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Bioactive compounds, antioxidant activity and minerals of 'Cajuí' (*Anacardium humile* St. Hill) during the ripening

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The present study aimed to determine the content of minerals, bioactive compounds and the antioxidant activity of 'Cajuí' (*Anacardium humille* St. Hill) during the ripening. The fruits were collected in three maturity stages. The content of the minerals, total phenolics, proanthocyanidin and carotenoids contents as well as the antioxidant activity, ABTS (2,2-azinobis-3-ethylben-zothiazoline-6-sulphonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) were determined, with results expressed on Trolox equivalent antioxidant capacity (TEAC). The Student's t-test was employed to analyse the data, based on a significance level of $p < 0.05$. The total phenolic contents were determined as 1424.33 ± 28.62, 991.16 ± 21.52 and 867.08 ± 28.56 mg GAE.100 g^{-1} for the immature, semi-mature and mature stages, respectively. Regarding the carotenoid content, 18.05 ± 1.01 was obtained for the immature stage, 8.62 ± 0.96 was obtained for the semi-mature stage, and 8.32 ± 0.97 mg-β-carotene.100 g^{-1} was obtained for the mature stage. Regarding the antioxidant activity; it was particularly relevant for the fruits in the immature stage in the three methods used: DPPH (30399.63 ± 27.06 µM TEAC.100g^{-1}), ABTS (40860.62 ± 9.32 µM TEAC 100 g^{-1}) and FRAP (6118.22 ± 83.04 µM TEAC.100 g^{-1}). The 'cajuí' exhibited high antioxidant activity and content of bioactive compounds, especially for the immature stage. Among the minerals, 'cajuí' showed high levels of K (potassium), P (phosphorus), and Mg (magnesium). Thus, the cajuí presents itself as a good source of antioxidant compounds which can be exploited by the food industry as an ingredient for the development of a food with functional properties and the pharmaceutical industry, as phytochemical.

Key words: Minerals, phenolic compounds; maturation stages; antioxidant activity.

INTRODUCTION

The Cerrado's flora comprises a variety of fruit tree species with great potential for agricultural use, which are traditionally used by the local population, who consume them *in natura* or processed as juice, liquor, ice cream, jams and sweets (Silva et al., 2008). Among the compounds present in foods with functional properties, antioxidants have received special attention because they protect the human body against oxidative stress, thus avoiding a great number of chronic non-communicable diseases (Canuto et al., 2010; Yahia, 2010).

Anacardium humile St. Hill. (Anacardiaceae) is commonly known as 'cajuzinho-do-cerrado' or 'cajuí' (Hoehne, 1979). This pseudo-fruit can be consumed in the form of juice, sweets and wine. The 'cajuzinhos' ("small cashews") are popularly recognised as having excellent taste and low astringency. The high sugar and total soluble solids contents, which influence the level of sweetness of these small cashew apples, are apparently responsible for the good sensory acceptance (Agostini-Costa et al., 2004).

During fruit maturation, physical, chemical, biochemical and physiological changes occur, which result in detectable transformations in the colour, flavour, aroma and texture (Romojaro et al., 1996). Some authors have demonstrated the existence of significant differences in the levels of bioactive compounds and in the antioxidant capacity of other tropical fruits at different maturation stages (Park et al., 2006; Zhang et al., 2006). Therefore, the present study aimed to determine the influence of the maturation stage on the bioactive compounds content and the antioxidant activity of the 'cajuí' fruit.

MATERIALS AND METHODS

Samples preparation

The *A. humile* St. Hill fruits were provided in two lots in the months of August and September of 2012 by the Brazilian Agricultural Research Corporation (Empresa Brasileira de Pesquisa Agropecuária – Embrapa), located at latitude 05°05'21'' and longitude 42°48'07'' in the city of Teresina, capital of the State of Piauí, Brazil. The fruits were collected directly and were in three different maturation stages (immature, semi-mature and mature), as determined by physical characteristics such as fruit colour and shape(immature - green fruit and firm texture; semi-mature - yellowish green fruit and soft texture; and ripe - yellow fruit and soft texture)with the satisfactory integrity of the samples based on the fruit conservation state. Once sanitised, the pulp of the fruit was removed, and the fruits were dried in a ventilated oven at 45°C for 12 h. After drying, the fruits were ground in a mill and stored in plastic bags at -20°C for later analysis of the levels of bioactive compounds and the antioxidant properties.

