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CENTESIMAL AND MINERAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF THE BACABA FRUIT PEEL

COMPOSIÇÃO CENTESIMAL, MINERAL E POTENCIAL ANTIOXIDANTE DA CASCA DO FRUTO DE BACABA

Bruna Mendes CORRÊA[1], Luana BALDISSERA[2] , Flavia Rodrigues BARBOSA[3] , Elton Brito RIBEIRO[4], Carla Regina ANDRIGHETTI[4], Juliana da Silva AGOSTINI[4], Dênia Mendes de Sousa VALLADÃO[5]

1. Aluna de mestrado do Programa de Pós-Graduação em Ciências Ambientais, Universidade Federal de Mato Grosso - UFMT, Sinop, MT, Brasil; 2.Aluna de graduação em Farmácia, Universidade Federal de Mato Grosso - UFMT, Sinop, MT, Brasil; 3. Professor, Programa de Pós-Graduação em Ciências Ambientais, Instituto de Ciências Naturais, Humanas e Sociais, Universidade Federal de Mato Grosso - UFMT, Sinop, MT, Brasil; 4. Professor, Instituto de Ciências da Saúde, Universidade Federal de Mato Grosso - UFMT, Sinop, MT, Brasil; 5. Professor, Programa de Pós-Graduação em Ciências Ambientais, Instituto de Ciências da Saúde Universidade Federal de Mato Grosso - UFMT, Sinop, MT, Brasil. deniavalladao@gmail.com.

ABSTRACT: *Oenocarpus bacaba* Mart. is a palm tree native from Amazon with highlighted antioxidant activities. Its fruit (bacaba) processing dismisses the peel, which has nutrients that can collaborate for its antioxidant activity and preventing diseases. Thus, this study assessed the centesimal and mineral composition, physicochemical characterization (acidity, total soluble solids, pH, and color), total phenolics (Folin-Ciocalteu method), anthocyanins and antioxidant activity by DPPH (2,2 difenil-1-picril hidrazil) method in bacaba peels. For the centesimal composition, it was found 4.87, 1.42, 29.13, 1.08 and 63.32 g 100 g^{-1} for water content, ashes, lipids, proteins, and total carbohydrates, respectively. For the mineral composition, it was found 582.97, 79.00, 51.79, 0.625, 37.02, 2.37 and 77.12 mg 100g^{-1} contents for potassium, sodium, magnesium, copper, calcium, manganese, and phosphorus, respectively. The physicochemical characterization showed pH 5.66, titratable acidity 0.306% of citric acid, total soluble solids 9,75, and coordinates L* a* e b* of 19.03, 8.07 and 9.25, respectively. Phenolic contents were 42.07 mg EAG g^{-1}. The antioxidant potential IC_{50} was 1.07 mg mL^{-1} and anthocyanins 37.31 mg 100 g^{-1}. Results show that bacaba peels are an alternate source of nutrients suggesting their use in food as well cosmetic industries, especially for their antioxidant activity and mineral composition.

KEYWORDS: Anthocyanins. Nutritional composition. DPPH. *Oenocarpus bacaba* Mart., Total phenolic.

INTRODUCTION

Brazil is the third biggest country in fruit production, with great potential of expansion due to its large amount of native and exotic fruits not economically exploited (PARANÁ, 2015). Bacabeira (*Oenocarpus bacaba* MART.) is a palm tree native from Amazon biome with great antioxidant properties. It produces a purple fruit (bacaba), very similar to açaí (*Euterpe oleracea* Mart.) (FINCO et al., 2012; PUERARI et al., 2015).

The economically potential use of bacaba is based on its fruit pulp as a beverage, heart of palm extraction and its edible oil, as well as the use of its leaves in handicraft, fiber and tile production, and its stem in construction industry (FERREIRA, 2005; GUIMARÃES, 2013). Despite its local importance, low attention has been addressed to the functional and nutritional potential of bacaba (GUIMARÃES, 2013).

The fruit processing of bacaba for its pulp and edible oil extraction from the nut generates the peel as a by-product. These tropical fruit by-products have high contents of ingredients that can be extracted and used in nutraceuticals (GORINSTEIN et al., 2011).

The development of nutraceuticals from these by-products contributes to the improvement of tropical cultures processing economy, due to the great social seek for products that offer improvements in quality of life, stimulating industries on research for new technologies that aim the reduction of economical losses and environmental impact of industry, as well to promote consumer health (MELO et al., 2011). Also, studies have proven that fruit peels can have more nutrients and bioactive compounds than their pulps (CÓRDOVA et al., 2005; GONDIM et al., 2005), as an example the phenolic compounds, a bioactive compound often found in greater amounts in the peels than the pulps (KALT, 2000).

