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Antioxidant Capacity of Acai Alcoholic Beverages Elaborated by Different Processes

ANNE CAROLINE DE ALBUQUERQUE SALES
LIZETH MERCEDES GARCIA JAIMES
ALESSANDRA SILVA FERREIRA
Institute Federal of Education, Science and Technology of Amazonas (IFAM)
Campus Manaus Center (CMC), Department of Chemistry, Ambient and Food (DQA), Manaus, AM, Brazil
JOSIANA MOREIRA MAR
EDGAR APARECIDO SANCHES
Federal University of Amazonas (UFAM)
Laboratório de Polímeros Nanoestruturados (NANOPOL)
Manaus, AM, Brazil
JAQUELINE DE ARAÚJO BEZERRA
LÚCIA SCHUCH BOEIRA[1]
Institute Federal of Education, Science and Technology of Amazonas (IFAM)
Campus Manaus Center (CMC), Department of Chemistry, Ambient and Food (DQA), Manaus, AM, Brazil

Abstract

Technological alternatives for the development of new products, as a strategy to add value to native fruits, is a great demand in the interior of the Amazonas state, Brazil. Acai, a native Amazon fruit, has received great interest due to the concentration of its antioxidant compounds. With this in mind, it was developed an alcoholic beverage from native acai using two different processes: acai maceration (AMP) and acai pulp (APP). In this study, it was determined the antioxidant capacity and quantification of total phenolic content (TPC) of alcoholic beverages produced by AMP and APP. The antioxidant capacity was determined using the DPPH, ABTS and FRAP assays and total phenolic content determined by the Folin-Ciocalteu method. For the APP, the results obtained for the

[1] Corresponding author: lucia.boeira@ifam.edu.br

filtered juice were significantly higher than in the unfiltered juice. Considering both processes employed, for the acai alcoholic beverages produced, the minimum and maximum values obtained for DPPH (μM TE) was 8,893.3 and 11,398.9, for ABTS (μM TE) was 14,596.7 and 18,068.0, for FRAP (μM Fe(II)) was 15,258.9 and 22,094.0 and for TPC (mg GAE/L) was 902.2 and 1,178.2. Comparing the employed processes, the results of the antioxidant capacity tended to be slightly higher in the beverages produced by the APP. However, in the decision make of which process should be used to elaborate the acai alcoholic beverage, our results showed that the antioxidant capacity is not an important parameter to consider, as the acai alcoholic beverages elaborated by both processes presented approximate results.

Key words: *Euterpe precatoria*, alcoholic beverage, production processes, acai pulp, acai *in natura,* DPPH, ABTS, FRAP, Total Phenolic Content

1. INTRODUCTION

Acai, a native fruit from the Amazon region, has received great interest due not only to its exotic taste but also to its potential health benefits. About 90 bioactive substances have already been reported in its fruit, such as phytosterols and phenolic compounds, including anthocyanins, cyanidin 3-glycoside and cyanidin 3-rutinoside (Yamaguchi et al., 2015). The *Euterpe* genus has about 28 species located in Central and South America and distributed throughout the Amazon basin. However, only the fruits of *E. olearacea* and *E. precatoria* have been commercially used for food purposes. The major difference between these species is related to palms growth. *E. oleracea* is popularly known as "acai-do-Pará" and *E. precatoria* is native from the state of Amazonas and popularly known as "acai-do-Amazonas" (Henderson, 1995). In the Amazonas state, the acai production is based on the extractivism process, which represents an important economic, social and cultural activity. The acai is not consumed *in natura* and the fruit must be submitted to a process to obtain the pulp, which is a viscous and dense liquid with creamy

texture (Rogez, 2000). As both fruit and pulp are highly perishable, a conservation process is required after processing.

Different types of foods are produced from the acai pulp and commercialized as pulp, juice, beverages, powder, jelly, liqueur, etc. Currently, despite being a well-known fruit in Brazil, it is also exported and considered as a superfruit worldwide (Schreckinger *et al.*, 2010).

