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Estabilidade de compostos bioativos, atividade antioxidante e microbiológica de açai em pó
(*Euterpe oleracea* Mart.)

Stability of bioactive compounds, antioxidant and microbiological activity of açai powder
(*Euterpe oleracea* Mart.)

Estabilidad de compuestos bioactivos, actividad antioxidante y microbiológica del polvo de açai
(*Euterpe oleracea* Mart.)

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Resumo

O açaí é uma fruta tropical, típica da Amazônia e vem chamando atenção por sua capacidade antioxidante e alto teor de antocianinas, responsáveis por diversas propriedades farmacológicas e medicinais, além de potencial uso como corante natural. Devido à alta perecibilidade, o desenvolvimento de novos produtos a partir do açaí, que ofereça maior estabilidade e tempo de estocagem, como um pó, estão sendo produzidos. O objetivo dessa pesquisa foi avaliar a estabilidade do pó de açaí por um período de 270 dias à temperatura de 25 °C. Avaliou-se a cada 30 dias os seguintes parâmetros: ácido ascórbico, clorofila, antocianinas totais, flavonoides totais, polifenóis totais e atividade antioxidante pelos métodos ABTS^{•+} e DPPH[•]. As análises microbiológicas foram realizadas para Coliformes à 35 e 45 °C, Mesófilos, *Estafilococos* coagulase positiva, bolores e leveduras, e *Salmonella* sp. Os conteúdos de ácido ascórbico, polifenóis totais e flavonoides diferiram estatisticamente ($p < 0.05$) entre si, ao longo do armazenamento. No entanto a atividade antioxidante pelo método de DPPH[•] se manteve estável e as análises microbiológicas ficaram dentro dos padrões legislativos vigentes. Conclui-se que o pó de açaí manteve suas propriedades ao longo do armazenamento, sendo uma excelente fonte de compostos bioativos e fenólicos.

Palavras-chave: Atividade antioxidante; Segurança microbiológica; Vida útil.

Abstract

Açaí is a tropical fruit, typical of the Amazon and has been drawing attention due to its antioxidant capacity and high content of anthocyanins, responsible for several pharmacological and medicinal properties, as well as potential use as a natural dye. Due to the high perishability, the development of new products from the açaí power, which present greater stability to the storage time are being produced. The evaluation period was 25 days at a temperature of 25°C. The following parameters were evaluated: ascorbic acid, chlorophyll, total anthocyanins, total flavonoids, total polyphenols and antioxidant activity by ABTS^{•+} and DPPH[•] methods. Microbiological analysis were performed for Coliforms at 35°C and 45°C, Mesophiles, *Staphylococcus* coagulase positive, Molds, Yeasts, and *Salmonella* sp. The

contents of ascorbic acid, total polyphenols and flavonoids differed statistically ($p < 0.05$) from each other, throughout the storage. However, the antioxidant activity by the DPPH[•] method remained stable and the microbiological analysis remained within the current legislative standards. It is concluded that the açai powder maintained its properties throughout the storage, being an excellent source of bioactive and phenolic compounds.

Keywords: Antioxidant activity; Microbiological safety; Shelf life.

Resumen

El açai es una fruta tropical, típica de la Amazonía y ha llamado la atención por su capacidad antioxidante y alto contenido de antocianinas, responsable de varias propiedades farmacológicas y medicinales, además de su uso potencial como colorante natural. Debido a la alta perecebilidad, se está desarrollando el desarrollo de nuevos productos a partir de açai, que ofrece mayor estabilidad y tiempo de almacenamiento, como un polvo. El objetivo de esta investigación fue evaluar la estabilidad del polvo de açai durante un período de 270 días a una temperatura de 25 ° C. Los siguientes parámetros se evaluaron cada 30 días: ácido ascórbico, clorofila, antocianinas totales, flavonoides totales, polifenoles totales y actividad antioxidante mediante los métodos ABTS^{•+} y DPPH[•]. Se realizaron análisis microbiológicos para coliformes a 35 y 45 ° C, mesófilos, *estafilococos* coagulasa positivos, mohos y levaduras, y *Salmonella* sp. Los contenidos de ácido ascórbico, polifenoles totales y flavonoides diferían estadísticamente ($p < 0.05$) entre sí, durante el almacenamiento. Sin embargo, la actividad antioxidante mediante el método DPPH se mantuvo estable y los análisis microbiológicos se encontraban dentro de los estándares legislativos actuales. Se concluye que el polvo de açai mantuvo sus propiedades durante el almacenamiento, siendo una excelente fuente de compuestos bioactivos y fenólicos.

