



# Concentration of hydroalcoholic extracts of graviola (*Annona muricata* L.) pruning waste by ultra and nanofiltration: Recovery of bioactive compounds and prediction of energy consumption



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## ABSTRACT

Concentrated extracts of graviola leaves, which are widely discarded during the pruning of trees in commercial crops, were obtained by different membrane concentration process. These leaves are commonly used in traditional medicine due to their pharmacological properties. In this study, hydroalcoholic extracts (with 50 or 70% of ethanol (v/v)) of graviola leaves were subjected to ultrafiltration (UF), nanofiltration (NF) and to combination of both processes (UF followed by NF). The concentrated fractions coming from these processes were analyzed for their total antioxidant capacity and total phenolic content in order to evaluate the selectivity of each concentration step. Processes performance were also evaluated for productivity (permeate fluxes) and energy consumption, which allowed the choice of the best membrane filtration system in terms of energy savings and of interest compounds retention. The combination of processes of UF and NF showed retention coefficients higher than 75% in concentration of phenolic compounds. This sequential design resulted also in higher fluxes of NF, the fluxes increased almost seven times for extracts with 50% of ethanol, and almost three times for extracts with 70% of ethanol when compared to direct processes of NF. Furthermore, the combined system of UF and NF promoted a significant reduction in the concentration time of the extracts by NF and the characterization of fouling mechanisms, which is important parameters to scale-up of this process, indicated that the strategy may increase the useful life of the membranes.

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## 1. Introduction

Graviola (*Annona muricata* L.), also known as “soursop” in English, “guanábana” in Spanish and “corossol” in French, belongs to the Annonaceae family, with about 119 genera and more than 2000 species. It is an ever green, terrestrial, erect tree that can grow to 5–8 m, having wide and dark green leaves and a mild and bright appearance (São José et al., 2014). It is native in the tropical regions of Central America and northern regions of South America, but has been distributed throughout all tropical regions (Freitas, 2012). The state of Bahia in Brazil is the world's largest graviola producer, and despite a scarcity of data on its planted area and production, the

estimated harvest in 2012 was 20,000 tons in a planted area of about 1,570 ha (ADAB, 2016; Lemos, 2014; São José et al., 2014). Its fruits are mainly processed as juices, ice cream, jams and sweets. The peels, leaves, stems, roots and fruit seeds are also well known and widely used in traditional medicine, containing a vast variety of chemical compounds known for their medicinal properties (Gajalakshmi et al., 2012; Gavamukulya et al., 2014; Machado et al., 2015; Matos and Lorenzi, 2008).

Graviola leaves are rich in bioactive compounds and have a high antioxidant capacity. Therefore, they can be used in an array of high-value-added products, such as dietary supplements and phytotherapies. Additionally, tons of leaves and twigs are discarded annually in producing countries because pruning is an indispensable practice for harvesting the fruits quickly.

Phytochemical screening of *A. muricata* leaves extracts revealed that they are rich in secondary metabolites such as alkaloids, saponins, terpenoids, flavonoids, coumarins and lactones,

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anthraquinones, tannins, cardiac glycosides, annonaceous acetogenins, phenols and phytosterols (Gavamukulya et al., 2014; Moraes et al., 2016). The high presence of phenolic compounds confers potent antioxidant capacity to the leaves and their extracts (Nawwar et al., 2012). They can prevent the onset and/or progress of oxidative disorders, as well as reduce the risk of diseases such as cancer, arthritis, diabetes and other diseases that occur during aging (Almela et al., 2006; Tabart et al., 2007).

Recovery of phenolic compounds from plant sources such as graviola leaves is generally made using methanol, ethyl acetate, acetone or other organic solvents which can compromise the final application of the extracts in food or pharmaceutical industries (Azmir et al., 2013; Moraes et al., 2016). Thus, the use of ethanol, a recognized GRAS solvent (*Generally Recognized as Safe*), have been extensively explored for this application. Nevertheless, the concentration of extracts obtained by hydroalcoholic extraction usually use heat to promote solvent evaporation, this process can harm a wide variety of thermally sensitive phenolic compounds present in graviola leaves and can influenced the antioxidant capacity of their extracts (Chipurura et al., 2010).