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The antioxidant activity of bacaba is due to its secondary metabolites in its fruits and leaves (FINCO et al., 2012; LEBA et al., 2016). The purple color of its fruit peel indicates the presence of anthocyanin, a flavonoid that belongs to the phenolic class, being responsible for the major colors in flowers, fruits, leaves, stems, and roots of plants, differing among red, purple, orange, pink, and blue, depending on the intrinsic conditions of the vegetables, like pH (TEIXEIRA et al., 2008).

In this context, this work aimed to analyze the centesimal, mineral, and physicochemical composition of bacaba peel for further natural products development.

MATERIAL AND METHODS

Samples

The fruits of bacaba were picked in the rural area of Sinop, Mato Grosso and processed in the Quality Control Lab at Federal University of Mato Grosso, *Campus* of Sinop. They were washed, sanitized and rinsed. Afterwards the cleaning process, fruits were submerged in ultra-pure water at 40 °C for 40 minutes to manually remove the peels. For conservation purposes, the peels were kept in oven with forced circulation of air at 40 °C for 24 hours to remove the water, then milled and stocked in freezer at -20 °C.

Determination of the peel yeld

The determination of the peel yeld was performed according to Brandão and Oliveira (2014). It was weighed fifty (50) fruits that were evaluated and characterized according to the following parameters: longitudinal diameter, transverse diameter, fruit weight, seed pulp weight and subsequently calculated the weight of peel and its yield by difference. Afterwards, the peels were dried at 50 °C for 4 hours and milled to obtain a fine powder.

Centesimal Composition

The water content was determined by drying the material in oven (Lucadema®) at 105 °C for three hours checking its weight. This process was repeated until the weight obtained was constant (IAL, 2008), the ash mineral composition by incineration in muffle (Autonics®) at 550 °C for four hours (IAL, 2008), the lipid fraction by Sohxlet method using ether as solvent (IAL, 2008). Total nitrogen (Nt) was determined by Kjeldahl method, and the crude protein by multiplying the Nt content by the conversion factor 6.25 (AOAC, 2002). The total carbohydrate content was estimated by means of the difference.

Physicochemical Characterization

The analyzed parameters were:

Total titratable acidity (ATT): determined by titration with NaOH (0.1 mol L^{-1}) using phenolphthalein as indicator and the result was expressed in percentage (%) of citric acid (m g^{-1}) (IAL, 2008).

Total soluble solids (SST): determined by straight reading of the peel (before drying) in Abbé refractometer (Polax®), previously calibrated with distilled water and the result was expressed in ^{0}Brix (IAL, 2008).

SST/ATT Ratio: it was calculated by dividing the total soluble solids by the total titratable acidity (SST/ATT) (IAL, 2008).

pH: 1 g of peel was macerated in 10 mL of water, filtered and measured in pHmeter (Tecnopon®) (IAL, 2008).

Color: it was directly analyzed the color spaces L* (brightness), a* (intensity of red versus green), and b* (intensity of yellow versus blue) in colorimeter (Kônica Minolta® - c220) at illuminant D65. The tone parameter (h*) and saturation were calculated from a* and b* parameters by the equations: $h^*=\arctan(b^*/a^*)$, and $c^*=\sqrt{a^{*2}+b^{*2}}$ (CIPRIANO, 2011).

Determination of Total Phenolics and Antioxidant Potential by DPPH (2,2-difenil-1-picrilhidrazil) Method

For the determination of phenolics and DPPH, a solution was prepared by the addition of 25 mL of ethanol 80% per one gram of peel. The prepared solution was kept into ultrasound for 30 minutes, centrifuged for 15 minutes at 5000 rpm, and filtered. An aliquot of the filtrate (5 mL) was diluted to 50 mL in ethanol 80%, obtaining the extract solution for the trials.

The quantification of total phenolics (FT) was carried according to Waterhouse (2002) methodology, with modifications. Aliquots of the extract solution were diluted in ethanol 80% and added 2.5 mL of the Folin-Ciocalteu (Vetec) solution, after 5 minutes standing it was added 2.0 mL of sodium carbonate solution 4% (mv^{-1}), standing still and protected from light for 1 hour. Thereafter, it was conducted the reading in spectrophotometer (PG Instruments Ltd®, T80 UV/VIS), at 750 nm wavelength.

