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Bioaccessibility of bioactive compounds and antioxidant potential of juçara fruits (*Euterpe edulis* Martius) subjected to *in vitro* gastrointestinal digestion

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ABSTRACT

An *in vitro* method involving simulated gastrointestinal digestion was used to assess the bioaccessibility of fifteen minerals, twenty-two phenolic compounds and the antioxidant capacity in juçara fruit during seven ripening stages. For minerals and phenolics, respectively, initial contents were up to 1325.9 and 22.9 mg 100 g^{-1}, whereas after *in vitro* digestion, the maximum values were 556.7 and 14.43 mg 100 g^{-1} (dry matter). Antioxidant capacity, determined by 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH) and ferric reducing antioxidant power (FRAP), after *in vitro* digestion decreased 51–78% when compared to the crude extract. Bioaccessible fractions of quercetin, protocatechuic and *p*-coumaric acids presented positive and significant correlation with results of DPPH and FRAP. Furthermore, our study demonstrated that the ripening stages of juçara fruit influenced the bioaccessibility of compounds and antioxidant capacity, which presented higher levels in purple fruits collected 42–69 days after the appearance of the red berries on bunches.

1. Introduction

Juçara fruit (*Euterpe edulis*), native to the Atlantic Forest, is a globose berry that weighs about 1 g and, when ripe, turns a shade of violet and closely resembles the fruit of *Euterpe oleracea* and *Euterpe precatoria* which are used in the production of açaí (De Brito et al., 2007; Schulz, Borges, Gonzaga, Costa, & Fett, 2016). For commercial exploitation, juçara fruit is macerated and mixed with different amounts of water in a depulping machine, where the epicarp and mesocarp are separated from the seeds. The process results in a liquid emulsion that is creamy with an intense dark purple color and characteristic flavor, which can be consumed in the form of pulp, juice, and as an ingredient in many foods (Bicudo, Ribani, & Beta, 2014; Borges et al., 2011; Schulz et al., 2016).

Bioactive constituents of juçara fruit (*E. edulis*) have been reported by many authors. The nutrients are mainly unsaturated fatty acids, protein, vitamins C and E, and dietary fibers (Borges et al., 2011; Inada et al., 2015; Rufino et al., 2010; Schulz et al., 2015). Moreover, the juçara fruit is a good mineral supplier for the human diet, and the main essential minerals found in these fruits are potassium, calcium, magnesium, iron and manganese (Inada et al., 2015; Schulz et al., 2015). On the other hand, heavy metals such as cadmium and nickel do not pose any risk of human intoxication through the intake of juçara fruit (Inada et al., 2015).

These fruits contain a substantial amount of phenolic acids such as ferulic acid, gallic acid, protocatechuic acid, and *p*-coumaric acid, and flavonoids especially quercetin, rutin (Borges et al., 2013; Guergoletto, Costabile, Flores, Garcia, & Gibson, 2016; Schulz et al., 2015), and anthocyanins (Bicudo et al., 2014; Da Silva,

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Rodrigues, Mercadante, & de Rosso, 2014; Novello et al., 2015). Due to the presence of the wide variety of phenolic compounds, studies have suggested that juçara fruits may 12exert antioxidants effects (Bicudo et al., 2014; Borges et al., 2011, 2013; Cardoso, Di Pietro, et al., 2015; Cardoso, Novaes, et al., 2015; Inada et al., 2015; Schulz et al., 2015). In the human body, antioxidant effects of phenolic compounds help protect cells against oxidative damage caused by free radicals, by stabilizing or deactivating free radicals before they attack cells (Pisoschi & Pop, 2015). Recently, phenolic compounds have attracted attention as potential agents for preventing many oxidative stress-related diseases (Celep, Charehsaz, Akyüz, Acar, & Yesilada, 2015; Mosele, Macià, Romero, & Motilva, 2016; Sengul, Surek, & Nilufer-Erdil, 2014).

Published data on dietary compounds of juçara fruit are an important quality attribute from a nutritional and commercial point of view. However, the possible effectiveness of bioactive compounds in the human body is greatly determined by the bioavailability of these molecules (Kulkarni, Acharya, Rajurkar, & Reddy, 2007; Swieca, 2016). Bioavailability is defined as the proportion of the ingested compound that is absorbed and metabolized through normal pathways (Cardoso, Afonso, Lourenço, Costa, & Nunes, 2015; Sengul et al., 2014). Among the most important factors determining bioavailability is bioaccessibility, which is defined as the fraction of a compound that is released from the food matrix and solubilized during digestion, i.e., it is the fraction of a compound potentially available for absorption (Alminger et al., 2014; Carbonell-Capella, Buniowska, Barba, Esteve, & Frígola, 2014). To the best of our knowledge, no studies have reported the bioaccessibility of juçara fruit. Therefore, determination of the bioaccessibility of the dietary compounds of juçara fruit is important because only bioaccessible constituents can be available for absorption and able to exert their beneficial effects.

In addition to bioaccessibility, fruit maturity can influence the content of nutrients and compounds that will be utilized by the body, since the ripening stage influences the content of compounds in plants (Taiz & Zeiger, 2009). The juçara fruits have a long ripening cycle, since it is possible to obtain visually similar edible purple fruit for a period longer than two months after the appearance of the red fruits in a bunch, a property typical of tropical palms (Bicudo et al., 2014; Schulz et al., 2015).

Given the above, the aim of this study was to determine the minerals and phenolic contents, as well as antioxidant capacity in juçara fruit (*Euterpe edulis* Martius) before and after the *in vitro* gastrointestinal digestion in order to provide data on bioaccessibility of these compounds and antioxidant potential in juçara fruits during ripening cycle.

2. Materials and methods

2.1. Chemicals and reagents

Hydrochloric acid (37% w/w), methanol, formic acid, hydrogen peroxide (30% w/w), sodium bicarbonate, 2,4,6-tris-(2-pyridyl)-1, 3,5-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), pepsin, pancreatin, glycodeoxycholate, taurodeoxycholate, taurocholate and ultra-pure phenolic standards, were obtained from Sigma–Aldrich (St. Louis, MO). Nitric acid (65% w/w) was purchased from Merck (Darmstadt, Germany) and purified by double sub-boiling distillation in a quartz still (Kürner Analysentechnik, Rosenheim, Germany). A standard multi-element, ICP III solution and Rh, Ca, Mg, K, and Na stock solution (Sigma–Aldrich, Buchs, Switzerland) were used. Diethyl ether, *n*-hexane, ascorbic acid, ferric chloride were obtained from Vetec (Rio de Janeiro, RJ, Brazil). Argon gas with a purity of 99.996%, acetylene, and nitrous oxide were purchased from White Martins (Sao Paulo, Brazil). All chemicals used in the experiments were of analytical reagent grade. Deionized water was obtained from a Milli-Q Plus system (Millipore, Bedford, USA).