The use of UF and/or NF polymeric membranes has been explored for many years to concentrate bioactive compounds from plant extracts (Conidi et al., 2012; Cruz et al., 2013; El-Abbassi et al., 2014; Ferrer-Polonio et al., 2017; Li and Chase, 2010; Murakami et al., 2011; Pinto et al., 2014; Prudêncio et al., 2012) and for recovering phenolic compounds from wastewater (Giacobbo et al., 2017; La Scalia et al., 2017; Romero-Dondiz et al., 2016). However, the application of these membrane processes using organic solvents such, as ethanol for example, is still a challenge (Weinwurm et al., 2016). The stability of the membranes with organic solvents is questionable and normally shows reduced permeated fluxes and high energy consumption, when compared to aqueous filtration system processes (Cassano et al., 2013; Alles et al., 2015; Cissé et al., 2011; Weinwurm et al., 2016). Thus the objective of present work is to evaluate the recovering of phenolic compounds from hydroalcoholic extracts of graviola leaves.

Although the phytochemical characteristics of graviola leaves are widely studied, to the best of our knowledge there are no reports on the concentration of bioactive compounds of graviola leaves in literature. The UF, NF and a combination of both concentration membrane processes were evaluated in order to optimize the choice of the best membrane filtration system in terms of energy saving and retention of the compounds of interest.

## 2. Material and methods

### 2.1. Raw material

Graviola (*A. muricata* L) leaves were obtained from a commercial cultivation located in the city of Trairi – CE, Brazil, latitude 3° 22' 15.98 "S and longitude 39° 17' 34.46" W. Voucher specimens are kept at Herbarium Prisco Bezerra under number 58,330, located at the Federal University of Ceará, Fortaleza, CE, Brazil. After harvesting, leaves were selected, washed and sanitized with a sodium hypochlorite solution (100 µg/mL) and then dried at 40 °C for 30 h in a forced air dryer at Embrapa Tropical Agroindustry, Fortaleza, CE, Brazil. Then, the leaves were ground into a powder using a knife mill and stored in metallized bags at room temperature.

The powdered graviola leaves were then sieved through a mesh 28 sieve (Tyler Standard Sieve Series - Granutest, Brazil) obtaining particles smaller than 0.6 mm in size. From these particles were prepared the hydroalcoholic extracts. The solid-liquid extraction was performed at a ratio of 1 g of sieved leaves/20 mL of solvent in a proportion of 50 or 70% of ethanol in water (v/v). The flasks were

capped to prevent solvent evaporation. The extraction was conducted in a shaker in a thermostatic bath (TE-052 Dubnoff Fibra-limantar, Tecnal, Brazil) at 80 rpm and 50 °C for 15 min, and the extracts were then filtered through a Büchner funnel with a vacuum pump. The experimental conditions used (Ethanol/water concentrations, temperature and extraction time) were optimized from a previous study by our research group (Moraes et al., 2016), but the final work with optimization planning has not yet been published.

The ethanol (EtOH) was from LS Chemicals (Analytical grade, Purity: 99%, Mumbai, India), while the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) diammonium salt, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), fluorescein sodium salt and Folin-Ciocalteu reagent were from Sigma Aldrich (St. Louis, MO), and potassium persulfate was from Acros Organics (Geel, Belgium).

### 2.2. Ultrafiltration (UF) and nanofiltration (NF) processes

UF and NF processes were carried out in a tangential filtration system (INVICT, MENTEST, Brazil) in a flat sheet module. The system is comprised of a jacketed stainless steel feed tank with a capacity of 1.5 L, attached to a refrigeration unit to control process temperature, and a direct drive piston pump (Model 2SF05SEEL, Cat pump, Minneapolis, USA) for feed circulation, a valve for pressure adjustment and a pressure gauge to control the module's output pressure. The filtration unit has a rectangular shape with dimensions of 0.11 m × 0.07 m, making a permeation membrane area of 0.0077 m<sup>2</sup>.

The filtrations were carried out in a continuous mode where the feed solution was recirculated in the system. The fixed operational parameters were: outlet pressure: 0.7 MPa (UF) and 2.0 MPa (NF); recirculation flow: 70 L/h (UF) and 60 L/h (NF); operating temperature 35 °C.

The processes were interrupted when the volume concentration factor (VCF) achieved 2.0 (UF) and 1.7 (NF). These VCF were defined based on the maximum process time of 6 h for UF and 8 h for NF. All experiments were performed in duplicate and the results were expressed as the average between them. Feed, permeate and concentrate stream samples were taken for further analysis of total phenolic (TP) content and antioxidant capacity (AC) and evaluation of the retention of these compounds. The amount of permeate that passes through the membrane in a given time was also registered to calculate the permeate flux.