Reagents

The Folin-Ciocalteu phenol reagent, catechin, β-carotene, 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonicacid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Chemical (St. Louis, MO, USA). Gallic acid, sodium carbonate (Na_2CO_3), hydrochloric acid (HCl), anhydrous ferric chloride ($FeCl_3$), methanol, ethanol, hexane and acetone were purchased from Vetec (Rio de Janeiro, state of Rio de Janeiro-RJ, Brazil). All of the reagents used were of analytical degree, and deionised water was used.

Procedure

The bioactive compounds analyses were performed in the Food Chemistry Laboratory of the Federal University of Santa Catarina (Florianopolis - SC), and minerals analyses in Institute of Food Technology (Campinas – SP).

Preparation of extracts

The extracts were prepared with a dry sample according to Rufino et al. (2010). Two solvents were used: 80% acetone and a methanol/acetone/water mixture (4:4:2 by volume). The extraction was conducted with an ultrasound device (USC-1400-Original ®) for a period of 60 min, followed by centrifugation at 4000 rpm. Subsequently, the supernatant was used to determine the total phenolic and proanthocyanidin contents and the antioxidant activity.

Determination of total phenolics content

For this analysis, 2 ml of deionised water was added to a 10 ml flask, 100 µl of sample (extract) was pipetted with an automatic pipette, and the sample was transferred to a 10 ml volumetric flask. Next, 0.5 ml of the Folin-Ciocalteu reagent was added, followed by vigorous agitation. After 5 min, 1.5 ml of sodium carbonate at 20% m/v was added, and the mixture was well agitated and diluted with deionised water to a volume of 10 ml. After a 2 h rest at room temperature with the sample kept in the dark, the absorbance was measured at 765 nm with a spectrophotometer Hewlett-Packard model HP 8452A (Cheadle Heath, Stockport Cheshire, UK)in a 10 mm cuvette (Singleton and Rossi, 1965). The total phenolic content was expressed in mg gallic acid equivalent (GAE) per 100 g of sample. Each determination was performed in triplicate and repeated at least three times.

ABTS method

The ABTS (2,2-azinobis-3-ethylben-zothiazoline-6-sulphonic acid)

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method used was previously described by Re et al. (1999). The $ABTS^+$ cation was produced by mixing ABTS (7 mM) with 2.45 Mm potassium persulphate (final concentration) and keeping the mixture in the dark and at room temperature for 16 h. For analysis, the reagent was diluted in ethanol until the absorption at 734 nm reached the value of 0.700 ± 0.02. The absorbance was measured in a Hewlett-Packard 8452A spectrophotometer 7 min after (kept at room temperature and in the dark) the addition of the extract. The results were expressed in µM Trolox equivalent antioxidant capacity (TEAC) per 100 g of sample. Each determination was performed in triplicate and repeated at least three times.

DPPH (2,2-diphenyl-1-picrylhydrazyl) method

For this analysis, 0.0394 g of the radical was weighted and dissolved in 10 ml of methanol. After this procedure, the solution was diluted 1:100 in 80% methanol v/v, adjusting the initial absorption to 0.800. Once the solution was prepared, 2.9 ml of the radical was pipetted into a 10 mm cuvette. The initial absorption was then measured. A 100 µl sample of extract was added, and a reaction time of 30 min was allowed at room temperature with the sample kept in the dark with the spectrophotometer Hewlett-Packard model HP 8452A. The results were compared to the standard antioxidant Trolox and expressed in µM TEAC 100 $^{-1}$g of sample. The method was conducted according to Kim et al. (2002). Each determination was performed in triplicate and repeated at least three times.

FRAP (ferric reducing antioxidant power) method

For the sample, 200 µl of the 3 M ferric chloride solution was pipetted into 10 ml test tubes in triplicate. Next, 200 µl of the sample extract was added to each test tube, followed by homogenisation with a vortex agitator. At this point, a chronometer was started to time the reaction. The reaction occurred for 30 min in a water bath at 37°C. The sample was then removed from the water bath, and 3.6 ml of the previously prepared TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) solution was added. Ten min after the addition of TPTZ (kept at room temperature and protected from light), the absorbance was measured at 620 nm with a spectrophotometer Hewlett-Packard model HP 8452A in 10 mm cuvettes. The results were expressed in µM TEAC per 100 g of sample, according to the methodology described by Benzie and Strain (1999), with modifications by Arnous et al. (2002). Each determination was performed in triplicate and repeated at least three times.

Determination of total carotenoid content

The carotenoid content was determined according to the methodology by Alvarez-Suarez et al. (2011) and Ferreira et al. (2009). In brief, 1 g of sample was weighed in a 30 ml beaker, and 10 ml of the acetone/hexane (4:6) solvent, which was kept under agitation for 10 min, was added. Next, the content was filtered, and the absorbance was determined at 450 nm with a spectrophotometer Hewlett-Packard model HP 8452A. The results were expressed in mg of β-carotene per 100 g of sample, for comparison with the standard β-carotene curve previously constructed. Each determination was performed in triplicate and repeated at least three times.