Palabras clave: Actividad antioxidante; Seguridad microbiológica; Vida alimentaria.

1. Introduction

Açai is a fruit whose plant is a tropical palm that develops in the north of Brazil, more precisely in the Amazon region. The fruits are round, dark purple, measure from 1 to 2 cm in diameter and are harvested mainly between July and December. The edible fruit pulp of açai is commonly macerated with water to produce a thick, purple drink with a creamy texture, oily appearance and characteristic taste (Boeira et al., 2020).

In a study of the compounds present in açai, Peixoto et al. (2016) and Rufino et al. (2007) emphasized the presence of bioactive substances, especially those who related to phenolic compounds and pigments, such as anthocyanins, flavonoids, and carotenoids, which are a potential source of natural antioxidants for human consumption.

Due to its highly perishable nature, consumption and marketing of açai were restricted to Brazil. However, increased international interest and expanded distribution made the açai pulp and various products made from the açai pulp widely available to the general public. The considerable interest has been generated by its high anthocyanin and antioxidant capacity (Garzón et al., 2017).

Most foodborne diseases are caused by pathogenic microorganisms present in the food due to contamination during its useful life, especially during processing and storage under inadequate conditions, or by toxins produced by these contaminants. Certain species, including *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella*, may even cause life-threatening infections (Paz et al., 2015).

Among the methods used for fruit preservation, the use of spray dryer represents a viable alternative, since the powder products have low water activity, which hinders or even prevents the growth of microorganisms and the physicochemical reactions and enzymes responsible for its deterioration, thus increasing its useful life. Furthermore, the production of powdered fruit pulp also has the advantage of greater ease in the transportation, storage and handling of the final product, either for consumption directly or as an ingredient in the preparation of other food products (Cavalcante et al., 2017).

Therefore, the objective of this study is to determine the content of bioactive compounds, antioxidant capacity, by ABTS^{•+} and DPPH[•] methods, and verify the microbiological safety of açai powder during 270 days of storage.

2. Material and Methods

The present research characterized as a laboratory and quantitative study as presented by Pereira et al. (2018). The acai powder was supplied by a fruit processing industry located in Ubajara, Ceará, Brazil. After the açai powdering process, the samples were stored in metallic rolled bags and transported to the Fruit and Vegetable Laboratory and the Food Microbiology Laboratory of the DTA/CCA/UFC in Fortaleza, Ceará, where they were stored at a temperature of $25 \pm 2^\circ\text{C}$.

Açaí powders were evaluated every 30 days for 270 days of storage for the bioactive compounds present and antioxidant capacity (ascorbic acid, chlorophyll, total anthocyanins, total flavonoids, total polyphenols (TPs), antioxidant activity by ABTS^{•+} and DPPH[•] methods) and microbiological analysis.

2.1. Bioactive compounds and antioxidant capacity

2.1.1 Ascorbic acid

The ascorbic acid content was determined according to the methodology of Strohecker & Henning (1967) using the standard solution of Tillman (0.02%). To prepare the extract, 1 g of açaí powder was used and, after homogenization, in 250 mL of oxalic acid (0.5%). A 2 mL aliquot was taken for titration with Tillman's solution. The results were expressed as mg of ascorbic acid/100 g of açaí powder.

2.1.2 Chlorophyll

The chlorophyll content was determined, according to Bruinsma (1963). In the procedure, about 1 g of the powder was added with approximately 10 mL of acetone solution (80%) and homogenized. It was transferred to a 50 ml volumetric flask and the volume was made up of acetone (80%). The obtained extract was filtered and the reading was carried out at 652 nm. Chlorophyll levels were expressed in mg/100 g of powder using Equation 1.

$$\text{Chlorophyll} = \frac{(\text{abs} \times 1000 \times V)}{\frac{1000 \cdot w}{34.5}} \times 100 \quad (1)$$

Where: V: final volume of the extract; w: powder weight (g); abs: absorbance.