2.2. Sample preparation

Juçara fruits were harvested in Florianopolis (latitude 27°35′48″ S, longitude 48°32′57″ W), Santa Catarina, Brazil, from August to November of 2013 at seven ripening stages: 0, 17, 23, 30, 42, 56 and 69 days after the red fruits appeared on bunches. At each ripening stage, 100 g of fruits were picked manually and randomly in two different bunches of three juçara palms The fruits collected were subjected to manual pulp removal, blanched for 10 min at 85 °C and then dried at 40 °C for 12 h (Borges et al., 2013). The dried pulp was ground in an ultracentrifugal mill (Z200 Retsch, Haan, Germany) with 1 mm sieve at 5700*g*.

2.3. Mineral analysis by ICP-MS and HR-CS AAS

Samples (200 mg) were digested with 6 mL of nitric acid and 1 mL of hydrogen peroxide using a microwave oven (MLS-1200, Milestone, Sorisole, Italy), with applied power varying from 250 to 600 W for 25 min in closed perflouroalcóxi Teflon® (PFA) vessels.

The minerals iron, copper, cobalt, zinc, manganese, selenium, aluminum, cadmium, nickel, lead, and arsenic were determined by inductively coupled plasma mass spectrometry (ICP-MS) using a Perkin Elmer SCIEX, model ELAN 6000 (Massachusetts, USA). The instrumental conditions were 15 L min^{-1} of argon main gas flow rate, 1 L min^{-1} of auxiliary gas flow rate, 0.9 L min^{-1} of nebulizer gas flow rate, and 1100 W of radio frequency. Given the inherent interferences for calcium, magnesium, potassium, and sodium for ICP-MS determination, the determination of these elements was performed using a high-resolution continuum source atomic absorption spectrometer (HR-CS AAS, ContrAA 700, Analytik Jena AG, Jena, Germany) using an air-acetylene flame for potassium, sodium, and magnesium and acetylene-nitrous oxide flame for calcium. Rhodium (10 μg L^{-1}) was used as an internal standard for all determinations using ICP-MS. External calibration was carried out using aqueous solutions prepared from a multi-element stock standard solution containing all analytes for determination using ICP-MS and single stock solutions for determination using HR-CS AAS.

To verify the accuracy of measurements, mineral contents were analysed in two certified reference materials (CRM)—namely, NIST SRM 8433 Corn bran and NIST SRM 1515 Apple leaves—under the same conditions as the juçara fruit samples.

2.4. Extraction procedure

For determination of the phenolic compounds and evaluation of the antioxidant capacity, defatted juçara pulp powder (1 g) was extracted from acid hydrolysis with methanol and hydrochloric acid at 85 °C for 30 min. Afterwards, the solution (pH 2) was subjected to partition extraction (thrice) with diethyl ether. After centrifugation, the supernatants were combined and, the organic solvent was vaporized using a rotary evaporator (Fisatom 802, São Paulo, Brazil). The dried extract was reconstituted in 1 mL of methanol and for injection in LC–ESI–MS/MS was diluted in methanol: water (70: 30) (Schulz et al., 2015).

2.5. Phenolic composition analysis by LC–ESI–MS/MS

Identification and quantification of phenolic compounds were performed according to Schulz et al. (2015). Forty-seven phenolic compounds were searched by liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI–MS/MS) on a 1200 high-performance liquid chromatography system (Agilent

Technologies—Waldbronn, Germany) coupled with a hybrid quadrupole linear ion trap mass spectrometer QTRAP® 3200 (Applied Biosystems/MDS Sciex, Concord, Canada) equipped with a TurboIonSpray™ source (electrospray-ESI). Separation was performed in a Synergi column (4.6 μm particle size, 150 mm, 2.0 mm). Liquid chromatographic analysis was carried out using a mobile phase gradient consisting of (A) 95% methanol in water and (B) 0.1% formic acid in water. The flow rate was 250 μL min^{-1}. The separation was carried out at 30 °C using segmented gradient elution as follows: 0–5 min, 10% A; 5–7 min, 90% A; 7–10 min, 90% A; 10–17 min, 10% A. The experiments were performed in negative ion mode, and the capillary needle was maintained at −4500 V. The MS/MS parameters were: curtain gas, 10 psi; temperature, 400 °C; gas 1.45 psi; gas 2.45 psi; and CAD gas, medium. The Analyst version 1.5.1 software was used for the LC–ESI–MS/MS system control and data analysis. Parameters for cone and collision energy for the 47 phenolic compounds tested are listed in Supplementary material (Table S1). Phenolic compound contents were expressed in mg per 100 g of dry matter. Total phenolic content was obtained from the sum of the contents of all quantified phenolic compounds and expressed in mg per 100 g of dry matter.

2.6. Antioxidant capacity

The determination of the *in vitro* antioxidant capacity was based on the capacity of the antioxidants present in the juçara fruit pulp extract to isolate/delay the DPPH radical according to the method of Brand-Williams, Cuvelier, and Berset (1995). In addition, the antioxidant capacity of the juçara fruit pulp extract was determined by the ferric reducing ability (FRAP) according to the method described by Benzie and Strain (1996). For both assays, a standard curve was prepared and the results were expressed in mg ascorbic acid equivalents to 100 g^{-1} of dry matter. Curve equation and determination coefficient obtained are shown in Supplementary material (Table S2).

2.7. In vitro digestion procedure

To assess the possible bioaccessible fractions of minerals, phenolics and antioxidant capacity, juçara fruit pulp was subjected to an *in vitro* gastrointestinal digestion procedure performed according to the method adopted by Ryan and Prescott (2010) with adaptations.

2.7.1. Gastric phase

Gastric solution was prepared by dissolving 0.32 g of pepsin with 0.7 mL of HCl, followed by the addition of deionized water up to a final volume of 100 mL. Afterwards, the pH was adjusted to 1.2 (Digimed DM-20, Campo Grande, Brazil).

About 200 mg of each sample was weighed and added to 3 mL gastric solution. The resulting solutions were placed in a thermostat shaker (Tecnal TE820, Piracicaba, Brazil) at 37 °C for 2 h.