Determination of proanthocyanidin content

The level of proanthocyanidin (condensed tannins) was determined by colorimetry using the vanillin method and using catechin as a standard (Price et al., 1978). For this procedure, 5.0 ml of the vanillin reagent (0.5 g of reagent and 200 ml of methanol HCl at 4%) was added to 1.0 ml of the methanol/acetone extract. After 20 min of reaction in the dark and at room temperature, the reading was taken at an absorbance of 500 nm. Measurements were performed in triplicate, and the results were expressed in mg of catechin equivalent (CE) per 100 g of dry sample. Each determination was performed in triplicate and repeated at least three times.

Determination of minerals

To determine the mineral, all glassware used after washing with soap Extran (Merck) previously remained immersion in nitric acid (HNO_3) 25% (v/v) for 24 h. Then, the glassware was rinsed with distilled water and demineralized (MΩ resistivity of 18.2 cm^{-1}). To determine the role of calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), sodium (Na), potassium (K), phosphorus (P) and zinc (Zn) in the samples, we used as a method of sample preparation by dry digestion, according to Horwitz and Latimer (2005) were weighed in a porcelain capsule 2.5 g sample in triplicate. Then, the samples were pre-calcined in the heating plate and incinerated in a muffle furnace at 450°C until formation of ash-free black dots. The ashes were quantitatively transferred to a volumetric flask with 25 ml of hydrochloric acid 5% (v/v) and the solution was filtered through quantitative filter paper before reading on emission spectrometer with inductively coupled plasma (ICP-OES). Analytical blanks were prepared by omitting the samples. The quantification of inorganic elements was performed using an emission spectrometer (ICP-OES) of Varian (Mulgrave Victoria, Australia) model Vista MPX axial view, equipped with a source of radio frequency (RF) of 40 MHz, one simultaneous multi-element detector solid state type Charge Coupled Device (CCD), a peristaltic pump, a spray chamber and nebulizer sea spray. The system is fully controlled by the ICP Expert software using the plasma gas liquid argon with purity of 99.996% (Air Liquid, SP, Brazil).

Statistical analysis

For the statistical analysis, a database was created with the statistical package for the social sciences (SPSS, 2010) software, version 17.0. The results are presented in tables with their respective means and standard deviations for each variable studied. To determine if there was a significant difference between means, Student's t-test and Tukey's test of means were used, Analysis of variance (ANOVA) and normality Kolmogorov Smirnov test were also applied. The significance level adopted for all the tests was $p < 0.05$ (5%) (Andrade and Ogliare, 2010).

RESULTS AND DISCUSSION

These results (Table 1) indicate that the total phenolics content decreased throughout the maturation process. Lopes et al. (2012) studied the antioxidant potential of the *A. occidentale* from different early dwarf clones during their ripening and obtained values lower than those in the present study, varying from 26.70 to 375.79 mg GAE.100 g^{-1}. Rocha et al. (2013) obtained sequential extraction values of 51.15 and 11.58 mg GAE.100 g^{-1} in alcoholic and aqueous extracts, respectively. This inconsistency

Table 1. Total phenolic content, Carotenoids content and proanthocyanidins content in 'cajuí' (*Anacardium humile* St Hill) at three maturation stages, expressed on a dry basis.[1]

Maturation Stages	Total Phenolics (mg.GAE.100 g^{-1}) [2]	Carotenoids (mg-â-carot.100 g^{-1})	Proanthocyanidins (mg CE.100g^{-1}) [3]
Immature	1424.33±28.62[a]	18.05±1.01[a]	37.41±0.00[a]
Semi-mature	991.16±21.52[b]	8.62±0.96[b]	5.88±1.64[b]
Mature	867.08±28.56[c]	8.32±0.97[b]	4.96±1.65[c]

[1]Data was expressed as average ± SD, n=3; [2]Equivalent to gallic acid (GAE); [3]Catechin Equivalent (CE) ;The same letters in the row represent non-significant difference between means according to Student's t-test $p<0.05$.

can be explained by differences in soil, climate, growing season, postharvest storage and extraction methods (Melo et al., 2006; Barreto et al., 2007; Rufino et al., 2010). Plants may also activate polyphenol synthesis in response to stress, such as injury, pathogens or low nutrients (Dixon and Paiva, 1995). Nevertheless, the variation between samples in different maturation stages is expected because maturation can be defined as a sequence of alterations in colour, flavour and texture of fruits and vegetables in general.

