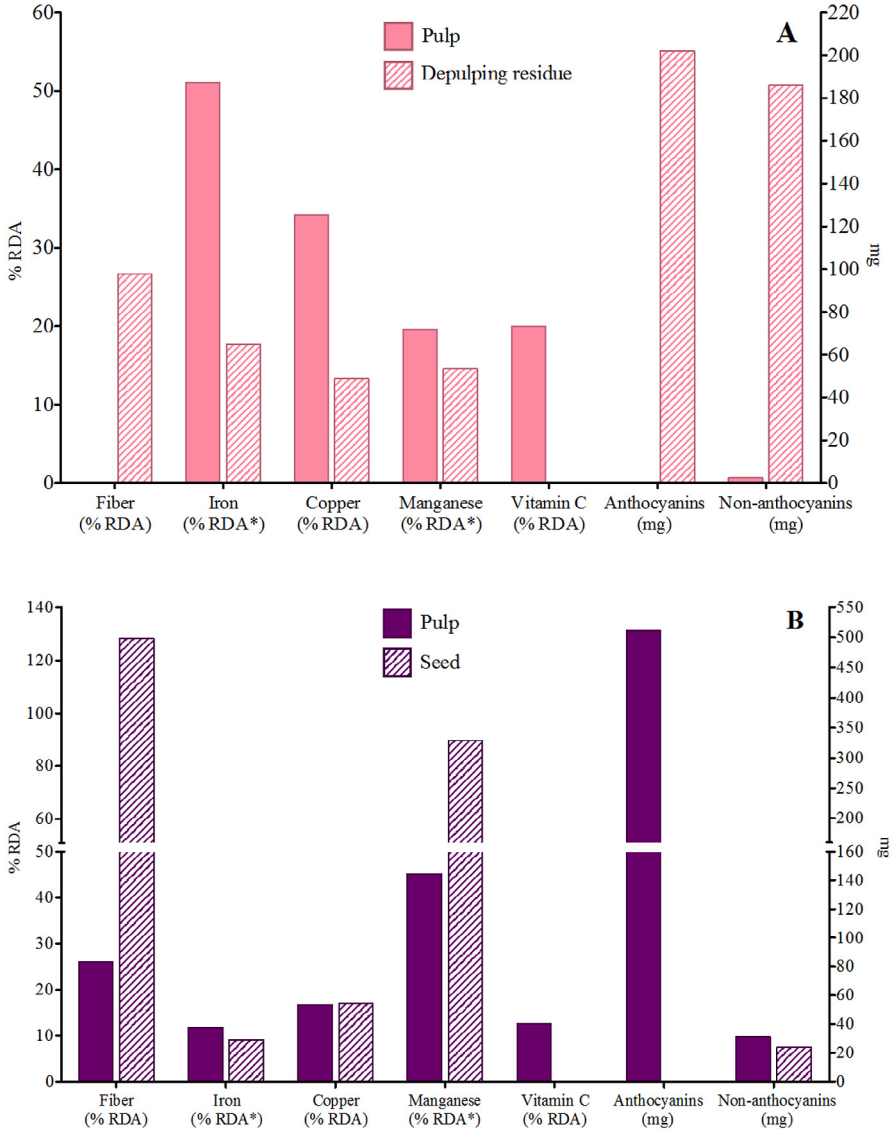

Fig. 1 – Nutritional and functional values of jabuticaba (A) and jussara (B) edible (expressed for 200 mL of pulps on fwb) and non-edible fractions (expressed for 100 g of jabuticaba depulping residue or jussara seed on fwb). % RDA plotted on left Y axis and content of anthocyanin and non-anthocyanin compounds plotted on right Y axis. *Average RDA for male and female adults.

to açaí pulp, which is marketed as a "super-food" with bioactivity in humans (Yamaguchi, Pereira, Lamarão, Lima, & Veiga-Junior, 2015). Although jussara seed did not show significant nutritional and functional values (Fig. 1B), its recovery after the depulping process is of great importance for the reforestation of the Brazilian Atlantic Forest. In this sense, ongoing reforestation efforts, such as "Projeto Amável," aim at using jussara seeds from depulping pilot-scale plants to plant new trees.

In conclusion, our results indicate that jabuticaba and jussara have high commercial potential due to their nutritional and functional properties. Therefore, these are fruits with promising economic valorization.

Acknowledgments

The financial support of Brazilian funding agencies (FAPERJ, E-26/010.001634/2014, E-26/111.664/2013; E-26/111.387/2012. Conselho Nacional de Desenvolvimento Científico e Tecnológico 481325/2012-3 and CAPES) is greatly acknowledged. We also thank Mr. George Braille (Juçaí Co., Rio de Janeiro, Brazil) for kindly supplying the jussara samples.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jff.2015.06.002.

