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Is the bioaccessibility of minerals affected by the processing steps of juçara fruit (*Euterpe edulis* Mart.)?

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ABSTRACT

The objective of this study was to verify if the processing steps of juçara fruit (*Euterpe edulis* Martius) affect mineral bioaccessibility using *in vitro* gastrointestinal digestion (IVG) followed by inductively coupled plasma optical emission spectrometry (ICP OES) analysis. The processing of the juçara fruit affected the content of calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn), and their bioaccessibility. It was observed an increase in the content of all minerals after the pulp extraction and reduction in bioaccessibility. It was possible to determine the bioaccessibility with the use of the IVG method and ICP OES. The bioaccessibility of Mg, Fe, Mn, and Cu decreased in the last processing step (pulp extraction), with the exception of Ca and Zn that could not be determined.

1. Introduction

The biodiversity richness of the Atlantic Forest makes this biome a hotspot (Tabarelli, Pinto, Silva, Hirota, & Bedê, 2005). However, this richness contrasts with the socioeconomic reality of the Ribeira Valley, Brazil, where is observed low Human Development Index (HDI) and the high rates of infant mortality and illiteracy (Romão, 2006). The population of the Ribeira Valley is composed mainly of quilombola (Afro-Brazilian people), caiçaras (people native to the coast) and indigenous communities. The income of these populations derives from the sustainable management of natural resources from the Atlantic Forest, such as fishing, mining, tourism, and agricultural activities (Nunes et al., 2016). Miguel and Bom (1974) reported that the inhabitants of this region have an inadequate dietary pattern, with deficiency of almost all nutrients, mainly of calcium (Ca), vitamins A, B_{12} and C, due to the low consumption of staple foods and nutrients such as milk and dairy products, fruit, vegetables, and meat.

An alternative to improve the food security of Ribeira Valley populations is the consumption of local foods derived from species native from the Atlantic Forest, such as juçara (*Euterpe edulis* Mart.), also known as the jiçara, ripeira or palmiteiro (Henderson & Galeano, 1996). Juçara was extensively exploited in the 50 and 60 decades due to its excellent palm heart quality, which led the juçara palm to be considered an endangered species (Silva-Matos, Frecklenton, & Watkinson, 1999). Currently, the pulp of the fruit produced by this palm, which is very similar to açaí (*Euterpe oleracea* Mart.), has been consumed due to its nutritional composition, and to make up the traditional Ribeira Valley population feeding, besides serving as a source of income for these families (Schulz et al., 2017).

The juçara pulp contains many essential micronutrients for the homeostasis of the human organism, such as potassium (K), iron (Fe), zinc (Zn), phosphorus (P), copper (Cu), calcium (Ca), and magnesium (Mg). Also it has the macronutrients: carbohydrates, proteins and lipids, the last two in greater quantity when compared to the açaí pulp, making the consumption of this food an extremely healthy and energetic option (Schulz et al, 2015, 2017). Despite the nutritional richness of juçara pulp, it is necessary to evaluate the bioavailability of the nutrients, since the presence of them in the food or its ingestion does not guarantee its utilization. Several factors interfere like chemical form of the nutrient, presence of binding agents, besides homeostatic regulating absorption mechanisms in the case of the micronutrients (Cozzolino, 2012). It is also indispensable to know the bioaccessibility of the nutrients present, fraction of a certain nutrient that is released from its alimentary matrix during the digestion and available for

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absorption, since this is a factor that also directly interferes in the bioavailability (Cilla, Bosch, Barberá, & Alegría, 2016).

Bioavailability and bioaccessibility is specially affected by food processing which can affect positively or negatively the nutrients content (Cilla et al., 2016). In order to obtain the juçara pulp, the fruit are submitted to several unit operations that can cause the quantitative reduction of nutrients; similar to what was observed by Krishnan, Dharmaraj, and Malleshi (2012), who reported reductions in mineral content in processed finger millet. On the other hand, processing can increase mineral content as reported by Briones-Labarca, Venegas-Cubillos, Ortiz-Portilla, Chacana-Ojeda, and Maureira (2011) in 'Granny Smith' apples and Gabaza et al. (2017) in millet. In this regard, it is important to verify the effect of the processing of juçara fruit on its micronutrients bioaccessibility.

The objective of this study was to verify if the processing of the juçara fruit affects the bioaccessibility of the minerals calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn), and the specific objectives were: *i.* verify the effect of processing steps on the content of minerals present in the mesocarp and pulp of juçara fruit, and *ii.* determine the *in vitro* bioaccessibility of the micronutrients by the gastrointestinal digestion (IVG) method followed by inductively coupled plasma optical emission spectrometry (ICP OES) analysis.

2. Material and methods

2.1. Plant material

Juçara (*Euterpe edulis* Mart.) fruit were harvested in five different locations: batches 1 and 2 were obtained from a cooperative of the Institute of Permaculture and Ecovillage of the Atlantic Forest (IPEMA), whose property was located in the rural area of the Serra do Mar in Ubatuba-SP (23°26′02″ South, 45°04′16″ West, altitude of 3 m). Batches 3 and 4 were collected in Jaboticabal-SP (21°15′17″ S, 48°19′20″ W, and 605 m altitude), at the São Paulo State University (UNESP), Faculty of Agrarian and Veterinary Sciences (FCAV), Jaboticabal Campus, and, batch 5 was obtained at the Araraquara Nautical Club located in Américo Brasiliense-SP (21°47′40″ S, 48°10′32″ W and altitude 664 m).

