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Chemical composition, bioactive compounds and antioxidant capacity of juçara fruit (*Euterpe edulis* Martius) during ripening

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Chemical compounds studied in this article:
Gallic acid (PubChem CID: 46780424)
Protocatechuic acid (PubChem CID: 72)
p-Coumaric acid (PubChem CID: 637542)
Taxifolin (PubChem CID: 439533)
Rutin (PubChem CID: 5280805)
Quercetin (PubChem CID: 5280343)
Myricetin (PubChem CID: 5281672)
Aromadendrin (PubChem CID: 122850)
Hispidulin (PubChem CID: 5281628)
Kaempferol (PubChem CID: 5280863)

ABSTRACT

The aim of this study was to evaluate the effect of ripening on the individual phenolic compounds, total anthocyanins and antioxidant capacity of juçara fruit. The color, moisture, total protein, total lipids, fatty acids and mineral contents were also evaluated. A total of eleven phenolic compounds were identified during the ripening of juçara fruit by HPLC–ESI-MS/MS. Rutin and quercetin showed highest levels at stages 6 and 7, with 0.27 to 0.28 mg 100 g^{-1} and 1.25 to 1.48 mg 100 g^{-1}, respectively. The skin color of the fruit during ripening changed from red to black, favored by the accumulation of anthocyanins (507.57–634.26 mg cyanidin 3-glucoside 100 g^{-1} fresh matter). In addition, these compounds (quercetin, rutin and anthocyanins) showed positive correlation with the antioxidant capacity, which explains the high antioxidant potential of juçara fruit observed in DPPH and FRAP assays. The results revealed that the pulp of the juçara fruit collected at the end of ripening (stages 5 to 7) also presents adequate nutritional potential for consumption, providing higher levels of protein, lipids, oleic and linoleic acids, potassium, calcium, iron and manganese.

1. Introduction

The juçara palm (*Euterpe edulis*) belonging to the Arecaceae family is native to the Brazilian Atlantic Forest and is found mainly from the southern part of the state of Bahia to the northern part of the state of Rio Grande do Sul (Lorenzi, 2006). The fruit of the *E. edulis* palm has the form of globose berries and the color evolves from green to black during the ripening process, similarly to açaí (*Euterpe oleracea* and *Euterpe precatoria*) (Rogez, 2000; Rogez, Pompeu, Akwie, & Larondelle, 2011). Juçara berries can be consumed in the form of juice, pulp and as an ingredient in many foods (Felzenszwalb, Marques, Mazzei, & Aiub, 2013). Juçara is a "super fruit" with a high nutritional value, that is, it is rich in nutrients. Oleic and linoleic acids are the fatty acids found in highest proportions, the lipid content is high (18.45–44.08%) and other components include proteins (5.13–8.21%) and ash content

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(1.55–3.32%) (Borges et al., 2011). The protein and lipid contents are higher than those of other berries and similar to that of açaí (*E. oleracea*) (De Souza et al., 2014; Schauss et al., 2006).

However, interest in these berries has arisen particularly due to their high in vitro antioxidant properties, attributed to their high content of phenolic compounds, such phenolic acids, anthocyanins and other flavonoids (Bicudo, Ribani, & Beta, 2014; Borges et al., 2013; Cardoso et al., 2015; De Brito et al., 2007).

Phenolic compounds improve the endogenous antioxidant activity, protecting the body against oxidative damage caused by free radicals. Therefore, phenolics are generally associated with health-promoting properties and the prevention of several degenerative diseases (Oroian & Escriche, 2015). The results of several studies have indicated that juçara fruit may exert antioxidant effects in vitro (Bicudo et al., 2014; Borges et al., 2011; Vieira, Cavalcanti, Meireles, & Hubinger, 2013) and in an animal model (De Castro et al., 2014). The extract from juçara berries also exhibits strong antioxidant activity and antiproliferative activity against Vero cells (Borges et al., 2013). A recent study indicated the positive effect of juçara juice consumption on the antioxidant status and cellular oxidative damage of healthy individuals, probably due to the absorption of polyphenols present in the juice (Cardoso et al., 2015). In addition, a toxicological assessment of juçara fruit revealed that it has no adverse effects on human health and could thus contribute to the development of new ingredients which could be incorporated into food products (Felzenszwalb et al., 2013).

The content of phenolic compounds in fruit is influenced by numerous factors including the ripening stage (Siddiqui et al., 2013). During ripening, several modifications (biochemical, physiological and structural) take place and these determine the attributes of the fruit quality (Tiwari & Cummins, 2013).

Literature sources indicate that the variation in the phytochemicals is mainly dependent on the biosynthesis of phytochemicals during plant growth and their changes during physiological maturity (Tiwari & Cummins, 2013).