The membranes were pre-conditioned by soaking them in ethanol/water solution (50 or 70% of ethanol in water (v/v) depending on the process) for 16 h at room temperature. This step aims to improve the permeate flux, thus ensuring that the membrane was thoroughly wetted by the solvent, which facilitates permeation. The main specifications of the membranes used are shown in Table 1.

Both membranes have hydrophilic characteristics and a good mechanical strength. The NF270 is a thin film composite membrane where the upper layer is of polyamide and the thicker bottom layer is of polysulfone. This membrane was used with the purpose of retaining the phenolic compounds of the graviola leaves that have a molar mass between 300 and 600 g/mol. The UH004 membrane was chosen due to its good permeability and ability to retain macromolecules by promoting partial purification of the extract prior to NF.

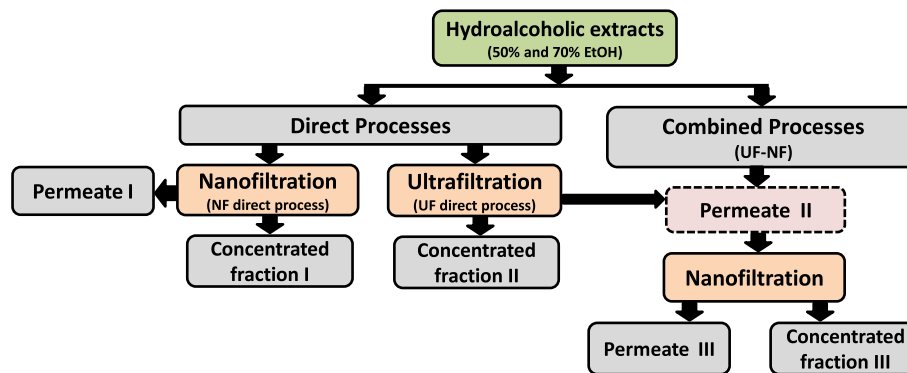
### 2.3. Schematic design of processes

The membrane filtration processes were carried out as shown

**Table 1**  
Specifications of ultra and nanofiltration membranes used in the experiments.

Parameters	UH004	NF270
Manufacturer	Microdyn-Nadir <sup>a</sup>	DOW FILMTEC <sup>b</sup>
Material <sup>j</sup>	PESH	Polyamide thin-film composite
MWCO (g/mol) <sup>j</sup>	4.000	200–300
Pore size (nm)	1.075 <sup>c</sup>	0.84 <sup>d</sup>
T <sup>MO</sup> (°C) <sup>j</sup>	95	45
p <sup>MO</sup> (MPa) <sup>j</sup>	1.0	4.1
pH <sup>j</sup>	0–14	3–10
Retention (%)	92–99 <sup>e, h</sup>	>97 <sup>f, i</sup>
Permeability to pure water (kg/(m <sup>2</sup> .h.bar))	>7 <sup>i</sup>	17 <sup>g</sup>
Membrane thickness (μm) <sup>k</sup>	120–130	210–250

MWCO (g/mol): Molecular weight cut-off, T<sup>MO</sup> (°C): Maximum operating temperature, p<sup>MO</sup> (MPa): Maximum operating pressure, PESH: Polyethersulphone with hydrophilic treatment, <sup>a</sup>Wiesbaden, Germany, <sup>b</sup>Illinois, USA, <sup>c</sup>Oatley-Radcliffe et al. (2015), <sup>d</sup>Nghiem and Hawkes (2007), <sup>e</sup>Mohammad et al. (2015), <sup>f</sup>Penha et al. (2015), <sup>g</sup>Cissé et al. (2011), <sup>h</sup>Retention of MgSO<sub>4</sub>, 2000 mg/L at 25 °C, <sup>i</sup>Retention of Dextran at 25 °C, <sup>j</sup>Data of Manufacturer, <sup>k</sup>Data experimentally obtained.



**Fig. 1.** Schematic diagram of filtration of hydroalcoholic extracts obtained from graviola leaves concentrated by UF, NF or by sequential process (UF-NF).

by the schematic diagram (Fig. 1). Briefly, UF and NF processes were firstly carried out separately (Direct Processes). In a second moment, a sequential membrane process using UF and NF was evaluated in order to concentrate the phenolic compounds from graviola extract. In this case, the UF was carried out for suspended solids reduction. In sequence, the permeate of UF was used as feed in a NF process, that was developed in order to concentrate the compounds that permeated of the UF membrane.