The FT content was determined by the interpolation of the samples absorbance against the

calibration curve previously set with Gallic acid (20 a 180 µg mL^{-1}) as standard. The calibration curve equation was y = 0.0005x + 0.0025, with x as the Gallic acid concentration, and y as the absorbance at 750 nm, determination coefficient r^2 = 0.994. Results were compared to the calibration curve and expressed as mg of EAG (equivalent of Gallic acid) for g of extract.

For the antioxidant potential assay, it was used the free radical DPPH (1.1-difenil-2-picrilhidrazil), according to Infante (2013) methodology, with modifications. Aliquots from the extract solution were diluted in ethanol 80%, and to 0.5 mL of every dilution it was added 3.0 mL of ethanol (99.5%) and 0.3 mL of the methanolic solution of DPPH at the concentration of 0,5 $mmolL^{-1}$. After 40 minutes standing still and protected against light, the absorbance of the samples were read in spectrophotometer (PG Instruments®, T80 UV/VIS) at 517 nm. According to the results it was determined the antioxidant ability of each concentration of the peel extract solution of bacaba by the equation: % DPPH inhibition = $[(A_0 - A_1) / A_0 \times 100]$, with A_0 as standard absorbance, and A_1 as sample absorbance. For the determination of IC_{50} (concentration of bacaba peel extract needed to reduce 50% of the DPPH radical) data were subjected to linear regression and the line equation was obtained (BRAND-WILLIAMS, 1995).

Anthocyanins

The extract was prepared with 1 g of bacaba peel added to 25 mL a mixture of ethanol 70% and hydrochloric acid 0,1 $molL^{-1}$ in a ratio of 3:1, this solution was kept into ultrasound for 30 minutes. Afterwards, it was centrifuged for 15 minutes at 5000 rpm. To 5 mL of the supernatant was added a mixture of ethanol 70% and hydrochloric acid 0.1 $molL^{-1}$ (3:1) up to 50 mL.

Total anthocyanins (AT) quantification was performed according to Lee and Francis (1972) methodology, with modifications. Aliquots of the extract solution were diluted to 10.0 mL in ethanol 70% and hydrochloric acid 0.1 $molL^{-1}$ (3:1), therefore read in spectrophotometer at 535 nm. Results were expressed in milligrams of cyn-3-glu (equivalent in cyaniding-3-glycoside) per 100 g of peel and calculated by the equation: A=a.b.c, with A as absorbance, a as coefficient of absorptivity (98.2 L cm^{-1} g^{-1}), b as light path (1 cm), and c as concentration (mg 100 g^{-1}).

Determination of Minerals

The determination of bacaba peel mineral profile was conducted after calcination of the sample in muffle (Autonics®) at 550 ^{0}C for 4 hours. Ashes were processed according to every metal determination (IAL, 2008; BRASIL, 2014). Magnesium, manganese, calcium, and copper were determined by Atomic Absorption spectrophotometry (Varian®, AA140), using a gas mixture of compressed air under a pressure of 50 psi, and acetylene at 11 psi (75 KPa – 0,76 Kgf/cm²). Phosphorous content was determined by spectrophotometry in the visible region at 420 nm (Biospectro®, SP-220). On the other hand, sodium and potassium contents were determined by flame photometry (Micronal®, B462), using a mixture of air gases and butane, under a pressure of 0.8 kg cm^{-1}.

Gas chromatography – mass spectrometry

Due to the great lipid content obtained, it was performed a qualitative analyzes of the oil extracted from the peel by chromatography – mass spectrometry (Shimadzu® CG-MS QP2010),equipped with an injector temperature of 280 °C, with a capillary column of fused silica DB-5 (0.25 mm x 30 m) and film thickness of 0.25 µm. The temperature of the column was held in 90 °C for 3 min., heating rate of 10 °C/min up to 240 °C, and maintained in isotherm condition during 20 min with flow rate of helium of 1.0 mL/min. The injection volume was 1.0 µL and split injection ration was 1:30. Mass spectra were obtained by electron impact ionization (EI) at 70 eV and scanned using full scan mode in the range 30-400 m/*z*. The ion source temperature was 200 ºC. The identification of volatile compounds was achieved by comparing the mass spectra against the data system library NIST.

