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Evaluation of nanofiltration membranes for the retention of anthocyanins of açai (*Euterpe oleracea Mart.*) juice

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ABSTRACT

The açai juice is an Amazonian product that has been exported to various countries around the world. Its main characteristic is the presence of antioxidant compounds especially anthocyanins. Nanofiltration is a membrane separation process that has the ability to separate compounds of low molar weight. Açai juice clarified by microfiltration was used as feed to evaluate nanofiltration membranes of different manufacturers regarding the permeate flux and the retention of anthocyanins. All the evaluated membranes were efficient in retaining the anthocyanins from the açai juice. NF 270 membrane, a composite membrane composed by a polyamide top layer and a polysulphone microporous support, presented the highest water permeability before and after the nanofiltration of açai juice. In addition, this membrane also presented the highest value of permeate flux in the nanofiltration process of açai juice as well as the anthocyanins retention above 99%. The effect of fouling for this membrane was approximately 28%. The observed results showed the potential of nanofiltration on the recovery of anthocyanins from açai fruit.

Keywords: Membrane separation; Microfiltration; Nanofiltration; Phenolic compounds; Açai pulp; Amazonian fruit

1. Introduction

Açai (*Euterpe oleracea Mart.*) is an Amazonian palm and its fruit is traditionally consumed in Brazil but has gained popularity abroad as a food and functional ingredient. Brazil is the first producer, consumer and exporter of açai pulp in the world. Açai pulp is dark purple in color with a high concentration of phenolic compounds, including the anthocyanins. The juice is obtained by cold-pressing the fruit to remove the edible part of the seed, which represents approximately 80% of the volume of the fruit [1].

Anthocyanins belong to the flavonoid family. They are water soluble compounds and natural pigments. They are widely distributed in nature, primarily observed in fruits and leaves. These pigments, together with the carotenoids, represent the largest class of colored substances in the plant kingdom [2]. Anthocyanins are the major phenolic compound in açai juice, being responsible for the color of the açai [3]. There are just two anthocyanins present in large quantities in this fruit, cyanidin-3-glucoside and cyanidin-3-rutinoside [4].

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The membrane separation processes have the ability to separate and concentrate compounds, which can therefore be added to products in the cosmetic, pharmaceutical and food industries as ingredients. Nanofiltration is a driven-pressure process that can be considered an intermediate technique between ultrafiltration and reverse osmosis and it can be applied to separate fine particles (up to 500 Da) [5].

One of the applications of nanofiltration in the food industry is the separation of bioactive compounds as anthocyanins that are present in fruit juices. The recovery of anthocyanins from aronia fruits by means of nanofiltration has been shown to be effective [6].

The major problem in the application of membrane processes is the permeate flux reduction caused by the concentration polarization phenomena, the formation of a gel layer on the top of membranes and fouling. Low transmembrane pressure and the use of high tangential velocities favor the minimization of the fouling effects. The fouling phenomena depend on the concentration of solutes in the region near the membrane surface, and these operating conditions minimize the reduction in permeate flux, allowing the permeate flux to stabilize faster and at higher fluxes when processed under higher pressure [7].

The use of combined techniques can help to reduce substances that favor fouling, thereby decreasing its effects. One technique that can be used to reduce the level of solutes in fruit juices is microfiltration, which has the ability to remove suspended matter and sterilize the processed product [7].

Selectivity, another important property of membranes, is related to the membrane's ability to retain specific solutes, which can be affected by the following factors: size and particle shape; material type; the configuration of the membrane; the concentration of substances retained; and the adsorption of solutes in the membrane. Apparent retention or rejection is a measure of selectivity by which it is assumed that the probability of a particle through the membrane is maximal when the rejection is 100% [7,8].

The aim of this study was to evaluate the retention of anthocyanins present in açai juice, previously clarified by microfiltration, using different commercial nanofiltration membranes and to determine the effects of fouling at these membranes.

2. Materials and methods

2.1. Raw material

Açai juice with 2.5% of solids was used as the raw material for this study. The juice was frozen at 18°C until its use.

2.2. Pretreatment of the juice

Açai juice was pre-filtered with a HAYWARD–LOEFFLER (South Iselin, USA) filter with 200 micrometer pore size. This filter was able to retain suspended solids and particles, resulting in a juice free from substances that could cause any damage to the microfiltration circulation pump.

