



Effect of microfiltration on bioactive components and antioxidant activity of *açaí* (*Euterpe oleracea* Mart.)

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ABSTRACT

Microfiltration of centrifuged *açaí* pulp was performed using two types of membrane (ceramic and polymeric), each one at two temperatures (25 and 35°C), aiming to evaluate the influence of these parameters on its chemical composition and antioxidant activity. Temperature and membrane material did not significantly influence the bioactive components contents and the antioxidant activity of the two obtained fractions. However, permeate flux was strongly dependent on both factors, reaching the highest mean value (117 l/h m²) with the ceramic membrane at 35°C. Clarified juice, the permeate fraction, contained about 16 mg/100 g of anthocyanins, 138 mg/100 g of total phenolics and 9 µmol Trolox/g of antioxidant activity. The retained fraction, with characteristics similar to the original *açaí* fruit, presented 75 mg/100 g of anthocyanins, 433 mg/100 g of total phenolics and 31 µmol Trolox/g of antioxidant activity. Therefore, microfiltration of *açaí* resulted in two fractions with distinct characteristics, both rich in bioactive components and with potential industrial application.

Keywords: Amazon fruits; Membrane processes; Bioactive compounds; Anthocyanins

1. Introduction

Açaí (*Euterpe oleracea* Mart.) is the name of the fruit from a palm plant native of the Amazon region and also the beverage obtained from it. The Pará State, in Brazil, is the main producer, most of it obtained extractively and it is also naturally found in other regions of the Amazon estuary floodplains due to their environmental conditions of high temperature, humidity and rainfall [1].

The *açaí* fruit is round-shaped dark-purple, presented in bunches constituted by weight of only 15% of edible

pulp. It is a very important species for the development of the Amazon region, as a major staple local food. It is also as a major economic resource for the local population due to its high potential use not only as food but also for handcrafts, raw material for cellulose industry and cover roofs of rural houses [2,3]. It is estimated that *açaí* chain business moves about \$18 million US dollars annually involving 25 thousand people, but the greatest production is still from extractive activity. In response to the growing market demand the actual tendency is to expand the cultivated areas [3].

The edible pulp is obtained from *açaí* after extraction of the pulp by mechanical processing and water addition,

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resulting in a thick and dark purple pulp with a creamy texture, oily appearance and characteristic flavor [1,3]. The Brazilian legislation classifies the *açaí* pulp according to its total solids content as thin, medium or thick, with 8–11%, 11–14% or more than 14% of total solids, respectively [4].

The pulp consumer market has been increasing due to its nutritional value, exotic flavor and composition in bioactive substances. In 2000, Brazil started international *açaí* commerce with USA and expanded it to other countries like France, Japan, Italy and Germany as frozen pulp, cream, ice cream and ready to drink with breakfast cereals. In the international market it is appreciated like an exotic beverage whereas in Brazil, around the country, as an energetic product and in the North region as part of the daily meal [5].

Açaí pulp is one of the highest nutritive food items of the Amazon region [3], with proximal composition, on dry basis, of 33 to 49% of total lipids, 31 to 49% total carbohydrates, and 14 to 16% of total proteins [6]. This composition associated to the relatively high pH is responsible for the highly perishable nature of the fruit and its products, an important problem that needs to be overcome by appropriate conservation processes, in order to reach the consumer market.

The hurdle technology was tested by employing acidification, pasteurization, reduced water activity and use of chemicals as preservation factors for obtaining microbiology and sensory satisfactory *açaí* pulp [7]. Acidification with citric acid and thermal preservation over 82.5°C for one minute was enough to reduce 99.8% of mesophilic bacteria and total coliforms from 21 to less than 3NMP/ml although these factors alone were not able to promote a good global acceptance.

High hydrostatic pressure has been tested [8] in order to inactivate *açaí* endogenous enzymes (polyphenoloxidase and peroxidase), which are responsible for undesirable changes in their original parameters. Partial inactivation of *açaí* pulp oxidases was verified.

Recently, *açaí* products have received increased interest of consumers and scientific community, due to its composition in bioactive compounds, especially phenolic compounds known for their antioxidant activity, and they can be considered as functional foods or functional ingredients [9]. The interest in antioxidants is due to their potential effects in the prevention of chronic and degenerative diseases such as cancer, cardiovascular and neurodegenerative diseases with primary causes related to oxidative stress [10].

Phenolic compounds, which comprise important classes of antioxidant components with potential benefits to health, are widely distributed in the plant kingdom and are part of human diet, with significant amounts present in vegetables, fruits and plant-based beverages [11]. These compounds are secondary metabolites

synthesized during the normal development of the plant and in response to stress conditions [12], and are responsible for major sensory characteristics of plant-derived foods and beverages, especially color and taste properties [13].