2.7.2. Small intestine phase

Intestinal solution was prepared from the solubilization of 0.2 g of bile salts (0.08 g of glycodeoxycholate, 0.05 g of taurodeoxycholate and 0.08 g of taurocholate) and 0.5 g of pancreatin in 3% $NaHCO_3$ aqueous solution; the volume of the solution was adjusted to 100 mL and the pH was adjusted to 6.8.

After gastric phase, the resulting solutions were treated with 3% $NaHCO_3$ aqueous solution to increase the pH to 6.8, subjected to intestinal solution, and placed in a thermostatic shaker at 37 °C for 2 h. Afterward, the samples were centrifuged for 20 min at 2850*g* (Fanem, Model 280R, Sao Paulo, Brazil).

2.8. Bioaccessibility

After *in vitro* digestion procedure, the supernatant was analysed by ICP-MS and HR-CS AAS for mineral determination. Sodium was not determined in the bioaccessible fraction due to the high content of this mineral present in bile salts. For phenolic compound determination, the supernatant (at a pH 2) was subjected to partition extraction (thrice) with diethyl ether. After centrifugation the organic solvent was vaporized, and the dried extract was reconstituted in 1 mL of methanol for analysis by LC–ESI–MS/MS. These extracts were also used for the determination of the antioxidant capacity.

The bioaccessibility (%) was defined as the content of the compound released in the simulated digestion process compared to the content of the compound in the sample, and the value was calculated according to the formula (Leufroy, Noël, Beauchemin, & Guérin, 2012):

$$\text{Bioaccessibility}\,(\%) = \left(\frac{\text{Content of the compound released in the simulated digestion}}{\text{Content of the compound in the sample}}\right) \times 100$$

2.9. Statistical analysis

All data are presented as mean ± standard deviation (S.D.) for six samples that were analysed in triplicate. Results were compared by one-way analysis of variance (ANOVA) and the Tukey test was carried out to identify significant differences between different ripening stages. The Student's *t*-test was performed to identify significant differences between the values found before and after *in vitro* gastrointestinal digestion in each ripening stage. Differences of $p < 0.05$ were considered significant. Correlations among data obtained for the antioxidant capacity and each individual phenolic compound were established using Pearson's correlation coefficient. Data were analysed using the Statistica 13.0 software (Statsoft Inc., Tulsa, OK, USA).

3. Results and discussion

3.1. Minerals

The mineral contents in certified reference materials (CRM) NIST SRM 1515 Apple leaves and NIST SRM 8433 Corn bran are summarized in Table 1. Recovery values were between 91% and 113%. Good agreement between determined and certified values of mineral contents indicated adequate accuracy of the method.

The content and the bioaccessibility of minerals for juçara pulp fruit are presented in Table 2. Among the 15 minerals analysed, potassium, calcium, magnesium, and sodium were the minerals present in greatest content (998.67–1325.88, 327.59–672.20, 161.40–189.70, 7.47–23.86 mg 100 g^{-1} dry matter, respectively), followed by manganese (49.81–89.16 μg 100 g^{-1} dry matter), iron (38.59–83.51 μg 100 g^{-1} dry matter) and zinc (23.89–32.88 μg 100 g^{-1} dry matter). With the exception of sodium, cobalt and heavy metals, minerals contents were higher at the end of the ripening cycle, i.e. the stages defined as 6 and 7.

The results of mineral content demonstrated that juçara fruits present considerable amounts of these nutrients. However, this content may differ from the bioaccessible fraction, which is the released content from the food matrix into the intestinal lumen after digestion and is available for intestinal absorption (Alminger et al., 2014; Fernández-García, Carvajal-Lérida, & Pérez-Gálvez, 2009). Thus, the *in vitro* method used to evaluate bioaccessibility is important for characterizing the nutritional quality of the fruit, since it is a useful tool for information

Table 1
Evaluation of accuracy using CRM NIST SRM 8433 (corn bran) and NIST SRM 1515 (apple leaves).

Mineral (LOD – LOQ) (μg g^{-1})	NIST SRM 8433			NIST SRM 1515		
	Found value (μg g^{-1})	Certified value (μg g^{-1})	Recovery (%)	Found value (μg g^{-1})	Certified value (μg g^{-1})	Recovery (%)
K (50–160)	602 ± 44	566 ± 75	106	1.65 ± 0.03 *	1.61 ± 0.02 *	102
Ca (100–330)	428 ± 35	420 ± 38	102	1.6 ± 0.4 *	1.53 ± 0.015 *	105
Na (20–70)	395 ± 8	430 ± 31	92	70 ± 9	64.4 ± 1.2	109
Mg (70–200)	864 ± 59	818 ± 59	106	0.3 ± 0.02 *	0.271 ± 0.008 *	111
Fe (0.6–0.2)	15 ± 1	14.08 ± 1.8	107	167 ± 2	183 ± 5	91
Zn (0.04–0.1)	19 ± 1.8	18.6 ± 2.2	102	14.11 ± 1.51	12.5 ± 0.3	113
Mn (0.04–0.1)	2.44 ± 0.07	2.55 ± 0.29	96	57.61 ± 0.57	54 ± 3	107
Se (0.1–0.2)	< LOD	0.045 ± 0.045	–	< LOD	0.05 ± 0.009	–
Co (0.01–0.02)	< LOD	0.006 ± 0.006	–	7.9 ± 0.01	8.7 ± 0.09	91
Cu (0.04–0.1)	2.71 ± 0.11	2.47 ± 0.4	110	6.04 ± 0.04	5.64 ± 0.24	107
Al (0.1–0.4)	1.11 ± 0.13	1.01 ± 0.55	110	272.09 ± 4.6	286 ± 9	95
As (0.04–0.1)	< LOD	0.002 ± 0.002	–	0.04 ± 0.05	0.038 ± 0.007	105
Pb (0.03–0.1)	0.14 ± 0.01	0.14 ± 0.034	100	0.44 ± 0.03	0.47 ± 0.024	94
Cd (0.03–0.1)	< LOD	0.012 ± 0.005	–	< LOD	0.013 ± 0.002	–
Ni (0.1–0.4)	< LOD	0.158 ± 0.054	–	< LOD	0.91 ± 0.12	–

Results expressed as mean ± S.D. (n = 3). *Concentration in %. LOD – Limit of detection.
LOQ – Limit of quantification.

Table 2
Content (μg g^{-1} dry matter) and the bioaccessible fractions (BF%) of minerals in juçara fruit pulp at different ripening stages.