REFERENCES

Abe, L. T., Lajolo, F. M., & Genovese, M. I. (2011). Potential dietary sources of ellagic acid and other antioxidants among fruits consumed in Brazil: Jabuticaba (*Myrciaria jaboticaba* (Vell.) Berg). *Journal of the Science of Food and Agriculture*, *92*(8), 1679–1687.

Acosta-Estrada, B. A., Gutiérrez-Uribe, J. A., & Serna-Saldívar, S. O. (2014). Bound phenolics in foods, a review. *Food Chemistry*, *152*, 46–55.

Alezandro, M. R., Dubé, P., Desjardins, Y., Lajolo, F. M., & Genovese, M. I. (2013). Comparative study of chemical and phenolic compositions of two species of jaboticaba: *Myrciaria jaboticaba* (Vell.) Berg and *Myrciaria cauliflora* (Mart.) O. Berg. *Food Research International*, *54*(1), 468–477.

Alves, G., & Perrone, D. (2015). Breads enriched with guava flour as a tool for studying the incorporation of phenolic compounds in bread melanoidins. *Food Chemistry*, *185*, 65–74.

Association of Official Analytical Chemists (2000). *Official methods of analysis* (17th ed.). Gaithersburg: AOAC International.

Batista, Â. G., Lenquiste, S. A., Cazarin, C. B. B., da Silva, J. K., Luiz-Ferreira, A., Bogusz, S., Jr., Hantao, L. W., de Souza, R. N., Augusto, F., Prado, M. A., & Maróstica, M. R., Jr. (2014). Intake of jaboticaba peel attenuates oxidative stress in tissues and reduces circulating saturated lipids of rats with high-fat diet-induced obesity. *Journal of Functional Foods*, *6*, 450–461.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, *239*(1), 70–76.

Bicudo, M. O. P., Ribani, R. H., & Beta, T. (2014). Anthocyanins, phenolic acids and antioxidant properties of juçara fruits (*Euterpe edulis* M.) along the on-tree ripening process. *Plant Foods Human Nutrition*, *69*(2), 142–147.

Borges, G. D. S. C., Vieira, F. G. K., Copetti, C., Gonzaga, L. V., Zambiazi, R. C., Filho, J. M., & Fett, R. (2011). Chemical characterization, bioactive compounds, and antioxidant capacity of jussara (*Euterpe edulis*) fruit from the Atlantic Forest in southern Brazil. *Food Research International*, *44*(7), 2128–2133.

Britton, G., & Khachik, F. (2009). Carotenoids nutrition and health. In G. Britton, S. Liaaen-Jensen, & H. Pfander (Eds.), *Carotenoids in food* (Vol. 5), 45–66. Boston: Birkhauser Basel.

da Silva, N. A., Rodrigues, E., Mercadante, A. Z., & de Rosso, V. V. (2014). Phenolic compounds and carotenoids from four fruits native from the Brazilian Atlantic Forest. *Journal Agricultural and Food Chemistry*, *62*(22), 5072–5084.

da Silva, P. P. M., do Carmo, L. F., Silva, G. M., Silveira-Diniz, M. F., Casemiro, R. C., & Spoto, M. H. F. (2013). Physical, chemical, and lipid composition of juçara (*Euterpe edulis* Mart.) pulp. *Brazilian Journal of Food and Nutrition*, *24*(1), 7–13.

de Brito, E. S., de Araújo, M. C. P., Alves, R. E., Carkeet, C., Clevidence, B. A., & Novotny, J. A. (2007). Anthocyanins present in selected tropical fruits: Acerola, jambolão, jussara, and guajiru. *Journal of Agricultural and Food Chemistry*, *55*(23), 9389–9394.

de Souza, V. R., Pereira, P. A. P., da Silva, T. L. T., Lima, L. C. D. O., Pio, R., & Queiroz, F. (2014). Determination of the bioactive compounds, antioxidant activity and chemical composition of Brazilian blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits. *Food Chemistry*, *156*, 362–368.

Darnet, S., Serra, J. L., Rodrigues, A. M. D. C., & da Silva, L. H. M. (2011). A high-performance liquid chromatography method to measure tocopherols in assai pulp (*Euterpe oleracea*). *Food Research International*, *44*(7), 2107–2111.

European Food Safety Authority (2006). *Tolerable upper intake levels for vitamins and minerals*. Retrieved from http://www.efsa.europa.eu/en/ndatopics/docs/ndatolerableuil.pdf.

Farah, A., Monteiro, M. C., Calado, V., Franca, A. S., & Trugo, L. C. (2006). Correlation between cup quality and chemical attributes of Brazilian coffee. *Food Chemistry*, *98*, 373–380.

Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *Journal of Nutritional Biochemistry*, *13*(10), 572–584.