Studies on the identification of phenolics in the *açaí* pulp indicate that anthocyanins are the major compounds [14,15] although there is no agreement in the identification of specific compounds and in their quantification [16,17,18]. In most of these studies, phenolic composition was related to a high antioxidant activity.

Membrane technology has been studied for juice processing due to their mild operation conditions that preserves its sensory quality and the thermosensitive compounds, besides generating two valuable fractions, the retentate and the permeate. Valliant et al. [20] submitted melon juice to microfiltration and observed a loss of 19% on phenolics compounds on the clarified juice and 100% of retention of carotenoids in the retained fraction. The clarified juice was very similar to the fresh juice in physico-chemical and nutritional properties.

Some operational conditions in microfiltration processes may be varied in order to reach the highest permeate flux, such as temperature, pressure, flow rate and some pre-treatment step. Wang et al. [20] verified that a temperature increase from 10 to 40°C in West Indian cherry juice microfiltration was enough to increase significantly the permeate flux and solids retention without affecting the distribution constants of glucose, fructose and ascorbic acid.

Data on the chemical composition of *açaí* are still scarce and the potential for development of new products has not been completely explored. There are potential consumers looking for the exotic *açaí* flavor that avoid consuming it due to its high caloric value caused by the high lipid content. Microfiltration of *açaí* pulp can provide two different products, a pulpy fraction (retentate), similar to the original pulp, and a clarified fraction (permeate), which is a lower calorie product that could be introduced to the international market, increasing its acceptance.

Therefore, the objective of this work was to evaluate the effect of microfiltration of *açaí* pulp, testing the influence of temperature and membrane type on the chemical composition, particularly anthocyanins, and on the *in vitro* antioxidant activity of the two obtained fractions, the permeate and the retentate.

2. Material and methods

2.1. Material

The *açaí* pulp used in the experiments was obtained directly from the industry located in Pará State (Brazil).

The reagents gallic acid, Trolox®, ABTS and potassium persulfate were purchased from Sigma-Aldrich (Germany). The Folin-ciocalteu solution was obtained from Merck (EUA). Methanol and formic acid used in chromatography analysis were HPLC grade (Tedia Brasil HPLC, Brazil).

2.2. Microfiltration tests

Açaí pulp was centrifuged prior to microfiltration in order to reduce total solids and lipid content. Pulp centrifugation was performed in a basket centrifuge (International Equipment Company, model SIZE 2) at 406 g, using a 150 µm nylon screen filter medium.

The microfiltration processes were conducted in duplicate at two different temperatures, 25 and 35°C, using two different tubular membranes systems, a TIA (Techniques Industrielles Appliquées) micro pilot ceramic system and a polymeric unit Protosep IV from Koch Membrane Systems.

The ceramic system was composed of four units of 0.1µm-alumina membranes with a total filtration area of 0.022 m². The polymeric system was composed by one 0.3 µm membrane with 0.05 m² of filtration area. Processes were conducted in batch mode at 3.0 bar of applied pressure up to a volumetric concentration factor of 2.0. Permeate flux was measured along the processes and samples were collected from the three fractions: feed, retentate and permeate.

2.3. Analytical methods

Soluble solids content, pH and acidity were determined in the three fractions AOAC, 2000 [21].

Total phenolic compounds were determined in all fractions by the Folin-Ciocalteu assay proposed by Singleton and Rossi [22] and modified by Georgé et al. [23]. The results were expressed in mg of gallic acid/100 g of sample.

The quantification of the total and monomeric anthocyanins was accomplished according to the differential pH methodology proposed by Giusti and Wroslad [24]. Samples were filtered to remove the suspension solids and the extracts were obtained with two different buffers, having their absorbance measured at 510 and 700 nm. The results were expressed in mg of cyanidine-3-glycoside/100 g.

Monomeric anthocyanins were identified by high performance liquid chromatography [25]. The extraction procedure consisted in vortex-mixing on vortex (1 min) and sonication (10 min) of test tubes containing the sample (±100 mg) and 2 ml of 10% methanolic formic acid, followed by centrifugation at 2500 g for 10 min at 20°C. This extraction stage was repeated three

more times and all supernatants were combined into a flask and dried under nitrogen gas. Before injection, the extract was diluted 1:9 with 10% methanolic formic acid. The chromatographic separation was performed in an HPLC-MS (model HP serialize 1100 MSD) using a SB-C18 column (3.5 µm, 4.6 × 150 mm) and a diode array detector (DAD) (GB15A) set at 530 nm. The mobile phase was constituted by 10% aqueous formic acid (Solution A) and 10% methanolic-formic acid (Solution B). The gradient followed the program: 12–25% B (32 min), 25–60% B (48 min), 60–100% B (50 min), 100–120% B (55 min). The volume injection was 50 µl and flux rate of 1 ml/min.