## 2.4. Process parameters

### 2.4.1. Determination of volumetric permeate flux ( $J_v$ )

Volumetric permeate flux ( $J_v$ ) measured in L/(m<sup>2</sup> h), is defined as the amount of permeate that passes through the membrane area in a given time, as expressed in Equation (1), where:  $m_p$  is the permeate collected mass (g);  $\rho$  is the solution density (g/L),  $t$  is the time (h), and  $A_p$  is the membrane permeation area (m<sup>2</sup>).

$$J_v = \frac{m_p}{\rho \cdot t \cdot A_p} \quad [1]$$

The average permeate flux corresponds to the slope of the straight line described by the equation  $V_p = f(A_p \times t)$  (Tylkowski et al., 2010).

### 2.4.2. Flux decrease (%)

The percentual flux decrease ( $D_f$ ) was calculated according to Equation (2), where  $J_i$  is the initial and  $J_s$  is the average permeate flux in L/(m<sup>2</sup> h).

$$D_f = \frac{(J_i - J_s)}{J_i} \cdot 100 \quad [2]$$

### 2.4.3. Fouling index ( $b$ )

The fouling index was estimated according to Equation (3), described by Cheryan and Merin (1981):

$$J = J_0 \cdot t^{-b} \quad [3]$$

Where  $J$  and  $J_0$  are the actual and initial permeate fluxes,  $t$  is time and  $b$  is the fouling index.

### 2.4.4. Volumetric concentration factor (VCF)

Volumetric concentration factor (VCF) was determined by Equation (4), where  $V_o$  and  $V_f$  are the initial and the final feed volumes (L), respectively.

$$\text{VCF} = \frac{V_o}{V_f} \quad [4]$$

### 2.4.5. Retention coefficient ( $R$ )

Retention coefficient ( $R$ ), expressed in %, for total phenolics (TF) and antioxidant capacity (AC) measured by ABTS and ORAC methods were determined according to Equation (5), where  $C_{p,i}$  is the concentration of solute  $i$  present in the permeate and  $C_{f,i}$  is the concentration of solute  $i$  in the feed solution.

$$R(\%) = \left(1 - \frac{C_{p,i}}{C_{f,i}}\right) 100 \quad [5]$$

#### 2.4.6. Energy consumption (E)

Equation (6) was used to estimate the energy consumption (E) of the processes in kWh/m<sup>3</sup> (or 0.001 kWh/L), as described by Jönsson and Wallberg (2009), where:  $\Delta p$  is the transmembrane pressure in kPa,  $Q_{feed}$  is the feed recirculation flow in m<sup>3</sup>/h,  $\eta$  is the pump efficiency factor (0.95),  $J_v$  is the volumetric permeate flux in m<sup>3</sup>/(m<sup>2</sup>h) or and  $A_p$  is the membrane area in m<sup>2</sup>.

$$E = \frac{\Delta p \cdot Q_{feed}}{\eta \cdot J_v \cdot A} \quad [6]$$

### 2.5. Extract characterization

#### 2.5.1. Total phenolic concentration

The concentration of total phenolic compounds was determined as described by Singleton et al. (1999), with slight modifications. First, 2.5 mL of the Folin-Ciocalteu reagent was added to 0.5 mL of sample extract. After 5 min, 2 mL of sodium carbonate solution (7.5% w/v) was added, followed by mixing in a shaker tube for 10 s. After 2 h protected from light at room temperature, the sample was read in an absorbance of 760 nm using a model DU70 UV/Vis spectrophotometer (Beckman Instruments, Inc., Fullerton, CA, USA). A six point calibration curve was constructed using gallic acid concentrations from 8 to 72  $\mu\text{g mL}^{-1}$ . Results were expressed in mg of gallic acid equivalent per g of dry extract, and were expressed as average  $\pm$  standard deviation (three replicates).

#### 2.5.2. Antioxidant capacity

The antioxidant capacity of extract fractions was evaluated by two different methods based on distinct mechanisms. The first involved the transfer of electrons (ABTS) using Free Radical Capture ABTS<sup>•+</sup>, according to the methodology described by Rufino et al. (2007), and quantified according to Re et al. (1999). The second was based on hydrogen transfer (Oxygen Radical Absorbance Capacity–ORAC), according to the methodology described by Dávalos et al. (2004) with slight modifications. The results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) per g of dry extract.