2.3. Açai juice clarification

The previously filtered açai juice was clarified using a tangential microfiltration process with α-alumina tubular membranes of 0.2 µm average pore size and total permeation area of 0.022 m². Microfiltration was carried out in a batch mode, at 2 bar transmembrane pressure and 35°C ± 2°C. Before each assay, the hydraulic permeability of the membrane was determined in order to verify the cleanliness and integrity of the membranes. After each assay, the equipment was washed and cleaned with a 2.5% NaOH solution and 400 ppm of chlorine at an average temperature of 50°C. After use the NaOH solution, the equipment was rinsed with distilled water until pH 7 to neutralize the sodium hydroxide.

2.4. Nanofiltration membrane tests for anthocyanins retention

The clarified açai juice was used as feed for the nanofiltration process. Nanofiltration was carried out in a plate and frame module with total permeation area of 150 cm² from GE Osmonics, (Minnetonka, USA) as shown in Fig. 1. Six different membranes were evaluated (Table 1): NF270 (Dow/Filmtec), UTC 60 (Toray), MPF 36 (Koch membrane systems), DK and DL (both from GE Osmonics) and NP010 (Microdyn Nadir).

Before the processes, the membranes were conditioned with water for one hour at 35°C and 20 bar and the water permeability determined. Nanofiltration was carried out at 35°C at 10, 15, 20 and 30 bar transmembrane pressures. The volumetric reduction factor (VRF)

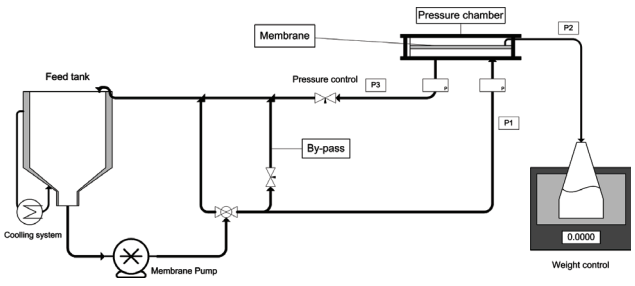

Fig. 1. Nanofiltration module GE Osmonics.

Table 1
Nanofiltration membranes utilized at the tests

Membrane	Manufacturer	Country	Membrane composition[1]	Nominal retention/cutoff[1]
NF270	Dow/Filmtec	USA	Semi-aromatic piperazine-based polyamide layer on top of a polysulphone microporous support	99.0% $MgSO_4$
UTC 60	Toray Industries Inc.	Switzerland	Aromatic polyamide	55% NaCl at 15 bar and 25°C
MPF 36	Koch Membrane Systems Inc.	USA	Not declared	~1000 Da
DK	GE	USA	Thin film	98% $MgSO_4$ at 25°C
DL	GE	USA	Thin film	96% $MgSO_4$ at 25°C
NP010	Microdyn Nadir	Germany	Polietersulfone	~1000 Da

[1]Specifications obtained from the manufacturers.

was maintained equal to 1, by recirculating both the retentate and permeate streams to the feed tank.

The VRF was calculated by using the Eq. (1):

$$\mathrm{VRF} = \frac{V_f}{V_f - V_r} \quad (1)$$

where V_f is the feed volume and V_r is the retentate volume.

For each process, approximately three liters of juice were used in a batch mode process. After each ten minute process, the permeate flux was determined in triplicate. A repeatability test (three assays) was conducted on the membrane that showed the highest permeate flux and the highest retention of anthocyanins. Samples of permeate were collected after the application of each pressure range to analyze the anthocyanins by the pH differential method and were expressed as cyanidin- 3-glucoside to check for the permeation of this compound through the membrane. Eq. (2) was used to calculate the retention of the membrane to this compound:

$$\mathrm{R}(\%) = \left(1 - \frac{C_p}{C_f}\right) \times 100 \quad (2)$$

where C_p represents the concentration of the compound at the permeate and C_f represents the concentration of the compound at the feed.

After this process, the material in the system was collected and the equipment was rinsed to remove the remaining product. The water permeability was then evaluated to determinate the flux decline and to determine how the açai juice functioned to reduce the efficiency of the membranes. Eq. (3) was used to determine this reduction in efficiency:

$$\mathrm{F}(\%) = \frac{\mathrm{PWF_b} - \mathrm{PWF_a}}{\mathrm{PWF_b}} \times 100 \quad (3)$$

where PWF_b represents the flux of water before processing and PWF_a represents the flux of pure water after processing. After each test, the membrane used in the test was discarded.

2.5. Analysis

Measurements of pH were performed in an automatic titrator, Metrohm® model 785 DMP–Titrino, after instrument calibration with buffers with a pH of 4.00 and 7.00. The total acidity was determined using an automatic titrator, Metrohm® model 785 DMP–Titrino, with sodium hydroxide reagent factored with sodium biphthalate. Values are expressed as mg of malic acid per 100 g of sample. The total solids were measured gravimetrically by determining the dry weight in the vacuum oven. The soluble solid content was determined using a Bellingham + Stanley Limited model hand refractometer with a correction for temperature (20°C), and was expressed in °Brix. All the analysis described above followed the A.O.A.C methods [9].