The identification was done by comparison of the sample chromatograms obtained in this study with a standard chromatogram of açaí obtained under the same conditions (extraction, mobile phase, column type, flow rate and detection at 530 nm) by Brito et al. [25]. The anthocyanins peaks of the standard chromatogram were identified by spectrometric mass data and confirmed by elution times of commercial standards of cyanidin-3-glucoside and cyanidin-3-rutinoside (Indofine Chemical Co., EUA).

Antioxidant activity of the pulps were determined after extraction in methanol/acetone solution [26] and quantification with ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic) acid) using Trolox® ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) as a standard [27]. The results were expressed in µmoles of Trolox equivalent per g of sample (TEAC).

The statistical analysis of data was accomplished by one-way analysis of variance followed by Tukey test, at 95% of probability, using the XLSTAT 7.5 software and variance analysis of two factors followed by Bonferroni test using PRISMA 4.0 version 4 [28,29].

3. Results and discussion

The permeate flux in the polymeric system was much lower than in ceramic system, at both temperatures, and remained essentially unchanged over time.

Statistical analysis of flux data indicates that temperature ($P = 0.0102$), membrane type ($P < 0.0001$) and interaction ($P = 0.0393$) had a significant effect, although it was more pronounced for the membrane type. At 25°C, average permeate flux in the ceramic membrane was 82 l/h m², whereas it was 18 l/h m² in the polymeric membrane. Similar behavior occurred at 35°C, with average flux values of 117.4 and of 25.3 l/h m², for the ceramic and polymeric membranes, respectively. The effect of temperature was higher in the ceramic membrane than in the polymeric one.

Fontes and Caminoto [30] have used refined and diluted açaí pulp for microfiltration in ceramic membranes

with two pore sizes. The permeate fluxes obtained by them were lower than the lowest flux (18 l/h m²) obtained in the present work in the polymeric system at 25°C, which shows that centrifugation, as used in this study, is more efficient as a pre-treatment than the solid content reduction by dilution. It is probably due to the removal of lipids and fibers during centrifugation minimizing concentration polarization phenomena and gel layer formation on the membrane surface, which was favorable to juice permeation.

Physicochemical parameters in the *açaí* pulp and in microfiltration fractions (permeate and retentate) are presented in Tables 1 and 2, respectively.

The characteristics of the clarified *açaí* juice did not significantly vary with temperature or membrane. Microfiltration was effective for clarifying centrifuged *açaí* pulp, decreasing its total solids content in 75% and soluble solids in 31%. Also acidity was reduced

30% in the clarified juice but pH did not change due to microfiltration.

Total solids of the retentate did not significantly differ as a function of the evaluated parameters. Although total solids increased about 70% compared to the centrifuged *açaí* pulp, all the retentate parameters, pH, acidity and total solids were according to the Brazilian legislation for thick *açaí* [4].

Membrane material influenced the total anthocyanin content of the *açaí* clarified juice ($P < 0.0001$) and the statistical evaluation indicated a tendency of interaction with temperature ($P = 0.0741$; Table 1). In the processes carried out in the ceramic system, total anthocyanin that permeated the membrane was 31% while in the polymeric system it was 25% of the total content in the feed *açaí* pulp. Although monomeric anthocyanins may be more sensitive to degradation, since they are not stabilized by

Table 1

Physicochemical parameters of *açaí* pulp and permeate (clarified juice) after microfiltration at 25°C and 35°C in ceramic and polymeric membranes