In the agricultural areas of the Amazonas state, technological sustainable alternatives have been employed aiming to aggregate value to native fruits by the generation of new products. The alcoholic fermentation, despite being considered a relatively efficient process of preservation, also reduces other forms of preservation technology. The alcoholic fermentation technology has been demonstrated worldwide as a significant contribution to the rural inhabitants livelihoods through income generation and enhanced food security (Marshall & Mejia, 2011). Currently, the elaboration of alcoholic beverages has been part of the culture, tradition and often characterizing their producing regions, apart from a source of capital and labor generation (Ribereau-Gayon, 2003).

Keeping in view the above-mentioned information, experiments were performed to evaluate the most appropriate operations to produce an alcoholic beverage using acai as raw material. The fermented beverages were prepared using two different processes based on the employed substrate: the first process was performed using the acai *in natura* (Sidrim *et al.,* 2018) while the second one was performed using the acai pulp (Boeira *et al.*, 2020).

Some reports have been found in scientific literature describing the antioxidant compounds in red wines according to the process of elaboration and also to the presence of these compounds in grapes. However, no reports have been found concerning the antioxidant capacity of acai alcoholic beverages produced by different processes.

In deciding which of the two studied processes to use, several parameters related to the quality of the final product should be considered. One of the parameters that should be considered is related to the antioxidant capacity of acai alcoholic beverages produced by both processes employed and this was the aim of this paper.

2. MATERIALS AND METHODS

2.1. Acai samples

Native acai samples (*E. precatoria)* were collected in two different regions of the Amazonas state (SISGEN Access Register Nº AE766AC): Anori **(**03º 46' 22" S and 61º 38' 39" W) and Codajás (03º 50' 12" S and 62º 03' 25" W).

2.2. Acai alcoholic beverage production processes

The acai alcoholic beverages were prodiced in the laboratory of Food Technology of the Campus Manaus Center/IFAM, Manaus, Brazil. The fruit was washed with potable water, then sanitized by immersion for 15 min in 0.2% peracetic acid and finally washed with potable water. The alcoholic beverages were prepared using acai *in natura* (known as acai maceration process – AMP), and also using the acai pulp (known as acai pulp process – APP), according to Figure 1.

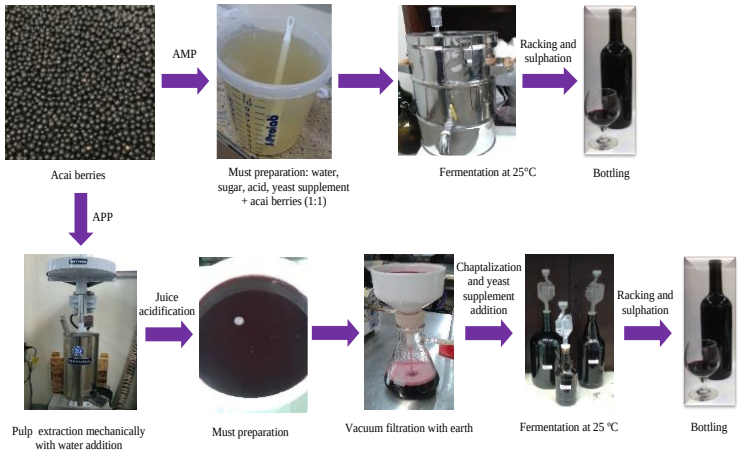

Figure 1 - Flowchart of the acai alcoholic beverage elaborated by the acai maceration process (AMP) and acai pulp process (APP)

In the AMP, the must was prepared using acai *in* nature and water added of sugar to reach a total soluble solids (TSS) content between 18º Brix and 22 º Brix, Thiazote® (Laffort) for the correction of nutrients and tartaric acid to reach a pH between 3.0 and 4.0. The proportion of acai *in nature* to water was 1:1. The fermentation of the

acai *in natura* was performed at 25 ºC in a stainless-steel tank equipped with an airlock valve and conducted by the use of Blastosel Grand Cru (Perdomini-ioc) yeast. The fermentation finished in about 8 days and the acai berries was maintained for 20 days in maceration (Sidrim et al., 2018). After fermentation/maceration, the alcoholic beverage was racked and potassium metabisulfite was added to reach about 50 mg/L of total SO_2. The beverage was bottled in glass bottles (760 mL) capped with a synthetic stopper.