The spectrophotometric quantification of phenolic compounds was performed according to the methodology of Singleton and Rossi [10], using Gallic acid as a standard. For the color reaction, it was used the Folin-Ciocalteu reagent at 10% and sodium carbonate at 7.5%. The result was expressed as mg of Gallic acid equivalents per 100 g of sample.

The content of monomeric anthocyanins was determined using a spectrofluorimeter Tecan Infinite 200, and measurements were performed with a microplate spectrofluorimeter (Infinite® 200, Tecan France SAS, Lyon, France) using 96-well polypropylene plates through the pH differential method described by Giusti and Wrolstad [11]. This method dissolves the sample in two buffer systems: potassium chloride/hydrochloric acid (pH 1.0) and sodium acetate (pH 4.5). The sample after extraction

is diluted and the absorbance is read in the range of 510 nm and 700 nm. The concentration is calculated from Eq. (4):

$$C\left(mg/100g\right) = \frac{A \times Mw \times DF \times 10^2}{\varepsilon \times L} \quad (3)$$

where C is the concentration of anthocyanins expressed as equivalent of cyanidin-3-glucoside per 100 g of sample; $A = (A_{510\ nm} - A_{700\ nm})pH_{1.0} - (A_{510\ nm} - A_{700\ nm})pH_{4.5}$; *Mw* (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside; *DF* (dilution factor) = 10 to 50; ε (molar extinction coefficient) = 26 900 l/mol/cm^{-1}; *l* (pathlength) = 0.52 cm (calculated for the specific well geometry with 200 µl of solution).

The analysis results were evaluated by analysis of variance (ANOVA), Tukey test and the statistical software XLSTAT 7.5.

3. Results and discussion

After carrying out two microfiltration tests, was observed an average initial permeate flux of 169 l/hm² and a final average permeate flux of 95 l/hm² (Fig. 2). The average permeate flux between both tests was 117 l/hm² with a total processing time of five hours yielding a VRF equal to 8.5. During the initial stages of the process there is a slight decrease in permeate flux and this behaviour continue until the end of tests. This behaviour is due to concentration polarization phenomena that occurrs by a deposit of particules on the membrane surface that difficult the passage of product and fouling that can obstruct the pores of the membrane reducing the permeation area.

The microfiltration of açai juice resulted in two fractions, retentate and clarified juice, both presenting anthocyanin concentration close to that of the feed content. The content of anthocyanin in the retentate and in the permeate fractions were 85.3 and 61.8 mg/100 g, respectively, representing 124% and 90% of the concentration of anthocyanin in the feed.

There were no significant differences between the filtered juice and the permeate of microfiltration regarding anthocyanins and phenolic content, dry matter and acidity. Anthocyanins are 60% of the total phenolic compounds present in açai samples evaluated. The reduction of phenolic and anthocyanins content in the clarified juice compared to the single strength juice can be explained by the retention of fibers and suspended particles, retained by the membrane, as indicated by the reduction in the dry matter content.

The hydraulic permeability of nanofiltration membranes was determined before and after the experiments with açai juice (Fig. 3). It was found that the permeate flux increased linearly with transmembrane pressure. Each membrane presented different permeate flux values in the range of transmembrane pressures applied. The NF270 membrane had the highest flux in all the pressure range, with a maximum flux at 30 bar, 30% higher than the permeate flux of the membrane NP010, the second highest permeate flux. The membrane MPF 36 exhibited the lowest value of water permeability.

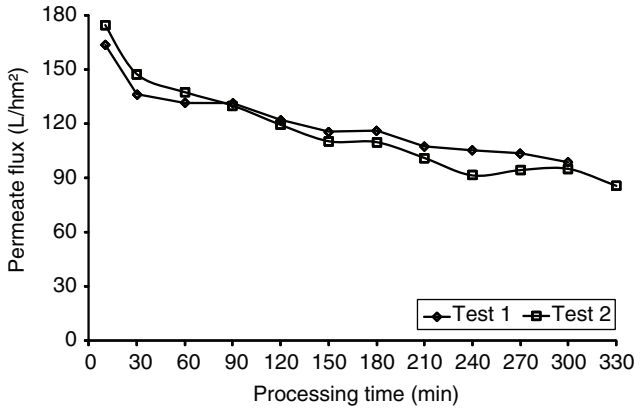

Fig. 2. Evolution of permeate flux during two microfiltration processes of açai juice (duplicate).