RESULTS AND DISCUSSION

Bacaba fruits used in this study had rounded edges, approximately diameter of 1.5 cm, and dark purple peels for the ripe fruits. Their mesocarp had about 1.5 mm of thickness, being white and oily, and the nuts were involved in a thin and fibrous endocarp, in agreement with data from Ferreira (2005) and Brandão and Oliveira (2014). The fruits average weight was 1.914 g, with the peel corresponding to 14.25% of the fruit. In addition to the color of the peel (purple), which indicates the presence of anthocyanins, it was noticed an oily appearance that may become interesting to pharmaceutical, cosmetic, and food industries.

Centesimal composition of the peels (Table 1) showed high content of lipids, 29.13 g $100g^{-1}$, an outstanding value if compared with other fruit peels. Gondim et al. (2005) obtained the highest lipid content in avocado peel, 11.04%, while fruit peels of pineapple, banana, papaya, passion fruit, melon, and tangerine did not exceed 1%. Other authors (OLIVEIRA et al., 2002; CÓRDOVA et al., 2005; MARQUES et al., 2010) studying fruit peels did not find lipid values higher than 1% as well. Thus, it was observed that the oil extracted from bacaba is not only concentrated in its nut and pulp but also in its peel. It was identified the presence of oleic and palmitic fatty acids, same ones found in the fruit, in agreement with Santos et al. (2017) who obtained 61.65% of oleic acid and 28.43% of palmitic acid in the fruit.

The water content was 4.87% performed in product after removal of excess water because it was necessary to submerge the fruit in water at 40 ° C to remove the fruit peel. Protein and ashes content were similar to other fruits as found by Gondim et al. (2005) and Marques et al. (2010).

Results of the physicochemical characterization of bacaba fruit peel are found in Table 2.

Table 1. Results of the centesimal composition of bacaba fruit peel after dried

Parameters	**Bacaba fruit peel (g $100g^{-1}$)**
Water content	4.87 ± 0.06
Ashes	1.42 ± 0.03
Lipids	29.13 ± 0.49
Proteins	1.08 ± 0.01
Total carbohydrates	63.32 ± 0.55

*Average ± standard deviation of the triplicates.

Table 2. Results of the physicochemical characterization of bacaba fruit peel

Parameters	
pH	5.66 ± 0.12
AAT (% of citric acid)	0.306 ± 0.00
SST (°Brix)	9.75 ± 0.15
Color (illuminant D65) L*	19.03 ± 0.07
a*	8.07 ± 0.01
b*	9.25 ± 0.39
c*	12.28
h*	48.89
SST/ATT Ratio	31.86

* Average ± standard deviation of the triplicates.

For the physicochemical characteristics there is no current legislation for the identity and marketing standards of bacaba fruit or its pulp, so it was compared to the parameters set for açaí pulp (*Euterpe oleracea* Mart.), a similar fruit to bacaba and widely marketed. The Normative Instruction n. 1 issued by MAPA- Ministério da Agricultura, Pecuária e Armazenamento (BRASIL, 2000) determines that the total titratable acidity must be between 0.27% and 0.45% of citric acid and pH 4.0 to 6.2, the acidity found in bacaba peel was 0.306% of citric acid and pH 5.66, meeting the legislation standards for the açaí pulp. Other studies demonstrate that the acidity of açaí pulp may vary from 0.19% to 0.94% of citric acid, and its pH between 3.55 and 5.20, emphasizing the similarity between these two fruits (ALEXNDRE et al., 2004; NASCIMENTO et al., 2008; CIPRIANO, 2011).

The total soluble solids (SST) content is used to estimate the sugar and organic acids content of the fruit, and bacaba peel showed a SST content of 0.975 °Brix, lower than its pulp, in which Neves et al. (2015) found 1.53 °Brix, and Canuto et al. (2010) found 2.0 °Brix. The SST/ATT ratio is considered an assessment factor of fruit taste, and in these peels it was 3.18. This value is lower than the one obtained from Brazilian grape (*Myrciaria jaboticaba* (Vell.) Berg.) (8.25), and açaí pulp (17.52) (CIPRIANO, 2011). Czelusniak et al. (2003) consider a threshold value of 20 for the SST/AAT ratio, meaning that lower values represent high acidity content, with greater relevance for industries (CIPRIANO, 2011).

In regard to the color, bacaba peel showed values of 19.03 for brightness (L*), 12.28 for color saturation (c*), and 48.89 ° for tone parameter (h*),

showing that the sample had low brightness and for that it is considered to be dark (ROCHA et al., 2017); with more saturated color similar to Brazilian grape peel studied by Cipriano (2011), who found values of c* around 11.32 and tone between red (0 °) and yellow (90 °). The variation to the yellow tone can be due to residual white pulp on the peel, agreeing with Canuto et al. (2010), who found a h* of 56.0 ° for bacaba pulp.