2.1.3 Total anthocyanin

For total anthocyanins, the methodology of Francis (1982) was followed with some modifications regarding the preparation of the extract. 0.5 g of açaí powder solubilized in potassium chloride (0.2 M) was used, which was kept in the dark for 2h. A 5 mL aliquot of the extract was homogenized with HCl solution (1.5 M) and ethanol (85%) in the ratio (15:85). The obtained extract was maintained for 15 hours at rest under refrigeration and absence of light. The extract was then filtered and the spectrophotometer (SHIMADZU,

model UV-1800) read at 535 nm. The results were expressed as mg of anthocyanins/100 g of *acai* powder, using Equation 2.

$$\text{Total anthocyanin} = \frac{F \times \text{abs}}{98.2} \quad (2)$$

Where: F = dilution factor; abs: absorbance.

2.1.4 Total flavonoids

The determination of flavonoids was according to Francis (1982), as well as for anthocyanins, but the reading was carried out at 374 nm in a spectrophotometer SHIMADZU, model UV-1800. The results were expressed in mg/100 g and calculated through Equation 3.

$$\text{Flavonoids} = \frac{F \times \text{abs}}{76.6} \quad (3)$$

Where: F = dilution factor; abs: absorbance.

2.1.5 Total phenolic

For determination of the total polyphenols, an extract was prepared from 2.5 g of *acai* powder added 20 mL of ethanol (50%), stood for 1 hour, followed by centrifugation at 3000 rpm for 15 minutes and filtration to a volumetric flask 50 mL. The obtained residue was added 20 mL of acetone (70%) for 1 hour, followed by centrifugation and filtration. The obtained acetone extract was added to the ethanolic extract with the calibration of the flask with distilled water. All these steps were performed in the absence of light. These extracts were used for quantification of total polyphenols (TPs) and total antioxidant activity (Larrauri *et al.*, 1997).

The total polyphenols in the extract were determined by using the Folin-Ciocalteu reagent, using a standard curve according to the methodology described by Larrauri *et al.* (1997) and reading at 700 nm. The results were expressed in mg equivalent to gallic acid (GAE)/100 g dry matter.

2.1.6 ABTS^{•+} radical scavenging assay

From the extract, the total antioxidant activity was determined according to Re et al. (1999) and Rufino et al. (2009). Initially, the ABTS^{•+} radical was prepared by mixing 5 mL of stock solution of ATBS with 88 µL of potassium persulfate solution, where it was kept in the dark for 16 hours. After this time, the absorbance was corrected to 0.70 ± 0.05 nm at 734 nm with ethyl alcohol. Aliquots of 30 µL, 20 µL and 10 µL of extract and 3 mL of the ABTS^{•+} radical was added and the absorbance read after 6 minutes. A standard curve of the Trolox standard solution (2mM) was used as a reference and the results were expressed in µM Trolox (TE)/g sample (dry weight).

2.1.7 DPPH[•] radical scavenging assay

The antioxidant activity by the DPPH[•] assay according to the methodology of Brand-Willians et al. (1995) with modifications of Rufino et al. (2007). The reduction of the DPPH[•] radical was verified at 515 nm, just after 120 minutes of rest of the extract, according to Rufino *et. al.* (2007). Through this methodology it is possible to calculate, after the establishment of the equilibrium of the reaction, the amount of antioxidant necessary to reduce 50% of the radical DPPH[•]. The determination of the time of evaluation of 120 minutes was based on the time of stabilization of the absorbance (IC₅₀) in previously realized kinetics.

2.2. Microbiological analyzes

Coliform counts at 35°C and 45°C (MLN/g), mesophilic counts (CFU/g), *Staphylococcus* coagulase-positive (CFU/g), yeast and fungal counts (CFU/g), and *Salmonella* sp./25 g (absence/presence) were performed according to the methodology proposed by the American Public Health Association (APHA, 2002).

2.3. Statistical analyzes

The experiments were conducted in a completely randomized design with ten sampling times (0, 30, 60, 90, 120, 150, 180, 210, 240, 270 days). The results were analyzed by an analysis of variance (ANOVA) and the comparison of means by Tukey's test ($p < 0.05$) using Statistica 7.0 software. The graphics were made in the Origin 8.0 program. Results are presented as a mean of five replicates \pm standard deviation.

3. Results and Discussion

The results of the quantification of the bioactive compounds (ascorbic acid, chlorophyll, total polyphenols) and antioxidant activity by the ABTS^{•+} and DPPH[•] methods are presented in Table 1.