In the APP, the acai fruit was macerated at 40 °C for 30 min and the pulp was extracted mechanically with 70% (w/w) potable water addition. In the pulp, 40% (w/w) potable water was added. Tartaric acid was used to correct the pH between 3 and 4, and it was vacuum filtered with diatomaceous earth filtration (DEF). Then, sugar was added in the acidified and filtered juice to reach 21 °Brix. After must preparation, the yeast Blastosel Grand Cru (Perdomini-ioc) and the yeast supplement Thiazote® (Laffort) were added according to the manufacturer's recommendation. The fermentations were performed in 2 L glass bottles fitted with an airlock valve and conducted at 25 °C. After fermentation, the alcoholic beverage was racked and filtered again. Potassium metabisulfite was added to reach about 50 mg/L of total SO_2. Then, the beverage was bottled in a glass bottle (760 mL), capped with synthetic stopper.

2.3 Antioxidant capacity

The antioxidant capacity was evaluated using the DPPH, ABTS and FRAP assays. Measurements were performed in triplicate using alcoholic beverage 1:10 in a Microplate Reader (Epoch 2, Biotek). The radical scavenging capacity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) radical based on Molyneux (2004) with slight modifications. Alcoholic beverage (10 µL) was added to 190 µL of $DPPH^{\cdot}$ methanolic solution (100 µM). The mixture was kept in the dark at room temperature for 30 min. The absorbance was measured at 515 nm and the results were expressed as micromolar of Trolox equivalent (µM TE).

The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\cdot+}$) radical scavenging assay was performed as described by Re et al. (1999) with modifications. ABTS (0.7 mM)

and potassium persulfate ($K_2S_2O_8$ at 2.4 mM) generated the radical by reacting in the dark for 16 h at room temperature. A volume of 2 µL of the alcoholic beverage and 200 µL of ABTS solution were added in a microplate. The absorbance was measured at 750 nm. After incubation in the dark for 6 min at room temperature, it was read at 750 nm. Trolox was used as positive control and the results were expressed as micromolar of Trolox equivalents (µM TE).

The Ferric reducing antioxidant power (FRAP) was performed with FRAP reagent freshly prepared adding acetate buffer (0.3 M, pH=3.6), TPTZ solution (10 mM) and $FeCl_3 \cdot 6H_2O$ (20 mM), 10:1:1. A sample aliquot (9 µL) was mixed with 27 µL of ultrapure water and added to 270 µL of FRAP reagent. After incubation for 30 min at 37 °C, the absorbance was measured at 595 nm. The results were obtained by the regression equation of ferrous sulphate and expressed as micromolar of Fe(II) Equivalents (µM Fe(II)) (Pulido et al., 2000).

2.4. Total Phenolic Content

It was used a modified Folin Ciocalteu method (Velioglu et al., 1998) to determine the Total Phenolic Content (TPC). Alcoholic beverage 1:10 (20 µL) was added to 150 µL phenol reagent and after 5 min it was added 150 µL of sodium bicarbonate (6%). The mixture was allowed to stand for 90 min in the dark and read at 750 nm using a Microplate Reader (Epoch 2, Biotek). The results were expressed as mg of Gallic Acid Equivalents per liter of alcoholic beverage (mg GAE/L). The assay was carried out in triplicate.

2.5. Statistical analysis

The One-Way ANOVA and Tukey test (95%) using the Minitab® 13.0 software was performed to compare the results.

3. RESULTS AND DISCUSSION

In a food matrix, the adequate separation and quantification of the antioxidant compounds is not a simple task due to its chemical diversity. Therefore, it is usually measured the total level of antioxidant activity. For the examination of multifunctional antioxidants, the performance of a single antioxidant test is

considered insufficient. Consequently, to provide adequate information more than one method is required (Lutz et al., 2011). In this work, three *in vitro* assays (DPPH, ABTS and FRAP) were employed to measure the antioxidant capacity. Also, it was analyzed the TPC as several works published in the literature has been demonstrating a correlation with the antioxidant capacity (Schauss et al., 2006; Ramos et al., 2015; Boeira *et al.,* 2020).