		Ripening stage						
		1	2	3	4	5	6	7
K*	Content	998.67 ± 52.17 ^{c}	1084.70 ± 71.11 ^{bc}	1129.32 ± 51.18 ^{bc}	1166.20 ± 42.68 ^{ab}	1160.70 ± 59.38 ^{ab}	1280.33 ± 34.18 ^{a}	1325.88 ± 54.70 ^{a}
	BF%	46.1	44.2	41.6	43.0	44.2	40.9	43.1
Ca*	Content	327.59 ± 13.11 ^{f}	404.58 ± 17.57 ^{e}	513.50 ± 11.02 ^{de}	544.56 ± 19.27 ^{d}	541.23 ± 21.43 ^{cd}	560.01 ± 12.33 ^{b}	672.20 ± 19.64 ^{a}
	BF%	14.3	25.4	23.6	18.9	35.6	47.3	65.5
Mg*	Content	161.40 ± 7.84 ^{c}	162.57 ± 11.22 ^{c}	161.87 ± 6.99 ^{c}	183.11 ± 8.42 ^{a}	170.29 ± 7.24 ^{b}	189.70 ± 3.28 ^{a}	181.62 ± 16.11 ^{ab}
	BF%	32.2	36.2	33.5	51.1	46.7	55.5	44.9
Na*	Content	23.86 ± 0.12 ^{a}	7.47 ± 0.26 ^{d}	19.47 ± 0.14 ^{bc}	18.43 ± 1.18 ^{c}	9.39 ± 0.14 ^{ef}	20.65 ± 0.83 ^{b}	19.25 ± 0.54 ^{bc}
	BF%	NA	NA	NA	NA	NA	NA	NA
Fe	Content	38.59 ± 1.19 ^{d}	55.67 ± 2.84 ^{c}	55.28 ± 2.92 ^{c}	56.38 ± 3.36 ^{c}	65.41 ± 4.37 ^{b}	68.64 ± 4.95 ^{b}	83.51 ± 3.57 ^{a}
	BF%	NB	NB	11.5	7.6	28.1	21.6	29.5
Zn	Content	31.56 ± 0.43 ^{a}	23.89 ± 1.12 ^{c}	29.59 ± 1.24 ^{ab}	25.87 ± 1.88 ^{b}	29.90 ± 1.65 ^{ab}	32.80 ± 1.24 ^{a}	32.88 ± 1.29 ^{a}
	BF%	51.7	54.6	56.1	35.8	56.1	66.9	69.2
Mn	Content	49.81 ± 2.82 ^{d}	56.92 ± 2.74 ^{c}	50.27 ± 3.56 ^{d}	78.24 ± 7.28 ^{b}	77.75 ± 6.82 ^{b}	74.52 ± 3.16 ^{b}	89.16 ± 8.17 ^{a}
	BF%	22.5	19.2	26.9	17.4	36.4	33.5	35.1
Cu	Content	7.83 ± 0.50 ^{c}	7.97 ± 0.64 ^{cd}	8.32 ± 0.63 ^{bcd}	9.54 ± 0.91 ^{b}	8.96 ± 0.74 ^{b}	8.65 ± 0.71 ^{b}	11.61 ± 1.48 ^{a}
	BF%	25.4	27.3	47.1	32.0	45.3	55.6	55.6
Co	Content	0.24 ± 0.01 ^{a}	0.14 ± 0.00 ^{ce}	0.12 ± 0.01 ^{d}	0.20 ± 0.01 ^{b}	0.15 ± 0.00 ^{e}	0.13 ± 0.01 ^{cd}	0.15 ± 0.00 ^{e}
	BF%	34.5	39.5	39.7	81.1	76.1	55.3	73.2
Se	Content	<LOD	<LOD	<LOQ	0.59 ± 0.03 ^{c}	0.96 ± 0.05 ^{b}	1.00 ± 0.05 ^{a}	1.07 ± 0.03 ^{a}
	BF%	NB	NB	NB	NB	48.9	64.7	59.8
As	Content	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	BF%	NB	NB	NB	NB	NB	NB	NB
Al	Content	1.47 ± 0.57 ^{bc}	2.87 ± 0.68 ^{c}	2.43 ± 0.26 ^{c}	2.66 ± 0.54 ^{bc}	2.73 ± 0.84 ^{bc}	2.88 ± 0.66 ^{a}	2.63 ± 0.70 ^{ab}
	BF%	NB	NB	NB	13.4	14.7	NB	NB
Pb	Content	0.40 ± 0.02 ^{d}	0.43 ± 0.01 ^{a}	0.39 ± 0.03 ^{d}	0.47 ± 0.02 ^{b}	0.30 ± 0.02 ^{e}	0.32 ± 0.01 ^{e}	0.24 ± 0.00 ^{c}
	BF%	NB	NB	NB	NB	NB	NB	NB
Cd	Content	0.07 ± 0.01 ^{a}	0.05 ± 0.00 ^{b}	0.04 ± 0.00 ^{c}	0.04 ± 0.00 ^{c}	0.03 ± 0.00 ^{d}	0.05 ± 0.01 ^{b}	0.04 ± 0.00 ^{c}
	BF%	NB	NB	NB	NB	NB	NB	NB
Ni	Content	2.10 ± 0.12 ^{a}	1.95 ± 0.13 ^{a}	0.88 ± 0.05 ^{b}	0.89 ± 0.06 ^{c}	1.24 ± 0.06 ^{c}	0.79 ± 0.04 ^{c}	0.81 ± 0.05 ^{c}
	BF%	7.4	9.1	NB	18.8	11.8	42.5	NB
Dry matter**		35.44	39.20	39.27	38.84	40.04	39.40	43.64

Ripening stages: 0 (stage 1), 17 (stage 2), 23 (stage 3), 30 (stage 4), 42 (stage 5), 56 (stage 6) and 69 (stage 7) days after the red fruits appeared on bunches.
Data are presented as mean ± S.D. (n = 6).
*Values expressed as mg 100 g^{-1}. $^{a–f}$Values in the same row followed by different letters indicate significant differences ($p < 0.05$).
**Values expressed as g 100 g^{-1}.
LOD – Limit of detection. LOD (μg g^{-1}) – Se: 0.1; As: 0.04; Al: 0.1; Pb: 0.03; Cd: 0.03; Ni 0.1.
LOQ – Limit of quantitation. LOQ (μg g^{-1}) – Se: 0.2; Al: 0.4.
NA – Not analysed.
NB – Not bioaccessible (value of the bioaccessible fraction < LOD).
Bioaccessible fractions (BF%) were calculated according to the formula: $BF\% = \left(\frac{\text{Content of the mineral released in the simulated digestion}}{\text{Content of the mineral in the sample}}\right) \times 100$.

acquisition on the influences of both the gastrointestinal system and the food matrix on the potentiality of nutrients that can be absorbed by the human organism (Hur, Lim, Decker, & McClements, 2011; Mosele et al., 2016).