Table 1 – Bioactive compounds and antioxidant activity of açai powder stability during 270 days of storage at 25°C.

Time (days)	Ascorbic acid (mg /100 g)	Chlorophyll (mg/100g)	Total phenolic (mg GAE/100g)	ABTS ^{•+} (µM TE/g)	DPPH [•] (g/g DPPH [•])
0	125.87 ± 26.42 ^a	7.41 ± 0.92 ^a	1287.73 ± 70.74 ^{abc}	68.50 ± 2.73 ^{ac}	1249.22 ± 95.74 ^a
30	153.79 ± 34.55 ^{abc}	5.75 ± 1.02 ^{ab}	1181.27 ± 133.33 ^{ac}	97.27 ± 4.18 ^b	1439.70 ± 308.52 ^a
60	174.75 ± 17.25 ^{abc}	4.92 ± 1.19 ^b	1659.16 ± 97.77 ^{bd}	75.41 ± 4.53 ^{cd}	1325.38 ± 64.07 ^a
90	174.49 ± 31.46 ^{abc}	6.14 ± 1.29 ^{ab}	1587.55 ± 89.18 ^{bcd}	93.29 ± 4.57 ^{bd}	1352.75 ± 221.49 ^a
120	194.63 ± 24.07 ^{bc}	6.06 ± 0.71 ^{ab}	1825.27 ± 200.89 ^d	68.86 ± 1.45 ^{ac}	1050.03 ± 261.49 ^a
150	208.67 ± 57.07 ^b	6.10 ± 1.54 ^{ab}	1290.50 ± 70.74 ^{abc}	63.98 ± 13.38 ^{ac}	1218.35 ± 390.35 ^a
180	208.90 ± 32.30 ^b	6.41 ± 0.65 ^{ab}	1177.39 ± 64.18 ^{ac}	56.38 ± 11.71 ^a	1204.13 ± 29.30 ^a
210	144.00 ± 18.09 ^{ac}	7.00 ± 0.58 ^{ab}	1446.66 ± 168.05 ^{abcd}	66.18 ± 15.05 ^{ac}	1940.67 ± 470.30 ^a
240	127.30 ± 14.39 ^a	5.71 ± 1.60 ^{ab}	1104.87 ± 162.21 ^a	54.47 ± 11.48 ^a	1351.67 ± 31.17 ^a
270	126.37 ± 16.10 ^a	7.12 ± 0.89 ^a	1422.21 ± 120.65 ^{ac}	59.49 ± 12.61 ^{ac}	1183.69 ± 54.07 ^a

Source: Research data (2020).

Mean ± standard deviation ($n = 5$ replicates). Different letters in the same column, indicate a significant difference between the samples ($p < 0.05$).

Ascorbic acid content varied over the storage period, 125.87 ± 26.42 mg/100 g (0 day) to 208.90 ± 32.30 mg/100 g (180 days). It was also observed with little statistical variation, showing that the sample remained stable during the period of storage.

The characteristic of the laminated packaging contributes to the maintenance of ascorbic acid content along with the days of stability, avoiding the action of light. Among other factors, such as care in packaging and weighing, as well as the presence of total polyphenols, which exert a protective effect on ascorbic acid.

Results for chlorophyll showed that; statistically, the samples remained stable throughout the storage. Rufino et al. (2010) studying the functional properties of Brazilian tropical fruits observed chlorophyll content for fresh *acai* of 20.8 mg/100 g of fresh matter. Therefore, a significant chlorophyll content is observed in açai powder, which seems to

guarantee, despite the losses, due to the processing applied, the presence of this substance was maintained.

The extracts of açai powder presented a high content of total polyphenols (Table 1), however, there was no upward or downward regularity of these values during storage. The TPs presented values ranging from 1104.87 ± 162.21 (240 days) to 1825.27 ± 200.89 (120 days) mg of gallic acid equivalent (GAE)/100 g of açai powder during storage.

Paz et al.(2015) in a study carried out with Brazilian fruit pulps and obtained in açai total polyphenol content with 1808 ± 28 mg GAE/100 g, followed by guava (1152 ± 52 mg GAE/100 g), graviola (817 ± 41 GAE/100 g), and tamarind (474 ± 47 GAE/100 g), similar to the content obtained in that study for 120 days of storage (1825.27 ± 200.89 mg GAE/100 g).