2.2. Processing and samples collection

The mesocarp and juçara pulp from the batches 1 and 2 were processed in a family agroindustry of Ubatuba-SP, and batches 3, 4 and 5, were processed in the Laboratory of Plant Production of FCAV-UNESP, according to the practices adopted by IPEMA. The fruit and pulp mesocarp samples were obtained in the following processing steps: *i.* at the time of harvest (fresh ripe fruit), *ii.* after selection and washing with potable water, *iii.* after immersion in hot water at ~100 °C for 2 min (soaking), and *iv.* after the pulp extraction using vertical pulping equipment. Subsequently the samples were frozen and stored at −20 °C until further laboratory analysis.

2.3. Quality analysis of the mesocarp and juçara pulp

Moisture content. The moisture content was determined using the method described by AOAC. (1997), which consisted of drying 10 g of the sample in an oven at 105 °C for 24 h. The results were expressed as percentage (%).

Hydrogenionic potential (pH). The pH was determined using a phmeter (Thermo Scientific, Orion 3 Star model) with the introduction of the electrode directly into the mesocarp and/or in the juçara pulp (AOAC., 1997).

Soluble solids content (*SSC*). SSC was determined using a digital refractometer (Alpha, Atago Co, Ltd, Japan), and expressed as percentage (%) (AOAC., 1997).

Titratable acidity (*TA*). The samples were titrated with 0.1 M sodium hydroxide solution to the equivalence point (pH 8.1) with the magnetic stirrer. TA was expressed as mg.100 g^{-1}, according to AOAC. (1997).

2.4. Minerals determination

2.4.1. Nitroperchloric digestion

Approximately 0.5 g of the mesocarp and/or the dehydrated pulp was weighed and 8 mL of a solution containing nitric/perchloric acids 2:1 (v/v) was added. Samples were digested at 100 °C for 2 h, filtered using ash-free paper filters (Unifil, Germany) into a 50 mL volumetric flask with the volume completed using Milli–Q® water, according to the methodology described by Sarruge and Haag (1974).

2.4.2. In vitro digestion

The bioaccessibility of micronutrients was assessed as described by Garret, Failla, and Sarama (1999) and Oomen et al. (2003). The fresh samples, without drying, were crushed and digested in three digestive solutions: basal saline (saliva), gastric juice and duodenal. Briefly, the *in vitro* digestion procedure consisted of weighing 3 g of sample of mesocarp and/or processed pulp of juçara which were placed in 50 mL Erlenmeyer containing 10 mL of the basal saline solution (150 mmol.L^{-1}) which consisted of NaCl (w/v), 5 mmol.L^{-1} of KCl (w/v) and 6 mmol.L^{-1} of $CaCl_2$ (w/v). To this solution was added 2 mL of Sigma-Aldrich α-amylase (0.075 g mL^{-1} - 3000 units; 20 units.mg^{-1}), the pH adjusted to 6.5 + 0.1 with 0.1 M $NaHCO_3$. The Erlenmeyers were then placed in a water bath with circular orbital motion at 95 rpm at 37 °C for 10 min. After this time the pH was adjusted to 2.5 + 0.1 with 0.1 M HCl and 2 mL of Sigma-Aldrich pepsin (0.04 g mL^{-1}; > 400 units.mg^{-1}) was added. Then the Erlenmeyers were placed in a water bath with circular orbital motion at 95 rpm at 37 °C for 1 h. After gastric digestion, the pH was adjusted to 6.5 ± 0.1 with 1.0 M $NaHCO_3$ and 10 mL of the solution containing the Sigma-Aldrich pancreatin (0.0006 g mL^{-1}; 8 × USP) and Sigma-Aldrich lipase (0.0036 g mL^{-1}; 30–90 units.mg^{-1}). Then the Erlenmeyers were placed in a water bath with circular orbital motion at 95 rpm at 37 °C for 2 h. After duodenal digestion, samples were placed in an ice bath and centrifuged (Beckman centrifuge, model Avanti J-25, Coulter, USA) at 20,000 × g for 20 min at 4 °C, to separate the supernatant (digestive fluid) from the solid phase. After centrifugation the digestive fluids were filtered on ash-free paper filter (Unifil, Germany) and stored at −20 °C until further analysis by inductively coupled plasma optical emission spectrometer (ICP OES). The entire digestive procedure was done in duplicate for each type of processing sample.

2.5. Quantification of chemical elements

The quantification of the chemical elements calcium (Ca), copper (Cu), Iron (Fe), manganese (Mn), magnesium (Mg), and zinc (Zn) present in the nitroperchloric and *in vitro* extracts (IVG) was performed by inductively coupled plasma optical emission spectrometry (ICP OES) with a radial viewing plasma configuration using a PerkinElmer Optima 8000 spectrometer (PerkinElmer, Waltham USA.) operating at 1400 W. The instrumental parameters can be observed in Table 1. To minimize the influence of salts and proteins which were added to simulate IVG digestion, all samples were diluted 100 times (v/v) using high purity deionized water (18.2 MΩ cm^{-1}) obtained by Milli-Q® water purification system (Millipore, Bedford, USA). The samples were introduced using Scott (Ryton®) cyclonic double pass spray chamber and an alumina (2 mm d.i.) injector was used. The Scott chamber was cleaned up with HNO_3 5% for 60 s every 10 readings. A multi-element standard solution (Merck, Darmstadt, Germany) containing all elements was used to plot the analytical curves of each element.

2.6. Determination of bioaccessibility

After determination of the micronutrient content in the samples