Rocha et al. (2011) obtained values of 170, 184 and 190 mg GAE.100 g^{-1} for three 'cajuí' varieties using a 70% acetone extraction. The variation in the content of these compounds can be explained by climate and soil change, among other environmental factors, because the fruits used for the study by Rocha et al. (2011) were collected in the states of Goias, Bahia and Federal District. There are no data in the literature concerning the total phenolic content in 'cajuí' at different maturation stages; however, studies have been conducted with other fruits. For instance, Lichtenthäler et al. (2003) and Pacheco-Palencia et al. (2009), demonstrated açaí (*Euterpe oleraceae* Mart.) to be a fruit rich in phenolic compounds and with high antioxidant activity.

Gordon et al. (2012) obtained total phenolics contents of 12317 ± 264, 3039 ± 149 and 3437 ± 154 mg GAE 100 g^{-1} for the immature, semi-mature and mature stages of açaí, respectively. Those results were similar to the ones obtained in the present study. Using a comparison with other typical fruits from the Cerrado region, Almeida et al. (2011) obtained 98.8 ± 5.6 mg GAE 100 g^{-1} for 'mangaba', 159.9 ± 5.6 mg GAE 100 g^{-1} for 'murici' (or golden spoon), 83.8 ± 6.1 mg GAE 100 g^{-1} for tamarind and 44.6 ± 2.7 mg GAE 100 g^{-1} for 'umbu'(or Brazil plum). The contents obtained in the cited fruits were lower than the ones observed in the present study. The carotenoids content decreased significantly as the fruit matured (Table 1): the immature stage exhibited the greatest carotenoid content (18.05 ± 1.01 mg 100 g^{-1}), followed by the semi-mature (8.62 ± 0.96 mg 100 g^{-1}) and mature (8.32 ± 0.97 mg 100 g^{-1}) stages.

Lopes et al. (2012) obtained the a minimum of 0.09 mg 100 g^{-1} and maximum value of 37.58 mg 100 g^{-1} of carotenoids in *A. occidentale* when studying different early dwarf clones during their ripening. Therefore, the carotenoids content obtained in the present study was higher in the three maturation stages in comparison to the data from the literature, especially in the immature stage. Carotenoids are present in chloroplasts and are normally masked by the presence of other dominant chlorophyll pigments. Therefore, although they are present in greater amounts in the immature stage, carotenoids are not visually perceived because chlorophyll is the dominant pigment. As the fruit matures and chlorophyll degrades, the carotenoids become visible in colour shades varying from orange-yellow to red.

Regarding the proanthocyanidin content, the values obtained also differed statistically among the immature (37.41 ± 0.00 mg CE 100 g^{-1}), semi-mature (5.88 ± 1.64 mg CE 100 g^{-1}) and mature (4.96 ± 1.65 mg CE 100 g^{-1}) stages. Rocha et al. (2011) obtained higher values (186, 242 and 132 mg CE 100 g^{-1}) for the three varieties of 'cajuí'. The carotenoid and proanthocyanidin content in plants can vary according to the environment, cultivation system, solar incidence, type of soil and even the type of extraction and methodology employed for analysis.

Table 2 shows the results for antioxidant activity using three different methods (DPPH, ABTS and FRAP). The immature stage exhibited the highest antioxidant activity.

The antioxidant activity of 'cajuí' decreased with maturation (Table 2), becoming lower in the mature stage for all the methods in different extracts. Due to the complexity of the composition of foods, their antioxidant power depends on the synergistic effects and redox interaction between the different nutrient and "non-nutrient" molecules, which together contribute to the possible health benefits. Therefore, attention has been given to the antioxidant activity of fruits, a parameter that allows a real evalution of the nutritional value of foods (Lenucci et al., 2006; Pellegrini et al., 2007).

In comparison to the present study, Gordon et al. (2012) obtained decreasing values throughout the maturation of açaí when determining the antioxidant activity using the ABTS radical-scavenging method,

Table 2. Determination of antioxidant activity (DPPH, ABTS and FRAP) of 'cajuí' (*Anacardium humile* St Hill) at three maturation stages, expressed ona dry-weight.[1]

Maturation stages	DPPH – TEAC[2] µM.100 g $^{-1}$	ABTS- TEAC µM.100 g^{-1}	FRAP – TEAC µM.100 g^{-1}
Immature	30399.63 ± 27.06[a]	40860.62 ± 9.32[a]	6118.22 ± 83.04[a]
Semi-Mature	18647.86 ± 61.65[b]	15107.08 ± 1.93[b]	2882.20 ± 29.01[b]
Mature	18498.16 ± 88.10[b]	12171.12 ± 3.87[c]	2125.48 ± 36.76[b]

[1]Results presented as mean ± SD, n=3; [2] TEAC value (antioxidant activity equivalent to Trolox).The same letters in the row represent non-significant difference between means according to Student's t-test p<0.05.