Neves et al. (2015) and Silva et al. (2017) obtained lower levels in açai pulps (558.56 mg GAE/100 g, 346.14 ± 8.62 mg GAE/100 g, respectively) than those obtained in this study. However, Silva et al. (2017) obtained 3437 ± 154 GAE/100 g in açai pulp, which is higher than those obtained in this study.

The Folin-Ciocalteu method does not measure the amount of phenolic compounds present, instead it measures the ability to reduce the compounds present in the extract relative to gallic acid and therefore expressed as gallic acid equivalents (GAEs). And although the determination of total polyphenols is simple, it should be considered that other compounds present, such as sugars and some amino acids, can lead to interference, thus underestimating the results (Paz et al., 2015).

Results of the antioxidant activity by the ABTS^{•+} method varied from 54.47 ± 11.48 $\mu\text{M TE/g}$ (240 days) to 97.27 ± 4.18 $\mu\text{M TE/g}$ (30 days) of açai powder. There is also an oscillation of the values with the storage time.

In the works of Garzón et al. (2017), Silva et al. (2017) and Gordon et al. (2012) the values of antioxidant capacity in açai pulps were around 15.7 ± 10.6 $\mu\text{M TE/100 g}$, 17.15 ± 0.24 $\mu\text{M TE/g}$ and 2.78 ± 0.10 $\mu\text{M TE/100 g}$, respectively.

The ABTS^{•+} method is an indicator of the antioxidant ability to extinguish the ABTS^{•+} radicals by an electron transfer reaction. Therefore, it can be concluded that the phenolic compounds present in açai are good electron donors and are capable of extinguishing the radicals ABTS^{•+} (Garzón et al., 2017). Furthermore, compounds with antioxidant properties may be significantly lost as a consequence of processing and storage, thereby affecting the antioxidant capacity of the food.

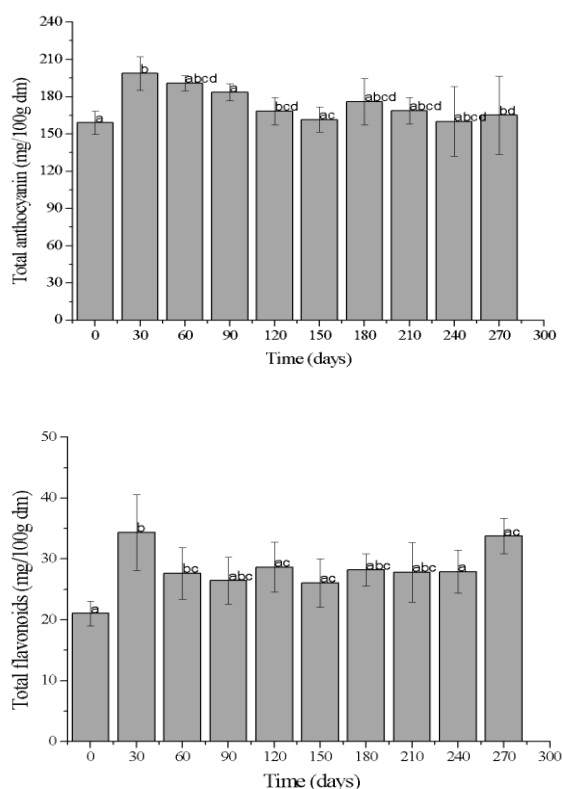
The antioxidant capacity of the organic açai powder quantified by the DPPH[•] free radical capture method did not present a significant difference ($p < 0.05$) in relation to the

storage times (Figure 1), with values between 1050.03 ± 261.49 g/g DPPH• (120 days) and 1940.67 ± 470.30 g/g DPPH• (210 days).

Results of the antioxidant activity by the DPPH• assay correspond to the amount of antioxidant present in the açai powder required to reduce the initial concentration of the DPPH• radical by 50% over a specified period of time thus lower the antioxidant capacity result by the assay DPPH• greater is the antioxidant activity of the sample. The values of this study can be considered low (Table 1), evidencing a high antioxidant capacity (Paz et al., 2015). The presence of high antioxidant activity through the DPPH• assay in açai was reported by Gonçalves et al. (2011), Paz et al. (2015) and Garzón et al. (2017).

The results obtained for total flavonoids and total anthocyanins during the stability of açai powder can be verified through Figure 1.