The FT content in bacaba peel was 42.07 mg EAG g^{-1}, most likely related to the presence of anthocyanins. Finco et al. (2012) and Canuto et al. (2010) found values of 17.59 mg EAG g^{-1} and 0.3 $mmolL^{-1}$ of Gallic acid in the fruit and pulp of bacaba, respectively. Also, when compared with other fruits, the value obtained is higher, as an example, Cipriano (2011) found in Brazilian grape 2.16 mg EAG g^{-1}, and in açaí pulp 3.10 mg EAG g^{-1}. On the other hand, Infante (2013) found in peels of bacupari-mirim (*Garcinia brasiliensis* Mart.) and araçá (*Eugenia leitonii* Lerg.), 36.35 mg EAG g^{-1} and 36.08 mg EAG g^{-1}, respectively. The FT content may be related to the antioxidant activity of fruits, which is of great relevance for industry due to the improvement of health through the prevention of diseases and delay of the ageing process, what turns bacaba into a fruit of great interest for the production of nutraceuticals, enriched foods, and cosmetics.

The DPPH test showed that it is necessary 1.07 mg mL^{-1} of bacaba peel extract to reduce DPPH in 50 %, higher value, with lower antioxidant activity than the one found by Finco et al. (2012), who obtained from the fruit a IC_{50} of 0.70 mg mL^{-1}. This variation in antioxidant activity, most of time, is due to the different experimental conditions. In addition, results showed that the peel has antioxidant activity, a promising feature for the development of products.

The high FT and anthocyanin contents, as well the antioxidant activity of bacaba peel agree with Finco et al. (2012), who found similar contents for either, FT and anthocyanins, in bacaba fruit and associated these high contents with its great antioxidant activity. Regarding the anthocyanins, previous studies showed that the fruits *Oenocarpus bataua* Mart. and *Oenocarpus bacaba* Mart. have high content of anthocyanins (FINCO et al., 2012; REZAIRE et al., 2014), collaborating for their antioxidant activity. Tauchen et al. (2016) correlated this antioxidant potential of anthocyanins from *Oenocarpus bataua* in the antiproliferative activity of cancer cells.

The peel of bacaba fruit was dark purple, indicating the presence of anthocyanins. The value of total anthocyanins (equivalent in cyanidin-3-glycoside) found in the peels, 37.31 mg of cyn-3-glu 100 g^{-1}, was similar to Finco et al. (2012), who obtained 34.69 mg of cyn-3-glu 100 g^{-1} from the fruit. In comparison with açaí pulp, Cipriano (2011) found 74.30 mg $100g^{-1}$ of total anthocyanins, greater than the value found in bacaba peel, although the fruit segment as well as the extracting technique were different.

Anthocyanins are found in several fruits, such as cherry, açaí, plum, strawberry, apple, and grape, however not yet widely marketed due to its tough extraction and low stability. In industry there is an interest about its use as dyeing agent, since the synthetic ones may harm consumer's health somehow, therefore stands the importance of verifying the composition and antioxidant activity of bacaba peel for its further use (CIPRIANO, 2011).

The minerals content: sodium, potassium, calcium, magnesium, copper, manganese, and phosphorous of the bacaba fruit peel are found in Table 3.

Table 3. Results of the minerals content of bacaba fruit peel

Minerals	**Bacaba fruit peel (mg $100g^{-1}$)**	**DRI (mg per day)**	**% DV (100 g portion)**	**% DV (15 g portion)**
Sodium	79.00 ± 0.39	2400.0	3.29	0.49
Potassium	582.97 ± 2.28	2500.0	23.32	3.50
Calcium	37.02 ± 1.38	1000.0	3.70	0.55
Magnesium	51.79 ± 0.04	260.0	19.91	2.99
Copper	0.625 ± 0.03	0.9	69.44	10.42
Manganese	2.37 ± 0.04	2.3	103.04	15.47
Phosphorous	77.12 ± 0.11	700.0	11.01	1.65

* Average ± standard deviation of the triplicates. Daily Recommended Index (DRI) and Daily Value (DV).