Table 3. Mineral composition (mg.100^{-1} g) of the *Anacardium humile* St Hill fruit in stage maturein relation DRI (Dietary Reference Intakes).

Minerals	DRI (mg/day;µg/day*)	Values**	%***
Calcium	1000	2.4 (± 0.2)	0.24
Copper	900	0.13 (± 0.01)	0.01
Iron	14	0.24 (± 0.01)	1.71
Phosphorus	700	27.0 (± 1)	3.85
Potassium	-	127.0 (± 5)	-
Sodium	-	8.1 (± 0.6)	-
Magnesium	260	9.8 (± 0.1)	3.77
Zinc	7	0.14 (± 0.01)	2.00

*Daily Intake: mg/day for calcium, magnesium, phosphorus, iron, zinc and manganese; mg / day for copper (Brasil, 2005); ** Results are expressed as averages ± SD (mg / 100 g); *** Percentage adequacy according to the IDR.

namely 17.0 ± 0.71, 4.04 ± 0.05 and 2.78 ± 0.10 µM TEAC 100 g^{-1}. Such values are lower than the ones obtained in this study. However, it is interesting to observe that the antioxidant activity decreased with a decrease in the total phenolics content, demonstrating once again that the presence of such compounds influences the antioxidant activity.

Bramorski et al. (2011) obtained similar values to the ones found in the present study for mature 'camarinha' fruit (*Gaylussacia brasiliensis* (Spreng) Meisn), with values of 2041.20 ± 4.03, 2110.62 ± 19.94 and 765.32 ± 2.60 µM TEAC 100 g^{-1} for a methanol extraction using the ABTS, DPPH and FRAP methods, respectively. For an extraction with acetone, the results were 2054.72 ± 1.54, 2256.64 ± 92.50 and 851.77 ± 21.59 µM TEAC 100 g^{-1} for ABTS, DPPH and FRAP, respectively. Thus, based on the higher values obtained in the present study, 'cajuí' exhibited high antioxidant activity.

Moo-Huchin et al. (2015) determined the antioxidant compounds, antioxidant activity and content of individual phenolic compounds of freeze-dried peel from three tropical fruits grown in Yucatan, México: purple star apple (*Chrysophyllum cainito* L.), yellow cashew and red cashew (*Anacardium occidentale*). The freeze-dried peels were good source of antioxidant compounds. ABTS and DPPH values in the peel from each fruit were 3050.95 to 3322.31 µM Trolox 100 g^{-1} dry weight (DW) or 890.19 to 970.01 mg of vitamin C 100 $^{-1}$g DW, and 1579.04 to 1680.90 µM Trolox 100^{-1} g DW or 340.18 to 362.18 mg of vitamin C 100^{-1} g DW, respectively.

Table 3 presents the contents of minerals (mg/100 g) and recommended daily intake (RDI) in the A. Humile (stage mature). The fruit is highlighted in the content K (127 mg 100^{-1}g), P (27.0 mg 100^{-1}g) and Mg (9.8 mg 100^{-1}g). Fruits in general are a good potassium source because this cation represents one of the most abundant minerals due to its diversity of functions in plants (Andola et al., 2011; Soares et al., 2004). Marc et al. (2011) conducted analysis in cashew apple juice (*A. occidentale*) and obtained values ranged from 2043.8 to 2189.5 mg L^{-1} for K, 213.9 to 215.8 mg L^{-1} for P, and 195.6 to 215.1 mg L^{-1} for the mineral Mg. Among macro minerals analyzed, these were obtained in greater quantity similar to the present study. Trace elements do not provide calories but they play an important role in the metabolic processes of the human body.

Conclusions

'Cajuí' exhibited a high content of phenolic compounds, especially at the immature stage. The acetone solvent

performed the best for the extraction of such compounds. The proanthocyanidin content was greatest in the immature stage and decreased during the maturation process. The cajuí showed high levels of K, P and Mg. 'Cajuí' displayed a high total carotenoids content, with the highest content at its immature stage. The fruit exhibited high antioxidant activity as confirmed by the three methods tested (ABTS, DPPH and FRAP), especially at the immature stage. Thus, the cajuí presents itself as a good source of antioxidant compounds which can be exploited by the food industry as an ingredient for the development of a food with functional properties and the pharmaceutical industry, as phytochemical.

Conflict of interests

The authors have not declared any conflict of interests.

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