#### 2.5.3. Particle size distribution

Particle size distribution of the extracts was determined by laser diffraction in a Laser Scattering Mastersizer (Malvern Instruments, Mastersizer 2000 UK). Diameter was determined based on the average diameter of a sphere with the same volume (Brouckere Diameter – D<sub>43</sub>). All samples were analyzed three times.

### 2.6. Characterization of the membranes

#### 2.6.1. Atomic force microscopy (AFM)

Analysis of the membranes' surface morphology was done before and after the concentration process. Membranes were analyzed using an atomic force microscope (AFM) NanoSurf model EasyScan 2 (Liestal, Switzerland). The scanning was performed on a membrane surface area of 75  $\mu\text{m} \times 75 \mu\text{m}$  in non-contact mode, and the images were processed using free Gwyddion software version 2.43 (Czech Metrology Institute, Czech Republic), which provided the parameters related to sample roughness. After

acquiring each original membrane image, new images with a side length of 5, 10, 22, and 41  $\mu\text{m}$  were obtained. Roughness mean square values (Rms) were obtained from the photomicrographs.

### 2.7. Statistical analysis

All the results were submitted to analysis of variance (ANOVA) and Tukey test was used to compare the means obtained from the membrane concentration processes. SAS software version 9.4 (SAS Institute Inc, USA) was used to perform the analysis. Differences were considered significant at a level of 5% ( $p \leq 0.05$ ). Mathematical modeling was performed with Statistica 8.0 (Statsoft, USA) in order to evaluate fouling mechanisms.

## 3. Results and discussion

### 3.1. Feed characterization

Moisture, ash, total protein, lipids, carbohydrates, solids, pH, density and antioxidant capacity extract values are shown in Table 2. The extract with 70% EtOH contained a higher amount of lipids and proteins than the one with 50% EtOH, which on the other hand showed a greater amount of ash and carbohydrates. This is reasonable since fats are hydrophobic, while carbohydrates and metals that result in ash are hydrophilic. The obtained higher protein content using more EtOH suggests that most of the recovered proteins were lipophilic, being membrane-bound proteins. It should be noted that proteins and enzymes are denatured by EtOH, causing suspended particles to form. These suspended particles had their size distribution determined and the results are presented in Fig. 2 (see Table 3).

The extracts with 50% EtOH showed a smaller particles size distribution, whereas the particles dispersed in extracts with 70% EtOH were larger and the particle size distribution graph still showed a bimodal profile with a second well-marked peak with particles around 500  $\mu\text{m}$ , which was not observed for the extract with 50% EtOH. The increase of ethanol percentage in the extract may have favored particle agglomeration.

According to Rabelo et al. (2016) this can happen because in polar solvents the suspended macromolecules in the extracts are generally electrostatically more stable and less susceptible to agglomeration or precipitation. Once the polarity is reduced by an increase on ethanol concentration, a greater destabilization of the system occurs, favoring cluster formation. This explanation suggests that the extract containing 70% EtOH is the most likely to form clusters and promote fouling.

### 3.2. Direct process (UF and NF) of membrane concentration

#### 3.2.1. Retention of bioactive compounds

Differences in composition of total phenolic compounds and also in antioxidant capacity between feed, concentrate and permeate were significant ( $p \leq 0.001$ ) for the two evaluated process (UF and NF) and for both extracts (50 and 70% EtOH), indicating the good retention of evaluated membranes. However, in case of UF, the retention coefficients related to ABTS and ORAC are nearly independent on the percentage of EtOH. Similar behavior was observed to NF membranes considering the total phenolic compounds (TP) and ABTS. These results may be associated with the fact that these membranes, regardless of the solvent composition or the filtration process evaluated, have formed a cake layer on their surface during the filtration process that contributed to a very close retention of phenolic compounds.

Finally, comparing the processes was observed that NF showed higher retention coefficients compared to UF, as expected.