Regarding the mineral composition of bacaba fruit peel (Table 3), it was found that potassium is the most abundant, being important to the neuromuscular activity regulation, such as weakness, fatigue, and cramps, followed by sodium, important in controlling absorption and transportation of nutrients such as chlorine, amino acids, water, and glucose. Besides, phosphorous, a mineral responsible for the maintenance of pH and nucleotide synthesis, presented a content close to sodium, followed by magnesium, a mineral involved in bone formation and with enzymes in the metabolism of cholesterol, amino acid, and carbohydrate. Beyond these, it was found calcium, essential mineral for the blood coagulation, neural transmission, teeth and bones formation, and muscular contraction, crucial to the organism (DOUGLAS, 2001). In comparison to açaí, Menezes et al. (2008) found in its lyophilized pulp higher concentrations of potassium, calcium, magnesium, manganese, and copper. Moreover, sodium and phosphorous were the minerals with higher concentrations in bacaba peel. Regarding other fruits, Marques et al. (2010) found lower contents of sodium, potassium, phosphorous, magnesium, and manganese in mango peel, while Gondim et al. (2005) found lower contents of copper, sodium, potassium, and magnesium in avocado, pineapple, banana, papaya, passion fruit, and melon peel than the contents found in bacaba peel.

Considering the daily intake recommendation for adults (BRASIL, 2005), the different mineral elements present in a 15 g (1 tablespoon) portion of bacaba fruit shell (Table 3) serve from 0.49 to 15.47% of the daily value.

The Brazilian legislation (BRASIL, 1998) determines that food to be considered sources of vitamins and mineral needs at least a daily value (DV) of 15 % in relation at daily recommended index (DRI) of reference per 100 g of solid food, and to be considered rich food in minerals and vitamins, the food needs have a DV of 30 % in relation of DRI of reference per 100 g of solid food. Thus according with Table 3, the peel of bacaba can be considered a potassium and magnesium source, and rich in copper and manganese (GRANATO et al., 2009).

Regarding potentially toxic micronutrients, the copper content (6.25 mg Kg^{-1}) did not exceed the permitted limit (10 mg Kg^{-1} for fresh or industrialized fruits) by the current legislation (BRASIL, 2013), which establishes maximum limits of tolerance for inorganic contaminants in food.

Thereby, bacaba fruit peel showed to be a valuable source of oil, anthocyanins, and minerals, able to be repurposed for the production of dye agents, nutraceuticals, and cosmetics.

CONCLUSION

Bacaba fruit peel showed to be a source of nutrients and have a good antioxidant potential, claiming the importance of its repurpose instead of be disposed as residue. Besides, taking sustainable advantage of these natural sources might contribute to the community development, not to mention the creation of new products.

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RESUMO: *Oenocarpus bacaba* MART. (bacaba) é uma palmeira nativa da Amazônia que se destaca por suas propriedades antioxidantes. O processamento de seu fruto gera a casca como produto de descarte, que possui nutrientes que podem colaborar na ação antioxidante e na prevenção de doenças. Assim, o presente trabalho avaliou a composição centesimal e mineral, caracterização físico-química (acidez, sólidos solúveis totais, pH e cor), fenóis totais (método de Folin-Ciocalteu), antocianinas e potencial antioxidante pelo método do DPPH (2,2 difenil-1-picril hidrazil) em cascas de bacaba. Para a composição centesimal verificou-se teores (%) de 4.87, 1.42, 29.13, 1.08 e 63.32 para o teor de água, cinzas, lipídeos, proteínas e carboidratos totais, respectivamente. Para a composição mineral foi encontrado teores ($mg100g^{-1)}$ de 582.97, 79.00, 51.79, 0.625, 37.02, 2.37 e 77.12 para potássio, sódio, magnésio, cobre, cálcio, manganês e fósforo, respectivamente. A caracterização físico-química apresentou valores de pH de 5.66, acidez titulável de 0.306% de ácido cítrico, sólido solúveis totais de 9.75 e cor nas coordenadas L* a* e b* com valores de 19.03, 8.07 e 9.25 . Para o teor de fenóis foi encontrado 42.07 mg $EAG.g^{-1}$. O EC_{50} para a potencial antioxidante foi de 1.07 $mg.mL^{-1}$ e o teor de antocianinas foi de 37.31 $mg.100g^{-1}$. Os resultados mostram que as cascas de bacaba são uma fonte

alternativa de nutrientes sugerindo seu aproveitamento na indústria alimentícia e cosmética, principalmente pela sua potencial atividade antioxidante e composição em minerais.

PALAVRAS-CHAVE: *Oenocarpus bacaba* Mart., Antocianinas. Fenóis totais. DPPH. Minerais.

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