Figure 1 – Total flavonoids and total anthocyanin of açai powder stability during 270 days of storage at 25°C.



Source: Research data (2020).

Mean \pm standard deviation ($n = 5$ replicates). Different letters indicate a significant difference between the samples ($p < 0.05$).

The data obtained for total flavonoids (ranging from 21.04 ± 2.02 mg/100 g to 34.35 ± 6.22 mg/100 g) and total anthocyanins (159.03 ± 9.45 mg/100 g to 198.75 ± 13.68 mg/100 g) showed a statistically significant difference over the storage, where it demonstrates that it does not present a characteristic sequence, with random behaviors.

Neves et al. (2015) and Silva et al. (2017) obtained in açai pulps contents of 43.25 mg/100 g and 73.54 ± 2.59 mg/100 g for total anthocyanins, respectively, lower data than that verified in this study during the 270 days of storage. However, Paz et al. (2015) verified pulps of açai contents of 672 ± 40 mg/100 g, given higher than those obtained in this study for total flavonoids.

The results of the microbiological analysis of açai powder during the 270 days of stability study are presented in Table 2.

Table 2 – Count of microorganisms of açai powder stability during 270 days of storage at 25°C.

Times (days)	Coliforms 35°C (MLN/g)	Coliforms 45°C (MLN/g)	Mesophiles (CFU/g)	<i>Staphylococcus</i> coagulase positive (CFU/g)	MOULDS/YEAS TS (CFU/G)	<i>Salmonella sp.</i> (in 25 g)
0	< 3.0	< 3.0	< 10	< 10	< 10	ausência
30	< 3.0	< 3.0	1.0×10	< 10	3.0×10	ausência
60	< 3.0	< 3.0	< 10	< 10	< 10	ausência
90	< 3.0	< 3.0	< 10	< 10	1.0×10	ausência
120	< 3.0	< 3.0	< 10	< 10	3.0×10	ausência
150	< 3.0	< 3.0	< 10	< 10	1.0×10	ausência
180	< 3.0	< 3.0	1.0×10	< 10	1.0×10	ausência
210	< 3.0	< 3.0	1.0×10	< 10	1.0×10	ausência
240	< 3.0	< 3.0	< 10	< 10	< 10	ausência
270	< 3.0	< 3.0	1.0×10	< 10	1.0×10	ausência

Source: Research data (2020).

CFU: Colony Forming Unit; MLN: Most Likely Number.

In this study, low counts of Coliforms were found at 35°C and 45°C, lower than 3.0 MLN/g, lower than that established by pertinent legislation (BRASIL, 2001). For the *Staphylococcus* coagulase positive count, a maximum value of 1.0×10 CFU/g was found during storage time being allowed up to 10^2 /g, by the legislation. An absence of *Salmonella sp* was found, as prescribed in RDC n° 12 of January 2 (2001) of ANVISA (National Agency of Sanitary Surveillance), which establishes the microbiological standards for food.

The counts of mesophilic bacteria, moulds and yeasts (Table 2) were low without compromising the microbiological stability of açai powder. And although the product was submitted to pasteurization before the drying process, the presence of mesophilic bacteria, moulds and yeasts during the storage period was verified, which may be due to thermoresistant forms incorporated at some point in the processing.

Results indicate that the organic açai powder meets the current standards, being in consumption conditions. Maintenance of characteristics over the storage period indicates that processing conditions, type of packaging and storage time did not favor microbial growth.

Lucas et al. (2018) in a study with açai pulps and they concluded that this product can be dried by different methods in order to extend the shelf life and still results in foods with high retention of bioactive compounds, therefore, the different drying methods that can be employed are effective in obtaining foods with preserved nutritional characteristics.

4. Conclusions

The açai powder maintained its antioxidant properties throughout the storage, being an excellent source of phenolic and bioactive compounds, mainly as far as the anthocyanin content is concerned. These results characterize it as a product with functional activity and can be used as an ingredient in food production. The high content of anthocyanins characterizes it as a potential natural dye, with the advantage that these pigments are not harmful to health like artificial ones.

The results of the microbiological analysis indicated a stable product in the 270 days of storage, indicating that the hygienic-sanitary conditions were maintained during the processing of the product. The results of this study contribute to the factors that influence the quality and stability of the bioactive compounds present in açai base food products.

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