The mineral bioaccessibility ranged from 0 to 81.1%. Those with the greatest bioaccessible fractions were cobalt (34.5%–81.1%), zinc (35.8%–69.2%), calcium (14.3%–65.5%) and magnesium (32.2%–55.5%). In general, as with the total concentration, the highest bioaccessibility values for essential minerals were found in stages 6 and 7 (Table 2).

Iron presented bioaccessible fractions from 0 to 29.5%, with rates increasing according to the advancement of ripening (Table 2). The low bioaccessibility of iron normally observed in most fruits was due to the low concentrations of protein, which increased bioaccessibility by reducing and chelating iron (Sandberg, 2002; Suliburska & Krejpcio, 2011). However, ripe juçara fruit presented considerable amounts of protein (5.13–8.21% on a dry matter basis) (Borges et al., 2011), which can explain the higher bioaccessibility values of iron in the pulp of fruits harvested at the end of ripening. The same occurred with zinc, since the proteins also promote greater bioaccessibility of this mineral.

In addition to the composition of food plants in terms of the quantity and quality of proteins, the lowest rates of bioaccessibility during early ripening stages might stem from the chemical form of the minerals, nutrient interactions, and the presence of compounds such as fibers, polyphenols, and phytates, which form insoluble complexes that can negatively affect mineral bioaccessibility in food plants (Fernández-García et al., 2009; Sandberg, 2002).

The bioaccessible levels of copper (1.99–6.46 μg g^{-1} dry matter) and manganese (11.21–31.34 μg g^{-1} dry matter) in juçara fruit pulp were higher than those found by Arpadjan, Momchilova, Venelinov, Blagoeva, and Nikolova (2013) for walnuts (2–2.5 and 8–12.1 μg g^{-1} dry matter, respectively), which are considered one of the main contributors in the supply of copper and manganese in the human diet. For calcium and magnesium, bioaccessible fractions found in juçara presented higher values than other vegetables, comparable to cereals, leguminous grains and nuts (Suliburska & Krejpcio, 2011).

Considering the dietary reference intakes of potassium (4700 mg day^{-1}), calcium (1000 mg day^{-1}), magnesium (420 mg day^{-1}), iron (8 mg day^{-1}), zinc (11 mg day^{-1}), manganese (2.3 mg day^{-1}), selenium (55 μg day^{-1}), and copper (900 μg day^{-1}) for an adult male (IOM – Institute of Medicine, 2001), the intake of 100 g of juçara fruit pulp in the later ripening stages (stages 6 and 7) supplies 45–53% of manganese, 38–50% of selenium, 24–43% of copper, 8–11% of magnesium, 10–18% of calcium, 6–12% of iron, 5–8% of zinc, and 4–5% of potassium. RDA values are unavailable for cobalt.

For metals, the average bioaccessible fractions of nickel and aluminum ranged from 7.4 to 42.5% and 13.4 to 14.7%, respectively (Table 2). Cadmium and lead did not present bioaccessibility during any ripening stage. The bioaccessible fractions of nickel and aluminum in the samples did not demonstrate a pattern during the ripening stages.

Among the metals studied, Codex Alimentarius Comission (1996) establishes maximum levels for lead (0.2 mg kg^{-1}), cadmium (0.05 mg kg^{-1}) and arsenic (0.1 mg kg^{-1}) in plant foods. For nickel the value of the tolerable upper intake level (UL) is 1 mg/day (IOM, 2001). No established limits of aluminum in food have been found, however the Agency for Toxic Substances and Disease Registry (ATSDR, 2016) proposed maximum consumption limit of 1 mg per kg of weight/day. In this way, the consumption of juçara can be considered safe for human health, since the maximum values found in these fruits, on a wet basis, were 0.18 mg kg^{-1} for lead, 0.02 mg kg^{-1} for cadmium, <0.01 mg kg^{-1} for arsenic, 0.76 mg kg^{-1} for nickel and 1.14 mg kg^{-1} for aluminum.

3.2. Phenolic compounds

The content of each phenolic compound in juçara fruit pulp is presented in Table 3. It was possible to find 22 phenolic compounds: 10 phenolic acids, 10 flavonoids, 1 stilbene and 1 phenol aldehyde. Information of mass spectra limits of detection and quantification, as well as curve equation and determination coefficient obtained from the analysis of these compounds are shown in Supplementary material (Table S3). Compounds of higher content were protocatechuic (8.57–22.94 mg 100 g^{-1} dry matter), aromadendrin (2.16–16.43 mg 100 g^{-1} dry matter), vanillic acid (8.51–16.51 mg 100 g^{-1} dry matter) and ferulic acid (2.59–18.12 mg 100 g^{-1} dry matter). Other studies that evaluated phenolics in juçara fruits presented lower values for these compounds: protocatechuic acid had values of 1.34–3.80 mg 100 g^{-1} dry matter (Bicudo et al., 2014; Schulz et al., 2015), aromadendrin 2.04–7.64 mg 100 g^{-1} dry matter (Schulz et al., 2015), while vanillic acid presented values of 2.66–3.97 mg 100 g^{-1} dry matter (Bicudo et al., 2014) and ferulic acid 1.48–8.16 mg 100 g^{-1} dry matter (Bicudo et al., 2014; Borges et al., 2011).

The ripening process influenced the phenolic compounds contents in juçara fruits, with significant statistical difference ($p < 0.05$) between the studied stages. Phenolic acids and vanillin showed higher contents in the pulp of most immature fruits (stages 1–3), in most cases, with values from 0.07 to 22.94 mg 100 g^{-1} on a dry weight basis. In contrast, flavonoids and resveratrol had the highest contents in the pulp of more mature fruit (stage 4–7), with values of up to 16.43 mg 100 g^{-1} on a dry weight basis. This behavior during the ripening cycle was similar to previous studies by Bicudo et al. (2014) and Schulz et al. (2015), however, the results of this study were higher for most compounds.

After simulation of the gastrointestinal digestion, 18 phenolic compounds were detected (Table 3). Gallic acid, resveratrol, myricetin and kaempferol were not detected after this step. As for the crude extract, the bioaccessible fractions of phenolic compounds were significantly influenced ($p < 0.05$) by ripening. In general, the highest bioaccessibility values were found in the pulp of more mature fruit (stage 4–7) for the compounds of all the phenolic classes studied. The highest bioaccessible fractions were found in caffeic acid, apigenin, rutin and epicatechin.