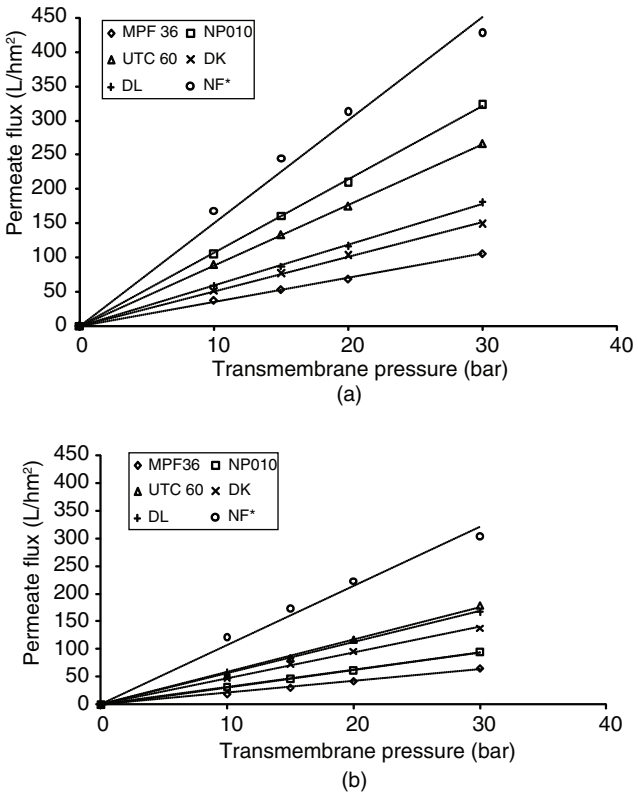

Fig. 3. Hydraulic permeability of nanofiltration membranes before (a) and after (b) tests with açai juice (*Means of three assays).

Table 2
Characterization of açai quality along the clarification process

Parameter	Açai juice	Filtered açai juice	Clarified açai juice	Açai juice retentate
pH	3.9 ± 0.04	4 ± 0.13	3.9 ± 0.01	3.9 ± 0.04
Acidity (g/100 g)[1]	0.30^{b} ± 0	0.28^{c} ± 0	0.28^{c} ± 0.01	0.33^{a} ± 0.01
Soluble solids (°Brix)	2.4^{b} ± 0.05	1.9^{c} ± 0.05	1.6^{d} ± 0.05	2.8^{a} ± 0.05
Dry matter (g/100 g)	$2.5^{a,b}$ ± 0.13	$2.0^{b,c}$ ± 0.02	1.8^{c} ± 0.05	3.0^{a} ± 0.21
Total phenolic (mg/100 g)[2]	138.8^{a} ± 4.5	111.5^{b} ± 2.37	104^{b} ± 4.17	140.4^{a} ± 12.05
Anthocyanin content (mg/100 g)[3]	83.9^{a} ± 9.33	68.7^{b}± 2.42	61.8^{b} ± 3.09	85.3^{a} ± 12.94

[1]expressed in malice acid; [2]expressed in Gallic acid equivalent; [3]expressed in cyanidin-3-glucoside equivalent. Identical letters in the same line do not differ significantly ($p > 0.05$) among themselves.

The behavior of the membrane with water, however, was unrelated to the membrane behavior observed when complex solutions with different solutes and macromolecules were present. The permeate flux with a real solution made up less than 5% of the flux with pure water. Nevertheless, the hydraulic permeability is the benchmark of integrity and efficiency of the cleaning process of a membrane, ensuring the reproducibility of results [8].

In the evaluation of water permeability after nanofiltration tests, the membrane NF 270 exhibited highest permeate fluxes, including values of 121 l/hm² at 10 bar, 173 l/hm² 15 bar, 222 l/hm² at 20 bar and 303 l/hm² at 30 bar. Membrane MPF 36 showed the lowest values of permeates flux for both water before and after the trial with açai juice.

The tests performed with açai juice in VRF = 1 showed that the NF270 membrane again exhibited the highest flux in all pressure ranges applied. This behavior was similar to that presented in the tests with pure water. The values of permeate flux were 67 l/hm² at 10 bar, 84 l/hm² at 15 bar, 93 l/hm² at 20 bar and 102 l/hm² at 30 bar (Fig. 4). The membrane MPF 36 exhibited the lowest permeability, with values of 8.9 l/hm² at 10 bar, 12 l/hm² at 15 bar, 17 l/hm² at 20 bar, 27 l/hm² at 30 bar.

