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Original Research Article

Characterization, bioactive compounds and antioxidant potential of three Brazilian fruits

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ABSTRACT

With the objective of stimulating the cultivation and consumption of native Brazilian fruits, the physicochemical composition and antioxidant potential of three native species, namely the *araticu-do-mato (Rollinia sylvatica* A. St.-Hil.), pindo palm (*Butia capitata* (Mart.) Becc.) and *mandacaru-de-três-quinas (Cereus hildmannianus* K. Schum.) were determined in this study. The pindo palm fruit stood out because of its elevated carotenoid content (39.6 μ g/g) and greater antioxidant capacity (26 μ M trolox/g of fresh sample) by the ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic) method, although by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, the pindo palm fruit (3847.5 g of fresh sample/g DPPH) and *mandacaru-de-três-quinas* fruit (3249.8 g of fresh sample/g DPPH) were considered to have the same antioxidant potential with no difference between them. The *mandacaru-de-três-quinas* fruit also showed the highest total phenolic compound content (1337.3 mg/100 g). Although the *araticu-do-mato* presented the highest vitamin C content (0.32 mg/g), it did not differ statistically from the *mandacaru-de-três-quinas* fruit (0.25 mg/g); on the other hand, it was considered to be equal to the pindo palm fruit (0.23 mg/g). The *araticu-do-mato* also showed the best result for the TSS/TTA (total soluble solids/total titratable acidity) ratio (41.92), thus it was adequate for *in natura* consumption and for processing as well.

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1. Introduction

Due to the incomplete efficiency of the human endogenous defense system, the influence of external factors such as smoking, pollution, UV radiation and food, as well as the existence of some physiopathological processes (aging, obesity, inflammation and ischemia), the importance of bioactive compounds obtained from diet has been well established, which can help overcome such deficiencies and also promote protection, prevention or reduction of the effects caused by oxidative stress (Pietta, 2000; Huang et al., 2005).

The association between a diet rich in fruits and vegetables and a decrease in the risk of cardiovascular diseases and certain types of cancer is based on epidemiological evidence and, by hypothesis,

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on their antioxidant contents (Alonso et al., 2004). The action of these antioxidant compounds is related to the attenuation of oxidative events that could contribute to the pathophysiology of these diseases (Pietta, 2000), and some vitamins, phenolic compounds and carotenoids stand out among them.

For instance, vitamin C captures oxygen radicals and the formed species following the loss of one electron act as free radicals, such as semidehydroascorbic acid or the ascorbyl radical. These species are relatively stable when compared to other free radicals, with half-lives of 10^{-5} s and are fairly unreactive (Padayatty et al., 2003). Moreover, they have the highest oxidizing power of recycling vitamin E in the lipid peroxidation process of membranes and lipoproteins (Murakami et al., 2006). The antioxidant effects of phenolic compounds are attributed to the reducing power of the aromatic hydroxyl group, which reduces reactive free radicals (Shahidi et al., 1992) and is capable of chelating transition metals. On the other hand, carotenoids protect biological systems from free radicals by transferring the energy of the excited oxygen molecule to the carotenoid molecule itself, reacting mainly with the peroxide radicals and molecular oxygen (Beutner et al., 2001).

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Brazil stands out in this context due to its elevated production of different native and exotic fruit trees as a result of its vast territorial extension and its insertion, mainly in tropical and temperate climate zones. However, despite the fact that agro-business is one of the most competitive sectors of the Brazilian economy (Brasil, 2009), large plantations that grow few species are taking over more area year by year, maintaining and increasing their productivity by means of fertilizers, herbicides and other chemicals. The population consequently loses the chance of varying their diet and knowing the use of native species capable of offering rich, nutritious alternatives, since there are innumerous economically under-explored species. Such species could be more widely used for *in natura* consumption or in the production of sweets, jams, juices and ice-creams.

Aiming to stimulate the cultivation and consumption of Brazilian native species, offering alternative foods that contribute to overall health, the current work determined the chemical composition, antioxidant potential, total phenolic compounds, vitamin C content and the carotenoid profile of three native species from the south of Brazil.

2. Materials and methods

2.1. Sample material

The araticu-do-mato (Rollinia sylvatica A. St.-Hil.), pindo palm fruit (Butia capitata (Mart.) Becc.) and mandacaru-de-três-quinas (Cereus hildmannianus K. Schum.), were the fruits used in the study, which were obtained from the active germplasm bank of native fruit trees of Embrapa Temperate Climate Station (Pelotas/RS/ Brazil) with the exception of the mandacaru-de-três-quinas, which came from the city Barra do Ribeiro (RS/Brazil). The araticu-do mato is an evergreen tree 6-8 m with fruits of sincarpo bacaceo, sweet and juicy pulp containing with many seeds, pindo palm fruit is a palm around 4–5 m and fruit has fibro-juicy mesocarp (pulp), acid flavor and the mandacaru-de-três-quinas is an arborescent cactus with sweet white pulp, black seeds, soft and edible. The fruits were incorporated in the Institute of Natural Sciences (ICN) Herbarium (UFRGS) under the number of 89236, 34139 and 115413 for araticu-do-mato, pindo palm and mandacaru-de-três-quinas, respectively.