As shown in Fig. 1, the total phenolic content obtained from the sum of the contents of all quantified phenolic compounds in this study was significantly higher than the bioaccessible levels ($p < 0.05$) in all ripening stages. Values up to 79.98 mg 100 g^{-1} dry matter were found in the crude extract of juçara fruit pulp. Schulz et al. (2015) and Bicudo et al. (2014), which also evaluated flavonoids and phenolic acids by LC–MS/MS of juçara fruits during ripening, found the maximum value of 27.38 and 44.27 mg 100 g^{-1} dry matter, respectively.

After simulating gastrointestinal digestion, the maximum value of the total phenolic content was well lower than in the crude extract (19.71 mg 100 g^{-1} dry matter). However, the bioaccessible values for caffeic acid in stages 5 and 6 presented values ten times higher than the crude extract. Rutin and epicatechin also presented higher bioaccessible contents than the crude extract in the pulp of more mature fruit (130–175%). Moreover, ellagic acid showed values five times higher after *in vitro* in the first ripening stage studied (Table 3). The other compounds studied presented values of 0.6–99% of bioaccessibility from the fourth stage of ripening.

These different values of bioaccessibility are due to chemodiversity of the phenolic compounds, which vary from simple molecules to highly polymerized molecules. Most of the phenolic

Table 3
Content (mg 100 g^{-1} dry matter) and bioaccessible fractions (BF%) of phenolic compounds in juçara fruit pulp at different ripening stages.

		Ripening stage						
		1	2	3	4	5	6	7
Phenolic acids								
Protocatechuic	Content	10.42 ± 0.27 ^{bc}	8.57 ± 0.51 ^{c}	12.13 ± 1.25 ^{bc}	12.34 ± 0.33 ^{bc}	14.58 ± 0.06 ^{b}	22.94 ± 1.99 ^{a}	22.51 ± 1.64 ^{a}
	BF%	5.1	55.0	71.9	17.3	15.0	62.9	57.0
p-Coumaric	Content	2.02 ± 0.05 ^{a}	0.53 ± 0.04 ^{e}	0.60 ± 0.06 ^{de}	0.60 ± 0.03 ^{de}	0.81 ± 0.07 ^{b}	0.78 ± 0.03 ^{bc}	0.67 ± 0.01 c^{d}
	BF%	NB	NB	NB	NB	NB	10.8	13.2
Vanillic	Content	16.51 ± 0.21 ^{a}	10.28 ± 0.47 ^{b}	9.87 ± 0.23 ^{b}	8.64 ± 0.47 ^{b}	9.43 ± 0.08 ^{b}	9.55 ± 1.11 ^{b}	8.51 ± 0.31 ^{b}
	BF%	13.3	16.3	15.4	16.7	12.9	11.0	11.2
Gallic	Content	0.48 ± 0.03 ^{b}	0.77 ± 0.03 ^{a}	0.20 ± 0.01 ^{e}	0.34 ± 0.02 c^{d}	0.25 ± 0.03 ^{d}	0.34 ± 0.06 c^{d}	0.37 ± 0.01 ^{c}
	BF%	NB	NB	NB	NB	NB	NB	NB
Caffeic	Content	0.39 ± 0.02 ^{a}	0.11 ± 0.00 ^{c}	0.36 ± 0.02 ^{a}	0.37 ± 0.02 ^{a}	0.16 ± 0.01 ^{bc}	0.15 ± 0.01 ^{c}	0.21 ± 0.00 ^{b}
	BF%	NB	262.4	252.2	568.2	956.0	1088.6	532.1
Ferulic	Content	18.12 ± 0.79 ^{a}	3.21 ± 0.00 ^{bc}	3.07 ± 0.37 ^{bc}	3.14 ± 0.15 ^{bc}	4.10 ± 0.69 ^{b}	4.57 ± 0.41 ^{b}	2.59 ± 0.20 ^{c}
	BF%	0.5	6.8	7.4	9.5	6.2	4.6	6.9
Syringic	Content	6.83 ± 0.91 ^{a}	3.72 ± 0.38 ^{b}	3.53 ± 0.18 ^{bc}	3.71 ± 0.42 ^{bc}	2.96 ± 0.03 ^{c}	3.47 ± 0.09 ^{bc}	3.76 ± 0.16 ^{b}
	BF%	8.7	11.2	17.6	17.0	15.7	14.0	10.6
Sinapic	Content	2.38 ± 0.16 ^{c}	3.01 ± 0.07 ^{c}	5.29 ± 0.11 ^{a}	4.08 ± 0.38 ^{b}	4.40 ± 0.30 ^{ab}	4.29 ± 0.10 ^{b}	4.69 ± 0.13 ^{ab}
	BF%	NB	NB	0.1	0.6	0.5	1.2	0.5
Ellagic	Content	0.07 ± 0.02 ^{e}	0.33 ± 0.00 ^{de}	0.53 ± 0.01 ^{d}	4.09 ± 0.06 ^{c}	7.13 ± 0.01 ^{a}	7.15 ± 0.15 ^{a}	6.08 ± 0.23 ^{b}
	BF%	488.0	13.3	3.8	1.1	NB	NB	NB
Chlorogenic	Content	ND	0.35 ± 0.02 ^{b}	0.33 ± 0.02 ^{bc}	0.57 ± 0.03 ^{a}	0.21 ± 0.01 ^{d}	0.27 ± 0.01 c^{d}	0.38 ± 0.05 ^{b}
	BF%	NB	NB	NB	NB	7.9	32.1	3.5
Flavonoids								
Apigenin	Content	0.03 ± 0.00 ^{a}	0.03 ± 0.00 ^{a}	0.03 ± 0.00 ^{a}	0.03 ± 0.00 ^{a}	0.03 ± 0.00 ^{a}	0.02 ± 0.00 ^{a}	0.02 ± 0.00 ^{a}
	BF%	76.6	87.5	NB	84.9	94.9	98.3	99.2
Kaempferol	Content	ND	0.17 ± 0.01 ^{e}	0.20 ± 0.01 ^{de}	0.24 ± 0.01 ^{d}	0.62 ± 0.02 ^{c}	0.68 ± 0.02 ^{b}	0.77 ± 0.02 ^{a}
	BF%	NB	NB	NB	NB	NB	NB	NB
Aromadendrin	Content	2.16 ± 0.51 ^{e}	7.83 ± 0.07 ^{d}	11.92 ± 0.38 ^{c}	15.28 ± 0.60 ^{ab}	15.09 ± 0.28 ^{ab}	13.78 ± 0.68 ^{bc}	16.43 ± 1.15 ^{a}
	BF%	2.1	2.8	1.9	3.1	2.2	1.6	0.9
Catechin	Content	<LOQ	0.36 ± 0.02 c^{d}	2.75 ± 0.23 ^{a}	<LOQ	0.35 ± 0.03 c^{d}	0.66 ± 0.00 ^{c}	2.34 ± 0.05 ^{b}
	BF%	NB	NB	NB	NB	NB	11.0	NB
Epicatechin	Content	ND	0.02 ± 0.00 ^{bc}	0.12 ± 0.01 ^{b}	0.16 ± 0.01 ^{ab}	0.06 ± 0.00 ^{c}	0.03 ± 0.00 ^{bc}	0.17 ± 0.02 ^{a}
	BF%	NB	NB	21.3	36.5	35.7	163.3	25.2
Quercetin	Content	0.17 ± 0.01 ^{f}	0.17 ± 0.00 ^{f}	0.33 ± 0.03 ^{d}	0.51 ± 0.02 ^{bc}	0.42 ± 0.03 c^{d}	0.57 ± 0.03 ^{b}	0.82 ± 0.03 ^{a}
	BF%	31.1	21.9	11.5	35.2	47.5	53.5	39.9
Taxifolin	Content	2.49 ± 0.01 ^{d}	2.57 ± 0.04 ^{d}	4.51 ± 0.39 ^{c}	5.87 ± 0.08 ^{ab}	5.60 ± 0.56 ^{b}	5.48 ± 0.42 ^{b}	6.50 ± 0.46 ^{a}
	BF%	NB	NB	1.7	3.0	3.2	2.2	1.1
Myricetin	Content	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	BF%	NB	NB	NB	NB	NB	NB	NB
Isoquercetin	Content	0.36 ± 0.00 ^{d}	0.74 ± 0.01 ^{c}	1.26 ± 0.05 ^{a}	0.88 ± 0.08 ^{bc}	0.96 ± 0.01 ^{b}	0.85 ± 0.06 ^{bc}	1.39 ± 0.02 ^{a}
	BF%	NB	4.4	8.4	19.5	19.1	42.3	30.0
Rutin	Content	<LOQ	0.13 ± 0.01 ^{d}	0.19 ± 0.00 ^{b}	0.27 ± 0.01 ^{a}	0.15 ± 0.01 c^{d}	0.11 ± 0.01 ^{e}	0.17 ± 0.01 ^{bc}
	BF%	NB	NB	NB	NB	NB	175.0	130.7
Stilbene								
Resveratrol	Content	<LOQ	<LOQ	0.02 ± 0.00 ^{bc}	0.01 ± 0.00 ^{bc}	0.03 ± 0.00 ^{b}	0.02 ± 0.00 ^{bc}	0.06 ± 0.01 ^{a}
	BF%	NB	NB	NB	NB	NB	NB	NB
Phenol aldehyde								
Vanillin	Content	3.14 ± 0.61 ^{a}	2.39 ± 0.33 ^{ab}	2.15 ± 0.18 ^{b}	2.04 ± 0.00 ^{b}	2.08 ± 0.07 ^{ab}	1.98 ± 0.17 ^{b}	1.50 ± 0.00 ^{b}
	BF%	6.7	10.6	4.5	7.5	6.3	4.4	6.2
Dry matter*	35.44	39.20	39.27	38.84	40.04	39.40	43.64	