**Table 2**  
Characteristics of hydroalcoholic extracts from graviola leaves with 50% and 70% EtOH (v/v).

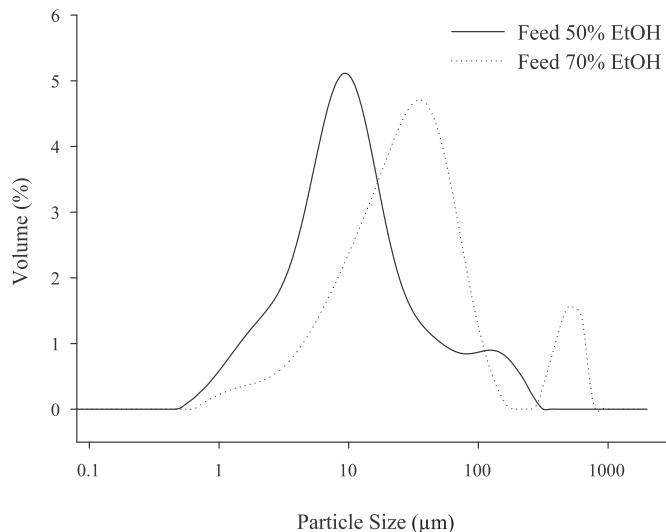
Parameters	Feed 50% EtOH	Feed 70% EtOH
Moisture (g/100 g, w.b.)	98.7 ± 0.5	98.6 ± 0.7
Ash (g/100 g, d.b.)	14.6 ± 1	11.6 ± 0.9
Protein (g/100 g, d.b.)	7.2 ± 1.7	10.2 ± 1.6
Lipids (g/100 g, d.b.)	15.4 ± 0.9	21.7 ± 1.1
Carbohydrates (g/100 g, d.b.)	37.7 ± 1.9	29.7 ± 1.6
Total solids (g/100 g, w.b.)	1.3 ± 0.1	1.4 ± 0.1
pH	6.1 ± 0.0	6.0 ± 0.0
Density (g/cm <sup>3</sup> )	0.93 ± 0.01	0.89 ± 0.01
Total phenolics (mg GAE/g, d.b.)	166.4 ± 8.9	132.8 ± 9.8
Antioxidant capacity ABTS (μmol TEAC/g, d.b.)	1516.0 ± 82.0	1088.4 ± 90.8
Antioxidant capacity ORAC (μmol TEAC/g, d.b.)	9256.1 ± 220.1	8683.8 ± 213.5

Data presented corresponds to an average of three determinations. w.b. = wet basis, d.b. = dry basis.

**Table 3**  
Retention coefficients of bioactive compounds of nano (NF) and ultrafiltration (UF) direct processes of hydroalcoholic extracts from graviola leaves with 50% and 70% of EtOH (v/v).

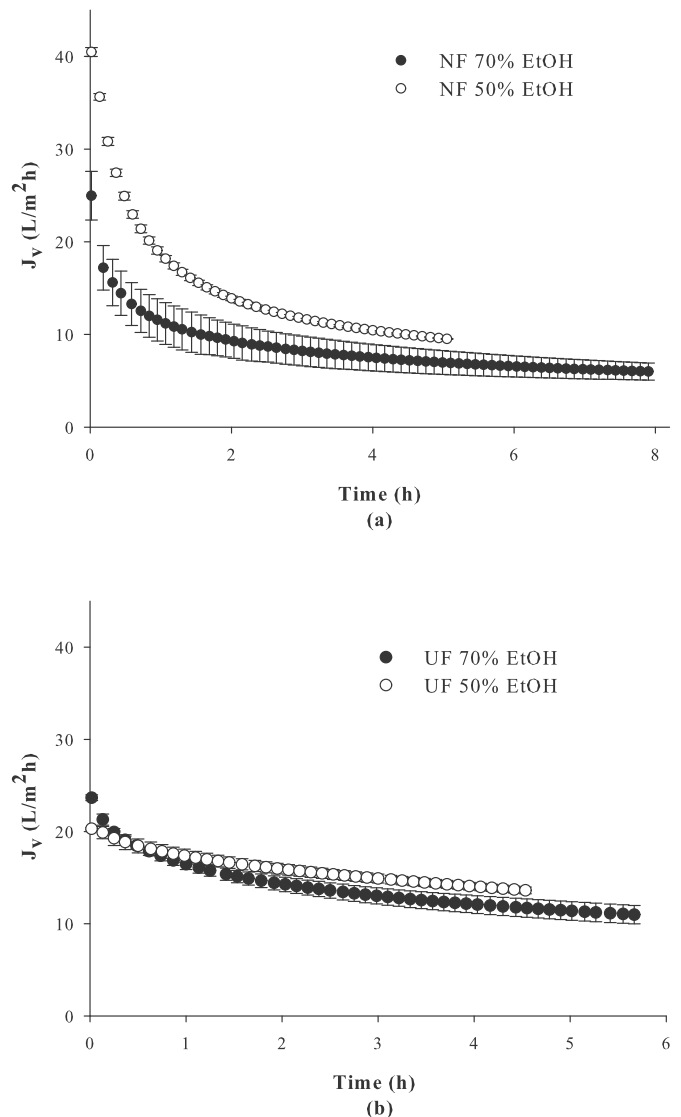
Direct process	Retention coefficients		
	TP (%)	ABTS (%)	ORAC (%)
NF 50% EtOH	97.9 ± 0.1 <sup>A</sup>	97.1 ± 0.3 <sup>A</sup>	96.5 ± 0.1 <sup>A</sup>
NF 70% EtOH	94.2 ± 3.2 <sup>AB</sup>	95.8 ± 0.7 <sup>A</sup>	91.8 ± 0.8 <sup>B</sup>
UF 50% EtOH	87.3 ± 0.4 <sup>B</sup>	86.8 ± 0.6 <sup>B</sup>	77.4 ± 2.0 <sup>C</sup>
UF 70% EtOH	75.1 ± 0.7 <sup>C</sup>	86.8 ± 1.0 <sup>B</sup>	77.2 ± 0.6 <sup>C</sup>