Parameters	<i>Açaí</i> pulp	<i>Açaí</i> permeate			
		Ceramic membrane		Polymeric membrane	
		25°C	35°C	25°C	35°C
Total solids (g/100 g)	8.5 ± 0.5 ^a	2.1 ± 0.1 ^b	2.1 ± 0.0 ^b	2.1 ± 0.1 ^b	2.0 ± 0.1 ^b
pH	4.5 ± 0.0 ^a	4.6 ± 0.1 ^a	4.3 ± 0.0 ^a	4.4 ± 0.3 ^a	4.6 ± 0.3 ^a
Soluble solids (°Brix)	3.6 ± 0.2 ^a	2.5 ± 0.2 ^b	2.5 ± 0.0 ^b	2.4 ± 0.4 ^b	2.5 ± 0.1 ^b
Acidity (g/100 g) ^x	0.25 ± 0.0 ^a	0.17 ± 0.0 ^b	0.17 ± 0.0 ^b	0.17 ± 0.0 ^b	0.19 ± 0.0 ^b
Total phenolics (mg/100 g) ^y	331.3 ± 19.5 ^a	141.5 ± 10.3 ^b	146.1 ± 1.9 ^b	129.4 ± 15.4 ^b	137.0 ± 9.1 ^b
Total anthocyanins (mg/100 g) ^z	58.0 ± 3.2 ^a	18.9 ± 1.9 ^b	17.6 ± 0.5 ^{bc}	14.0 ± 0.3 ^d	14.5 ± 1.2 ^{cd}
Monomeric anthocyanins (mg/100 g) ^z	33.2 ± 3.3 ^a	12.4 ± 1.6 ^b	11.2 ± 0.2 ^{bc}	8.0 ± 0.3 ^d	8.7 ± 0.6 ^{cd}
Antioxidant activity (µmol Trolox/g)	23.7 ± 2.5 ^a	9.9 ± 0.8 ^b	9.7 ± 0.8 ^{bc}	9.5 ± 0.6 ^{bc}	8.4 ± 0.9 ^c

^xmalic acid eq.; ^ygalic acid eq.; ^zcyanidine-3-glucoside eq.

Values (averages ± SD, $n = 3$) and different letters (a, b, c, d) in the same line indicate significant difference at 95%.

Table 2

Physicochemical parameters of *açaí* pulp and retentate after microfiltration at 25°C and 35°C in ceramic and polymeric membranes

Parameters	<i>Açaí</i> pulp	<i>Açaí</i> retentate			
		Ceramic membrane		Polymeric membrane	
		25°C	35°C	25°C	35°C
Total solids (g/100 g)	8.5 ± 0.5 ^b	15.1 ± 0.8 ^a	14.3 ± 0.8 ^a	15.1 ± 1.0 ^a	14.4 ± 0.1 ^a
pH	4.5 ± 0.0 ^a	4.6 ± 0.1 ^a	4.3 ± 0.0 ^a	4.4 ± 0.3 ^a	4.6 ± 0.3 ^a
Soluble solids (°Brix)	3.6 ± 0.2 ^c	4.4 ± 0.2 ^b	4.8 ± 0.2 ^a	4.7 ± 0.3 ^{ab}	4.7 ± 0.2 ^{ab}
Acidity (g/100 g) ^x	0.25 ± 0.01 ^b	0.29 ± 0.0 ^a	0.29 ± 0.0 ^a	0.29 ± 0.0 ^a	0.30 ± 0.0 ^a
Total phenolics (mg/100 g) ^y	331.3 ± 19.5 ^b	425.8 ± 22.5 ^a	426.6 ± 9.7 ^a	449.8 ± 16.8 ^a	428.5 ± 19.9 ^a
Total anthocyanins (mg/100 g) ^{z*}	58.0 ± 3.2 ^c	76.2 ± 3.5 ^{ab}	70.3 ± 2.0 ^b	80.4 ± 10.5 ^a	73.3 ± 3.8 ^{ab}
Monomeric anthocyanins (mg/100 g) ^{z*}	33.2 ± 3.3 ^b	39.7 ± 2.3 ^{ab}	35.6 ± 2.0 ^{ab}	42.8 ± 8.7 ^a	35.0 ± 2.8 ^b
Antioxidant activity (µmol Trolox/g)	23.7 ± 2.5 ^b	31.2 ± 1.2 ^a	31.3 ± 0.5 ^a	31.3 ± 1.4 ^a	31.2 ± 2.4 ^a

^xmalic acid eq.; ^ygalic acid eq.; ^zcyanidine-3-glucoside eq.

Values (averages ± SD, $n = 3$) and different letters (a, b, c, d) in the same line indicate significant difference at 95% and with * means $p < 0.05$.

interaction with other compounds [31], their behavior in the clarified juice was similar to that of total anthocyanins, presenting permeation of 35% and 25%, in the ceramic and polymeric systems, respectively. For monomeric anthocyanins there was also interaction between membrane material and temperature ($P = 0.0126$).

Pozo-Insfran et al. [16] identified and quantified as the major anthocyanidins in *açaí*, the cyanidine-3-glucoside with 1040 mg/l and perlagonidin-3-glucoside with 74 mg/l, both in fresh pulp. Moreover, Pacheco-palencia et al. [18] found 202 mg/l of cyanidine-3-rutinoside and 75 mg/l of cyanidine-3-glucoside in fresh *açaí* pulp. In this study, the same anthocyanins found by Gallori [17] were identified, cyanidine-3-glucoside and cyanidine-3-rutinoside, although the profile observed was opposite, being cyanidine-3-rutinoside in higher quantity than cyanidine-3-glucoside.