The acai alcoholic beverage prepared by the APP, the acai juice was submitted to a clarification process by DEF to remove suspended solid particles. The DEF was performed because it was demonstrated in previous works that when the juice is filtered for the must preparation the alcoholic beverage produced presented a cleaner and delicate flavor. The antioxidant capacity and TPC of the unfiltered and filtered juices concerning the acai collected in Anori is shown Table 1.

Table 1 - Antioxidant capacity and TPC results obtained for the juice filtered and not filtered from acai collected in Anori and used for the elaboration of acai alcoholic beverage by the APP

Juice	DPPH μM TE	ABTS μM TE	FRAP μM Fe(II)	TPC mg GAE/L
Not Filtered	7,693.3 ± 38.2^{b}	13,564.4 ± 83.9^{b}	17,147.8 ± 58.5^{b}	682.5 ± 0.8^{b}
Filtered	11,168.3 ± 38.2^{a}	17,175.6 ± 77.0^{a}	19,658.9 ± 25.5^{a}	1,207.5 ± 1.8^{a}

The data were mean values of triplicate samples. Different letters within rows indicate statistical differences according to ANOVA with a Tukey test at 5%

The results from DPPH, ABTS, FRAP and TPC assays were all significantly higher in the filtered juice when compared to the unfiltered one. The filtration process resulted in a decrease about 43% in the juice volume due to the removal of suspended particles. Interestingly, the result obtained by the TPC assay for the filtered juice was 43% higher when compared to the unfiltered one. For the DPPH, ABTS and FRAP assays the increase of the antioxidant capacity in the filtered juice were 31%, 21% and 13%, respectively, when compared to the results obtained for the unfiltered juice. These results demonstrated that the operation of DEF of the juice in the APP process for the elaboration of acai alcoholic beverage promoted an increase in the antioxidant capacity and TPC of the juice.

The results obtained for the antioxidant capacity and TPC in the acai alcoholic beverages produced by AMP and APP using the acai fruit collected from Anori are shown in Table 2. When DPPH and ABTS were performed, the results tended to be higher for the APP when compared to the AMP. However, for FRAP and TPC assays, the values obtained in the AMP were significantly higher than APP. In this case, greater difference between the results for both processes was observed in the results from the FRAP assay.

Table 2 - Antioxidant capacity and TPC results obtained for the acai alcoholic beverages elaborated from acai collected in Anori by both processes (AMP and APP) employed.

Process	DPPH µM TE	ABTS µM TE	FRAP µM Fe(II)	TPC mg GAE/L
AMP	10,802.0 ± 955.0[a]	17,113.0 ± 1,091.0[a]	19,374.0 ± 380.0[a]	1,178.2 ± 32.1[a]
APP	11,398.9 ± 198.9[a]	18,068.0 ± 337.0[a]	15,258.9 ± 95.7[b]	902.2 ± 35.4[b]

The data were mean values of triplicate samples. Different letters within rows indicate statistical differences according to ANOVA with a Tukey test at 5%

The results obtained for the antioxidant capacity and TPC in the acai alcoholic beverages produced by both processes using the acai collected in Codajás are presented in Table 3. The results show that, for all employed assays, the beverages prepared using the APP presented higher results when compared to the AMP, and the results obtained in the DPPH and ABTS assays were significantly different between both processes.

Table 3 - Antioxidant capacity and TPC results obtained for the acai alcoholic beverages elaborated from acai collected in Codajás and by both process (AMP and APP) employed

Process	DPPH µM TE	ABTS µM TE	FRAP µM Fe(II)	TPC mg GAE/L
AMP	8,893.3 ± 38.2[b]	14,586.7 ± 66.7[b]	19,131.1 ± 50.9[a]	1,166.4 ± 1.0[a]
APP	10,899.0 ± 960.0[a]	16,857.0 ± 1245.0[a]	22,094.0 ± 3,913.0[a]	1,144.6 ± 143.7[a]

The data were mean values of triplicate samples. Different letters within rows indicate statistical differences according to ANOVA with a Tukey test at 5%.