Values followed by the same letter in the same column are not significantly different at  $p \leq 0.05$ . TP = Total phenolics. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and ORAC (Oxygen Radical Absorbance Capacity) = antioxidant capacity measure methods.



**Fig. 2.** Particle size distribution of the feed extracts with 50% and 70% of ethanol.

Nevertheless, the high retention coefficients (above 75%) observed in the UF processes surprised us, since the compounds of interest in this work have a molecular mass between 100 and 700 g/mol. This is much smaller than the molecular mass cut-off of the membrane used (4,000 g/mol). This can be explained because phenolic compounds, which are responsible for antioxidant capacity, can be strongly bound to macromolecules found in a biological matrix, especially complex carbohydrates, thus hindering their passage through the membrane pores.



**Fig. 3.** Permeate fluxes of direct nanofiltration (3a, VCF = 1.7) and ultrafiltration (3b, VCF = 2.0) processes of extracts with 50% and 70% ethanol.

### 3.2.2. Permeate flux evaluation

Permeate flux curves in function of time (with standard deviations) are shown in Fig. 3(a) and (b).

The permeate flux profile obtained in UF (Fig. 3b) presented a



**Table 4**

Permeate fluxes evaluation and fouling index (FI) estimation of nano (NF) and ultrafiltration (UF) direct processes of hydroalcoholic extracts from graviola leaves with 50% and 70% of EtOH (v/v).

Direct process	Permeate fluxes		Fouling Index Estimation (FI)	
	Average (L/h.m <sup>2</sup> )	Decline (%)	FI	R <sup>2</sup>
NF 50% EtOH	7.1 ± 0.0 <sup>C</sup>	82.5 ± 0.3 <sup>A</sup>	0.2719	0.99
NF 70% EtOH	5.4 ± 1.0 <sup>D</sup>	80.2 ± 5.3 <sup>A</sup>	0.3106	0.96
UF 50% EtOH	12.1 ± 0.0 <sup>A</sup>	40.4 ± 0.4 <sup>C</sup>	0.1089	0.93
UF 70% EtOH	9.9 ± 0.2 <sup>B</sup>	57.9 ± 0.5 <sup>B</sup>	0.1671	0.98

Values followed by the same letter in the same column are not significantly different at  $p \leq 0.05$ .

smooth decline in comparison with NF. This result can also be observed in Table 4 when evaluating the percentage decline of permeate flux, and this profile suggests that a reduced number of particles were dragged into the UF membrane surface during the filtration process, which had a markedly less effect of concentration polarization and of fouling than the NF membranes (Table 4, fouling index). This behavior was already expected since the pressure applied to UF membranes is much lower than that applied to NF membranes.

In evaluating Table 4, the processes performed with the extracts containing 50% EtOH exhibited higher fluxes than those with 70% EtOH. The fact that hydrophilic membranes present higher fluxes with polar solvents is already well described in the literature (Yang et al., 2001; Bhanushali et al., 2001), but in the case of this study this fact was not the only reason to cause the observed difference, as it is possible to remark that the extracts with 70% EtOH were also the ones that presented the highest fouling index for both process (NF and UF).