All samples were collected when fully mature, in 2 batches containing about 3 kg of fruits. The fruits of pindo palm were harvested between the months of February and March, araticudo-mato between April and May and mandacaru-de-três-quinas between March and May 2010. The fruits were pre-selected considering the absence of visible injury and infections, and also color and size uniformity were taken into account as well, afterwards they were stored frozen $(-20 \degree C)$ until analyzed. In all the analysis, the normally edible parts of the fruits were used, that is, for the pindo palm fruit the skin and pulp were considered, but only the pulp for the araticu-do-mato and mandacaru-de-três-quinas. At the time of analysis, at least 10 fruits were thawed at room temperature and homogenized in an Ultra-turrax homogenizer (Ika, Artur Nogueira, São Paulo, Brazil) to determine the content of total soluble solids, total titratable acid, protein, sugars, ash, moisture, vitamin C, phenolic compounds and antioxidant activity. To analyze the content of lipids and fibers, the samples after homogenized, were freezedried (Apparatus Inc., EUA) and ground with a mortar and pestle. The results are expressed as fresh matter, except for proximate composition (proteins, lipids, carbohydrates, fiber) that was expressed as dry matter. All analyses were performed in triplicate and the results were expressed as mean and standard deviation.

2.2. Chemical composition

All analyses were carried out according to AOAC (1997). The protein concentration was determined by the Kjeldahl method using a conversion factor of 5.75. The lipid concentration was determined for Soxhlet extraction method, food fiber (total and insoluble) using the enzymatic-gravimetric method, the ash in muffle furnace controlled to 550 °C, moisture contents determination by gravimetry, the total carbohydrate content was determined by difference, and the reducing and non-reducing sugars were determined by Eynon–Lane method. Total titratable acidity (TTA) was determined by titration and the total soluble solids (TSS) by using a digital PAL-3 refractometer (Atago Co., Taiwan, China).

2.3. Total phenolic compounds

To extract these substances, five grams of fresh sample were homogenized in an Ultra-turrax homogenizer with 20 mL methanol, and centrifuged for 20 min at 25,400 × g in a refrigerated centrifuge at 4 °C. A 250 μ L aliquot of the supernatant was diluted in 4 mL of ultra-filtered water and a control was also prepared containing 250 μ L of methanol. The samples and the control were combined with 250 μ L of 0.25 N Folin–Ciocalteau Reagent (Swain and Hillis, 1959). After 3 min of reaction, 500 μ L 1 N Na₂CO₃ were added, the mixtures incubated for 2 h at room temperature and the absorbance read at 725 nm in an Ultrospec model 3100 pro UV-visible spectrophotometer (Amersham Biosciences, Sweden). A standard curve was constructed to quantitate the phenolic compounds, using chlorogenic acid in the concentration range from 0 to 0.50 μ g/mL. The results were expressed in mg chlorogenic acid equivalents/100 g fresh sample.

2.4. Antioxidant activity

Methodology based on sequestering the 2,2- diphenyl-1picrylhydrazyl (DPPH) radical was used to determine the antioxidant activity (Brand-Wiliams et al., 1995) and also the 2,2'-azinobis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) method (Kuskoski et al., 2005). The extract was obtained from 5 g of sample ground in methanol (50%) and acetone (70%) sequentially, using three different dilutions (1:5 (v/v), 1:10 (v/v), 1:15 (v/v)). The fresh samples were weighed in centrifuge tubes and extracted sequentially with 40 mL of methanol/water (50:50, v/v) at room temperature for 1 h. The tubes were centrifuged at $25,400 \times g$ for 15 min and the supernatant was recovered. Then 40 mL of acetone/ water (70:30, v/v) was added to the residue at room temperature, extracted for 60 min and centrifuged. Methanol and acetone extracts were combined, made up to 100 mL with distilled water and used to determine antioxidant capacity. For the DPPH method, a 0.1 mL aliquot of each dilution of the extract was reacted with 3.9 mL of DPPH radical (0.06 mM). The readings were made in a spectrophotometer at 515 nm after 30 min. The results were expressed in g of fresh sample/g DPPH. For the ABTS method, a 30 µL aliquot of each extract dilution was reacted with 3.0 mL of ABTS radical (5 mL ABTS stock solution with 88 µL of solution of potassium persulfate) and the reading taken at 734 nm after 6 min. The results were expressed as μM trolox/g fresh sample.

2.5. Vitamin C

The determination of vitamin C was based on the methodology proposed by Rosa et al. (2007) with some modifications. Each 5 g sample was ground in an Ultraturrax with 20 mL 0.05 M suprapure 96% sulfuric acid (Merck, Darmstadt, Germany) for 1 min, centrifuged at $25,400 \times g$ for 15 min and then filtered through a Teflon hydrophilic filter unit. The analyses were carried out in a

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