Ripening stages: 0 (stage 1), 17 (stage 2), 23 (stage 3), 30 (stage 4), 42 (stage 5), 56 (stage 6) and 69 (stage 7) days after the red fruits appeared on bunches.
Data are presented as mean ± S.D. ($n = 6$).
*Values expressed as g 100 g^{-1}.
$^{a-e}$Values in the same row followed by different letters indicate significant differences ($p < 0.05$).
LOD – Limit of detection. LOQ – Limit of quantitation. Data regarding LOD and LOQ of each phenolic compound are available in Supplementary Material (Table S3).
ND – not detected. NB – not bioaccessible (value of the bioaccessible fraction < LOD).
Bioaccessible fractions (BF%) were calculated according to the formula: $BF\% = \left(\frac{\text{Content of the mineral released in the simulated digestion}}{\text{Content of the mineral in the sample}}\right) \times 100$

compounds are found as glycosylated forms or as esters or polymers, which during gastrointestinal digestion are hydrolyzed as a consequence of the acidic environment of the stomach and of the alkaline environment of the intestine, as well as of the action of the digestive enzymes (Alminger et al., 2014; Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). These conditions result in several changes in the phenol structure such as hydroxylation, methylation, isoprenylation, dimerisation, and glycosylation, as

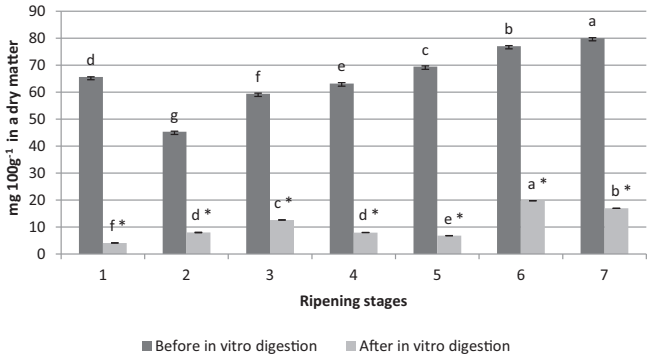

Fig. 1. Total phenolic content (mg 100 g^{-1} dry matter) before and after *in vitro* gastrointestinal digestion of juçara fruit pulp in seven ripening stages. Ripening stages: 0 (stage 1), 17 (stage 2), 23 (stage 3), 30 (stage 4), 42 (stage 5), 56 (stage 6) and 69 (stage 7) days after the red fruits appeared on bunches. Data are presented as mean ± S.D. ($n = 6$). [a–f]Different letters in the same series indicate significant differences ($p < 0.05$). *Denotes significant differences ($p < 0.05$) before and after *in vitro* gastrointestinal digestion in each ripening stage.

well as the formation of phenolic derivatives by partial degradation of the combined forms or by losing the moieties between phenols and sugars (Chen et al., 2016). Moreover, during the digestion the phenolics structures are susceptible to interaction with other dietary constituents released during this process, such as iron, other minerals, dietary fiber or proteins (Bouayed, Deuber, Hoffmann, & Bohn, 2012; Chen et al., 2016).