The membranes MPF 36, NP 010 and UTC 60 exhibited a linear increase in the permeate flux for the açai juice with an increasing transmembrane pressure, whereas the membranes DK, DL and NF 270 exhibited a trend toward stabilization of permeate flux with increasing pressure and may reach limit flux. However, this phenomenon was not confirmed due to a restriction on the equipment that did not allow the transmembrane pressure to be increased above 30 bar.

Samples were taken from the permeate streams for each evaluated transmembrane pressure in order to investigate the possible permeation of anthocyanins (Table 3). The analysis showed that the membrane NP 010 was the only one to allow the permeation through the membrane of a measurable quantity of anthocyanins. The value of 1.78 mg/100 g of juice at 10 bar can be considered low, although the membrane should avoid the permeation of any amount of anthocyanins compounds.

Note that by increasing the transmembrane pressure the retention coefficient of anthocyanins increases. This can be explained by the adsorption of the solute in the membrane. When adsorption occur inside the pores,

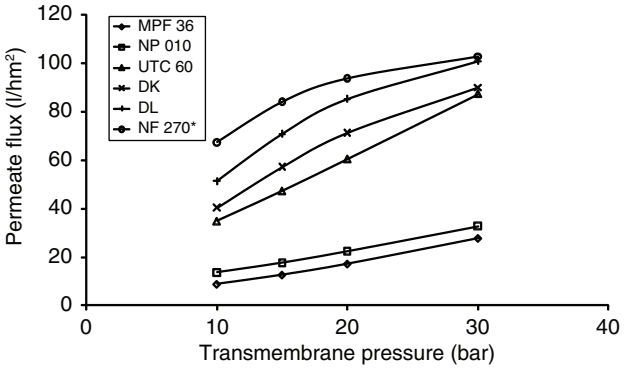

Fig. 4. Nanofiltration membrane behaviour in relationship of açai juice permeate flux (VRF = 1) using different transmembrane pressures (*Means of three assays).

Table 3
Anthocyanins retention coefficient of the 6 evaluated membranes

Membranes	ΔP (bar)	Retention coefficient (%)
MPF36	10–30	>0.99
UTC60	10–30	>0.99
DK	10–30	>0.99
DL	10–30	>0.99
NF270	10–30	>0.99
NP010	10	~0.97
	15	~0.97
	20	~0.98
	30	>0.99

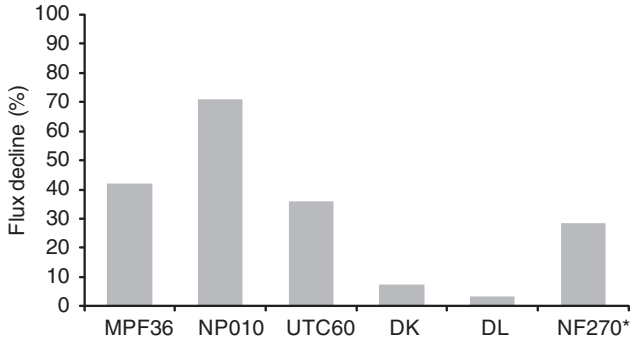

Fig. 5. Nanofiltration membranes flux decline (*Means of three assays).

there are more severe changes for the permeate flux and to the ability of the membrane selectivity. Consequently, the adsorption of molecules on the pore wall decreases the number of effective pores, which causes a drop of the permeate flux and an increase in the rejection by the membrane. The adsorption phenomenon depends crucially on the interactions between the solute in the solution being treated and the membrane material [7].

After evaluating the reduction of permeate flux, it was observed a clear decrease in the permeate flux of all membranes tested (Fig. 5). For NF 270, the reduction values were 27%, 29%, 28% and 29% at transmembrane pressure of 10, 15, 20 and 30 bar respectively, with an average of 28.6%.

The membrane NP 010 exhibited the highest decline in the flux, a reduction of 70%, 70%, 71% and 70% at transmembrane pressure of 10, 15, 20 and 30 bar respectively, with an average reduction of 70.9%. DK and DL membranes had values 10% below in comparison to the decline of permeate flux, averaging 7.4% and 3.5%, respectively. The DL membrane that exhibited intermediate values of permeates flux for pure water before and after tests with açai juice was the membrane that exhibited the lowest decline of permeates flux.

4. Conclusions

This study suggests that by couping microfiltration and nanofiltration it is possible to retain the anthocyanins present in açai juice. Microfiltration had retained only 10% of the total anthocyanins of the açai juice, indicating that this process does not severely affect the concentration of these compounds in the clarified juice. Regarding the nanofiltration, five between the six evaluated membranes showed the ability to concentrate de bioactive compounds of açai.

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