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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