Lima, A. D. J. B., Corrêa, A. D., Dantas-Barros, A. M., Nelson, D. L., & Amorim, A. C. L. (2011). Sugars, organic acids, minerals and lipids in jabuticaba. *Revista Brasileira de Fruticultura*, *33*(2), 540–550.

Lima, A. D. J. B., Corrêa, A. D., Saczk, A. A., Martins, M. P., & Castilho, R. O. (2011). Anthocyanins, pigment stability and antioxidant activity in jabuticaba [*Myrciaria cauliflora* (Mart.) O. Berg]. *Revista Brasileira de Fruticultura*, *33*(3), 877–887.

Liu, R., Li, J., Cheng, Y., Huo, T., Xue, J., Liu, Y., Liu, J., & Chen, X. (2015). Effects of ellagic acid-rich extract of pomegranates peel on regulation of cholesterol metabolism and its molecular mechanism in hamsters. *Food and Function*, *6*, 780–787. doi:10.1039/c4fo00759j.

Mattila, P., & Kumpulainen, J. (2002). Determination of free and total phenolic acids in plant-derived foods by HPLC with diode-array detection. *Journal of Agricultural and Food Chemistry*, *50*(13), 3660–3667.

Nile, S. H., & Park, S. W. (2014). Edible berries: Bioactive components and their effect on human health. *Nutrition (Burbank, Los Angeles County, Calif.)*, *30*(2), 134–144.

Otten, J. J., Hellwig, J. P., & Meyers, L. D. (Eds.), (2006). *Dietary reference intakes: The essential guide to nutrient requirements*. Washington: National Academies of Sciences.

Rahmani, M., & Csallany, A. S. (1991). Chlorophyll and β-carotene pigments in moroccan virgin olive oils measured by high-performance liquid chromatography. *Journal of the American Oil Chemists' Society*, *68*(9), 672–674.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, *26*(9–10), 1231–1237.

Reynertson, K. A., Yang, H., Jiang, B., Basile, M. J., & Kennelly, E. J. (2008). Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits. *Food Chemistry*, *109*(4), 883–890.

Rufino, M. D. S. M., Alves, R. E., de Brito, E. S., Pérez-Jiménez, J., Saura-Calixto, F., & Mancini-Filho, J. (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry*, *121*(4), 996.

Scartezzini, P., Antognoni, F., Raggi, M. A., Poli, F., & Sabbioni, C. (2006). Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparation of *Emblica officinalis* Gaertn. *Journal of Ethnopharmacology*, *104*, 113–118.

Singleton, V., Orthofer, R., & Lamuela-Raventós, R. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. *Methods in Enzymology*, *299*(1), 152–178.

Tan, B., & Brzuskiewicz, L. (1989). Separation of tocopherol and tocotrienol isomers using normal- and reverse-phase liquid chromatography. *Analytical Biochemistry*, *180*, 368–373.

United States Department of Agriculture, Agricultural Research Service. (2014). *USDA National Nutrient Database for Standard Reference* (release 27). Retrieved from http://ndb.nal.usda.gov/ndb/nutrients/index.

Winkel-Shirley, B. (2002). Biosynthesis of flavonoids and effects of stress. *Current Opinion in Plant Biology*, *5*, 218–223.

Wu, S., Dastmalchi, K., Long, C., & Kennelly, E. J. (2012). Metabolite profiling of jaboticaba (*Myrciaria cauliflora*) and other dark-colored fruit juices. *Journal of Agricultural and Food Chemistry*, *60*(30), 7513–7525.

Wu, S., Long, C., & Kennelly, E. J. (2013). Phytochemistry and health benefits of jaboticaba, an emerging fruit crop from Brazil. *Food Research International*, *54*(1), 148–159.

Yamaguchi, K. K. L., Pereira, L. F. R., Lamarão, C. V., Lima, E. S., & Veiga-Junior, V. F. (2015). Amazon acai: Chemistry and biological activities: A review. *Food Chemistry*, *179*, 137–151.

Zanwar, A. A., Badole, S. L., Shende, P. S., Hegde, M. V., & Bodhankar, S. L. (2014). Role of gallic acid in cardiovascular disorders. In R. R. Watson, V. R. Preedy, & S. Zibadi (Eds.), *Polyphenols in human health and disease* (Vol. 2), 1045–1047. San Diego: Academic Press.

Zhang, H.-M., Zhao, L., Li, H., Xu, H., Chen, W.-W., & Tao, L. (2014). Research progress on the anticarcinogenic actions and mechanisms of ellagic acid. *Cancer Biology and Medicine*, *11*(2), 92–100.

Zulueta, A., Esteve, M. J., & Frígola, A. (2009). ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. *Food Chemistry*, *114*(1), 310–316.