The acai collected in both regions, Anori and Codajas, demonstrated different profiles for FRAP and TPC. The values of FRAP and TPC of the beverages produced using the acai from Anori were higher for the

AMP, while the results obtained for the acai from Codajas were higher for the APP. These differences may be related to chemical composition in phenolic compounds of acai from both regions and also to the peculiarities in relation to the mechanisms of action, pH, time and temperature employed in both assays. Some criticism to the employed antioxidant methods are based on the absence in living organisms of such free radicals (DPPH/ABTS) and complexity of reaction mechanisms, as well as the biological activities due to the high *in vitro* antioxidant activity cannot be translated as the prevention of non-communicable diseases in humans (Granato et al. 2018). For the FRAP assay, for example, the critique of this method into effectiveness *in vivo* is related to the acidic pH (3,6), suggesting to use this method as only a screening method, allowing an idea of the antioxidant potential (Schaich et al., 2015).

According to Lv et al. (2017), colorimetric assays used to estimate the *in vitro* antioxidant capacity and TPC are used for quality control of food and natural products, as well as for an idea of the potential beneficial effects. In food technology, *in vitro* antioxidant assays together with TPC may be important to assess the effects of processing on the stability of phenolic compounds and represent an important tool to estimate the impact of processing or to extract more antioxidant compounds from raw materials (Hashemi et al., 2018; Touati et al., 2016; Asensio et al., 2017). The TPC and the interactions between antioxidants are important methods in wine and fruit wines studies to understanding beverage stability, aging processes and the impact of technological interventions (Gao et al 2012). Interferences in these nonselective methodologies exist and are well demonstrated by comparing HPLC results with TPC, where the latter are greatly overestimated. Nevertheless, despite their imperfect nature, the usefulness of *in vitro* results cannot be ruled out (Granato et al., 2018). Without a doubt, as these assays are easy to perform, low-cost and do not require sensitive equipment, they have been used to assess complex food matrices (Pérez-Burillo et al., 2018).

Comparing the results obtained by both processes (Tables 2 and 3), the results of the antioxidant capacity tended to be slightly higher in the beverages produced by the APP when compared to those produced by the AMP. The difference encountered in the results of the

antioxidant capacity between the acai alcoholic beverages produced by the two processes employed, measured by the *in vitro* assays to assess the impact of different operations and technological interventions used in both processes on the stability of phenolic compounds, was not enough to be considered as a parameter for choosing one process over the other. Certainly, in the decision make of which process to use to produce the acai alcoholic beverage, other parameters such as the sensorial characteristics related to the flavor and aroma attributes, as well as the process cost should be considered. Therefore, the results obtained in this work was important to clarify the question about the antioxidant capacity in relation to the process used to produce the alcoholic beverage using acai as raw material, as this fruit is well known worldwide by its antioxidant capacity and this capacity would be desired also to be stable during the manufacture of any product.

The results of this work are corroborated by those found by Bezerra *et al.* (2019), who evaluated the acai alcoholic beverages produced by both processes (AMP and APP) and observed that the antioxidant capacity of beverages depended greatly on the acai maturation degree and not on the manufacture process. These authors reported a high antioxidant potential with results of scavenging capacity in ABTS from 5,878.9 to 20,152.2 μM TE, FRAP from 6,750.0 to 17,710.0 μM Fe (II) and TPC from 636.5 to 2,432.8 mg GAE/L. Also, Boeira et al. (2020) demonstrated significant difference in the antioxidant capacity of alcoholic beverages produced by APP with acai from different regions. It was reported values for DPPH from 6,089.3 to 10,629.3 μM TE, for ABTS from 5,283.0 to 15,297.8 μM TE and for TPC from 638.1 to 2,983.8 mg GAE/L.

4. Conclusion

Considering the results obtained and experimental conditions employed, it can be stated that the results of the antioxidant capacity tended to be slightly higher in the beverages produced by the APP when compared to those produced by the AMP. However, the difference found between the results of antioxidant capacity in acai alcoholic beverage manufactured by both processes was not enough to

be considered as one parameter for choosing one process over the other.

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