Phenolic compounds (phenolic acids, flavonoids, tannins, stilbenes, xanthones, anthraquinones, etc.) represent one of the main groups of secondary plant metabolites. Phenolic acids are synthesized via the phenylpropanoid pathway through the action of the enzyme L-phenylalanine ammonialyase (PAL) with phenylalanine as a substrate (Tiwari & Cummins, 2013). Biosynthetically, the majority of flavonoids (anthocyanins, chalcones, flavones, flavanols, etc.) derive from malonyl-CoA and p-coumaroyl-CoA. The biosynthesis of flavonoids commences with the synthesis of chalcone, which is catalyzed by the enzyme chalcone synthase (CHS) (Meléndez-Martínez, Fraser, & Bramley, 2010). This enzyme undergoes isomerization through the action of chalcone isomerase to form a flavanone. Also, anthocyanidin flavinium ions are produced by anthocyanidin synthase (ANS) and the glycosylated UDP-GIc: flavonoid-3-O-glycosyltransferases, responsible for the transfer of glucoside to the 3-O position of flavonoids, is a key enzyme in regulating anthocyanin synthesis during red coloration (Inostroza-Blancheteau et al., 2014).

Diverse abiotic stress triggers the synthesis of a large class of secondary phenylpropanoid metabolites. It is important to note that the phenylpropanoid compounds induced by abiotic stress, including ultraviolet radiation, are accompanied by raised transcriptional levels of a network of genes, such as the enzymes PAL, CHS and ANS (Inostroza-Blancheteau et al., 2014).

To our knowledge, no authors have reported the changes in the composition of juçara fruit during the ripening process or investigated the relation between the bioactive profile during ripening and the antioxidant capacity. In this context, the objective of this research was to correlate the levels of anthocyanins, individual phenolic compounds and antioxidant capacity, as well as other nutritionally relevant components, with the ripening stages of juçara fruit and demonstrate the important parameters associated with the harvest time in relation to the producers' profits and consumer health.

2. Materials and methods

2.1. Collection of samples

Sampling of the fruit was conducted in Florianopolis in the state of Santa Catarina, Brazil. The experimental station consists of seven juçara palm trees and three of these were selected for the monitoring of the fruit ripening cycle. Fruit samples were harvested from each palm from late August until early December (n = 100 berries/harvest) including 0, 17, 23, 30, 42, 56 and 69 days after the red berries appeared in bunches, totaling seven ripening stages.

2.2. Chemical reagents

The chemical reagents used were methanol, hydrochloric acid, sodium hydroxide, hydrogen peroxide (30% m/m), ultra-pure phenolic standards and Rh, Ca, Mg, K and Na stock solutions purchased from Sigma-Aldrich Co. (St. Louis, MO). A standard multi-element ICP III solution was purchased from Perkin-Elmer (Shelton CT, USA). Nitric acid (65% m/m) was purchased from Merck (Darmstadt, Germany) and purified by double sub-boiling distillation in a quartz still (Kürner Analysentechnik, Rosenheim, Germany). N-hexane, potassium hydroxide, ethyl ether, sulfuric acid and ammonium chloride were obtained from Vetec (Rio de Janeiro, RJ, Brazil). A commercial mixture of fatty acid methyl esters (FAME): Grain Fatty Acid Methyl Ester Mix — analytical standard, 10 mg mL^{-1} in methylene chloride was purchased from Supelco (Bellefonte, PA, USA). All chemicals were analytical grade. Argon gas, acetylene and nitrous oxide were purchased from White Martins (São Paulo, Brazil). Deionized water was obtained from a Milli-Q Plus system (Millipore, Bedford, USA).

2.3. Sample preparation

The berries were subjected to pulp removal manually, and moisture, protein and anthocyanins were evaluated. For the other analyses the pulp was submitted to bleaching for 10 min at 85 °C, and was then dried in a dryer at 40 °C for 12 h (Borges et al., 2011). The dried pulp was ground in an ultracentrifugal mill (Z200 Retsch, Haan, Germany) with 1 mm sieve at 5700 *g*.

2.4. Chemical analysis

General parameters were determined following the methods recommended by the Association of Official Analytical Chemicals (AOAC, 2005): moisture content (925.09) was determined by drying the sample in an oven at 105 °C until constant weight and the crude protein (920.87) content was calculated from the total nitrogen content determined by the Kjeldahl method, using a conversion factor of 6.25. Total lipids (920.85) were determined according to the Soxhlet extraction method.

Skin color values for each fruit were calculated as the mean of twenty measurements taken at equidistant points on each fruit. For the color measurements, a Minolta CR-400 Chroma portable colorimeter (Konica Minolta, Italy) with C illuminant and equipped with an integrating sphere with a 10 mm opening, was used. The color measurements were taken using the $L^*a^*b^*$ system.

2.5. Mineral contents

The samples (200 mg of dried pulp) were digested in an MLS-1200 microwave oven (Milestone, Sorisole, Italy) with 6 mL HNO_3 and 1 mL H_2O_2 with applied power varying between 250 and 600 W for 25 min in closed PFA vessels. Zinc, iron and manganese were determined by inductively coupled plasma mass spectrometry (ICP-MS) using a Perkin-Elmer SCIEX, model ELAN 6000 (Canada) instrument. Potassium, calcium, sodium and magnesium were determined on a high-resolution

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