Considering that the same extraction procedure was used in the crude extract and in the supernatant resulting from the simulated gastrointestinal digestion, guaranteeing the complete extraction of the compounds in both phases, the changes in the structure of phenolics is one hypothesis that can justify the highest contents of some compounds in the bioaccessible fraction compared to the crude extract. Caffeic acid may have been generated from hydrolysis and/or changes during the digestion process in the phenol structure of *p*-coumaric and chlorogenic acids, for example (Chen et al., 2016; Shahidi & Ambigaipalan, 2015). In the case of epicatechin, the higher content after gastrointestinal digestion might be due to the partial hydrolysis (intestinal pH) of proanthocyanidins (Mosele et al., 2016). The high bioaccessible content of ellagic acid in the first stage studied is possibly related to the ellagitannins, which are more concentrated in more immature fruit (Taiz & Zeiger, 2009), and when exposed to acids or bases, ester bonds are hydrolyzed and the ellagitannins are rearranged into the ellagic acid (Alminger et al., 2014).

3.3. Antioxidant capacity

The antioxidant capacity of the crude extract and digested extracts was assessed using the DPPH and FRAP assays (Fig. 2). The inhibition of DPPH radical and ferric reducing ability was highest for extracts related to ripening stage 6 (123.06 and 504.60 mg ascorbic acid equivalents 100 g^{-1} in a dry matter, respectively). Other studies carried out with juçara fruits during the ripening cycle also showed higher antioxidant capacity in the final ripening stages (Bicudo et al., 2014; Schulz et al., 2015).

After simulating gastrointestinal digestion, a decrease of 64–78% in DPPH scavenging capacity and of 55–67% in FRAP was observed. In both assays, the highest antioxidant capacity was found in the stages defined as 5, 6 and 7 after *in vitro* digestion.

The digestion process affected the antioxidant capacity due to decrease in the content of phenolic compounds and/or transformation into different structural forms with other chemical properties. In addition, factors such as pH changes and interference of antioxidant phenolics with other constituents of the sample may also

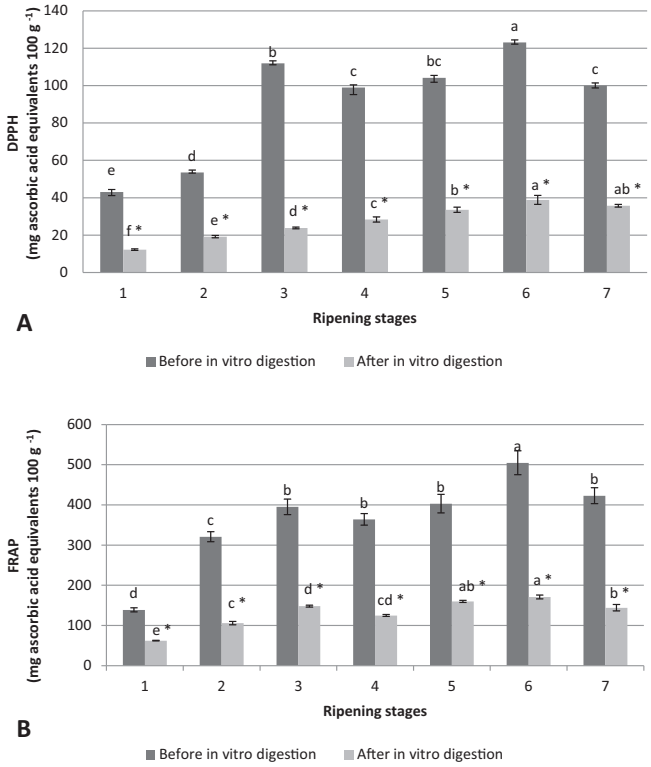

Fig. 2. Antioxidant capacity of juçara fruit pulp in seven ripening stages before and after *in vitro* gastrointestinal digestion by (A) DPPH and (B) FRAP assays. Ripening stages: 0 (stage 1), 17 (stage 2), 23 (stage 3), 30 (stage 4), 42 (stage 5), 56 (stage 6) and 69 (stage 7) days after the red fruits appeared on bunches. Data are presented as mean ± S.D. ($n = 6$). [a–f]Different letters in the same series indicate significant differences ($p < 0.05$). *Denotes significant differences ($p < 0.05$) before and after *in vitro* gastrointestinal digestion in each ripening stage.

cause changes in antioxidant capacity (Celep et al., 2015; Chen et al., 2016). Moreover, anthocyanins are the main contributors for the antioxidant capacity in juçara fruits according to Borges et al. (2013) and Schulz et al. (2015). The transition from the gastric acid to the mild alkaline intestinal environment results in a decrease in the amount of bioaccessible anthocyanins, because these pigments are highly unstable at intestinal pH contributing to decrease of the antioxidant capacity (Tagliazucchi et al., 2010).

The correlation between antioxidant capacity and phenolic and bioaccessible contents in juçara fruits pulp were calculated. A positive and significant correlation ($r > 0.68$; $p < 0.01$) was found between DPPH and protocatechuic acid, *p*-coumaric acid, rutin, quercetin and isoquercetin, while kaempferol, vanillic acid, ellagic acid and vanillin presented a negative correlation ($r > -0.85$; $p < 0.01$). In the FRAP assay, protocatechuic acid, *p*-coumaric acid, ferulic acid and quercetin presented positive and significant correlation ($r > 0.61$; $p < 0.01$). Vanillic acid, ellagic acid, kaempferol and vanillin were negatively and significatively correlated ($r > -0.69$; $p < 0.05$) with FRAP assay. These correlations indicated that polyphenol compounds contributed to the antioxidant capacities of the juçara fruit pulp play an important role in the beneficial effects of these fruits.

4. Conclusions

The content of minerals, phenolic compounds and antioxidant capacity was statistically lower after gastrointestinal digestion in relation to chemical extraction. Furthermore, the results indicated that the juçara ripening stages directly influenced the bioaccessibility of the compounds studied.

This study suggests that consuming 100 g of juçara fruit pulp can contribute greatly to the recommended daily intake of minerals, especially of manganese (45–53%), selenium (38–50%), copper (24–43%), calcium (10–18%), and iron (6–12%), as well as maximize the intake of phenolic compounds. Moreover, this preliminary bioaccessibility data reinforce that the optimal maturity for human consumption of juçara fruit are the ripening stages 5–7 (42–69 days after the appearance of red berries on bunches).

To our knowledge, this is the first study to evaluate the effects of *in vitro* gastrointestinal digestion on compounds and antioxidant potential of juçara fruit. This study is noteworthy because it provides data on the proportion of converted compounds to an absorbable form after *in vitro* digestion, which indicated an estimate of its availability for *in vivo* absorption. Future studies such as clinical trials are suitable in order to investigate the bioavailability and subsequent biological activity of the compounds.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.02.038.

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