The parameters evaluated in our study, type of membrane and temperature, influenced significantly total and monomeric anthocyanins in the clarified juice but did not have a statistically significant influence on total phenolics (Table 1). This is possible due to the fact that total phenolics comprise various classes of compounds, from simple structures such as phenolic acids to complex species as condensed tannins, the latter probably less sensitive to the evaluated factors.

Antioxidant activity of *açaí* pulp was evaluated by different studies [6,16,18]. In spite of the different values of phenolics content among these studies, the antioxidant capacity values were closer for [16,18], 54.5 $\mu\text{mol Trolox/ml}$ and 48.6 $\mu\text{mol Trolox/ml}$, respectively.

In the present study, membrane material affected the antioxidant activity of *açaí* clarified by microfiltration, possibly as a consequence of the contributing compounds that were also affected (Table 1). Although compared to the original *açaí* pulp, the clarified *açaí* juice had a lower value of antioxidant activity (9.4 $\mu\text{mol Trolox/g}$), this value was higher than that determined by [32] for frozen *açaí* pulp (6.9 $\mu\text{mol Trolox/g}$), but substantially lower than that of a clarified juice (44.5 $\mu\text{mol Trolox/g}$) obtained with filter aid [18]. These results suggest that besides the loss due to the different processing conditions, there is also the influence of the original fresh pulp quality.

The processes conducted in the polymeric system at 35°C and 25°C, and in the ceramic system at 35°C were statistically similar for preservation of the antioxidant activity in the clarified juice. However, considering anthocyanins content and the importance of these bioactive compounds for color quality, the polymeric system at 35°C was the less adequate process (Table 1).

As observed for clarified juice, total phenolics in the *açaí* retentate were not affected by the evaluated factors (Table 2). There was just a tendency ($P = 0.0908$) of an

effect due to membrane material. When the volumetric concentration factor ($\text{VCF} = 2.0$) was taken into account, the expected phenolics content of the retentate was about 662 mg/100 g. Since the obtained average value was about 430 mg/100 g, the increase was only 30%. This indicates that the distribution factor of phenolics compounds was not the same as the volumetric concentration factor, probably due to phenolic losses by the action of the endogenous enzymes polyphenoloxidase and peroxidase despite the nitrogen gas injection into the system.

Nevertheless, the anthocyanins content in the retentate fraction presented an average increase of 32% and 26%, in the polymeric and ceramic systems, respectively, compared to the feed fraction, being the process at 35°C in the ceramic system the less adequate condition for the anthocyanins retention. Even with significant differences in the anthocyanins content after processing, changes were not sufficient to affect the antioxidant activity of the retentate fractions (Table 2).

Although no statistical analysis could be performed due to insufficient number of replicates of each process, the mass balance estimate (data not shown) indicate that losses in bioactive compounds and in antioxidant activity measured by TEAC were very similar for all processes. Mass losses were 13–15% for total phenolics, 19–24% for total anthocyanins, 24–30% for monomeric anthocyanins, and 1416% for antioxidant activity.

Aiming the potential industrial use of both fractions, permeate and retentate, the best processing condition is the one that allows the lower losses, which means better preservation of bioactive compounds in the two fractions, and the higher productivity indicated by the permeate flux. In this sense, the condition of microfiltration in the polymeric membrane may be the less adequate since tended to have higher losses of anthocyanins compared to the other conditions. Moreover, based on process productivity, the results suggest that the best process among all the evaluated conditions was the microfiltration of *açaí* pulp in ceramic membranes at 35°C.

4. Conclusions

The two evaluated factors, membrane type and process temperature, did not influence significantly the total phenolic compounds and antioxidant activity of the obtained fractions, permeate and retentate, although the anthocyanins content was lower in clarified juice obtained using the polymeric membrane, probably due to a higher susceptibility of degradation using this membrane.

The membrane type had a strong influence on the permeate flux, which is the yield parameter, reaching

the highest value with the ceramic system at 35°C, being this the condition suggested as the most appropriate to microfiltrate *açaí* pulp.

Microfiltration showed to be efficient to clarify *açaí* juice and the two fractions obtained can be used for various purposes due to their different characteristics. The permeate or clarified juice may be used in the formulation of jellies, sports and soft drinks, mixed juices and others. As the retentate maintains many of the characteristics of the whole *açaí* pulp, it can be used in different products such as ready to drink nectars, yogurts, fillings and ice creams.

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