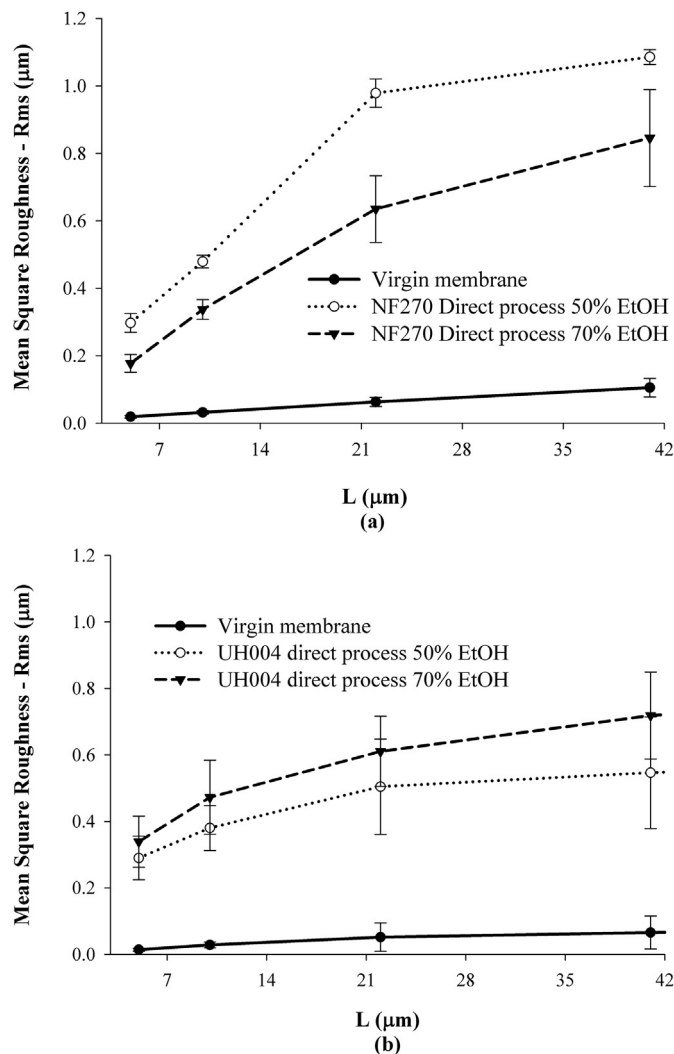
The fouling index measured in this work leads us to believe that particulate matter of the feed solution had great influence on the membrane fouling mechanisms. The extract with 50% EtOH produced smaller particles (around 10  $\mu\text{m}$ ) than extraction with 70% EtOH (around 35  $\mu\text{m}$  and 500  $\mu\text{m}$ ) (Fig. 2). Larger particles can increase fouling by cake formation, making the concentration difficult and reducing the flux. The effect of these bunched factors can be clearly evidenced by applying Hermia's blocking models (Hermia, 1982) (Tables 1 and 2 - Supplementary material) to predict fouling mechanisms. The analyses show that all fouling mechanisms were present during the UF and NF processes, and were dominated by cake formation, which showed the best fit ( $R^2 = 0.99$ ), followed by intermediate, standard and complete blocking of the pores.

### 3.2.3. Topography of membranes - AFM

Atomic force microscopy (AFM) was used to analyze the surface topography of the membranes. Fig. 4(a) and (b) show the variation of Mean Square Roughness (Rms) with the scale length (L) for the membranes before and after processes. Virgin membranes were conditioned in the same way as those used in filtrations.

It is important to emphasize that roughness is a measure that is directly linked to the scale; it means that the higher the scanned areas higher are roughness values (Boussu et al., 2005, 2007; Wong et al., 2009). Smaller areas of 5, 10, 22 and 41  $\mu\text{m}^2$  were analyzed from the images of a scanned area of 75  $\mu\text{m}^2$ .

NF and UF membranes had a roughness increase after processes (Fig. 4a and b), which is characteristic of the particles and aggregates' deposition on the membrane surfaces. There were not significant differences between membrane roughness for UF direct processes when 50% or 70% EtOH extracts were used. For NF, membrane roughness used in the filtration process of extracts with



**Fig. 4.** Mean square roughness (Rms) as a function of the scale length L (side of the scanned area in  $\mu\text{m}$ ) of NF (4a) and UF (4b) membranes, before and after filtration processes of extracts with 50% and 70% of ethanol.

70% EtOH was significantly higher than those used in the process with 50% EtOH. This is highly correlated to the greater fouling and lower permeate flux observed for processes with 70% EtOH.

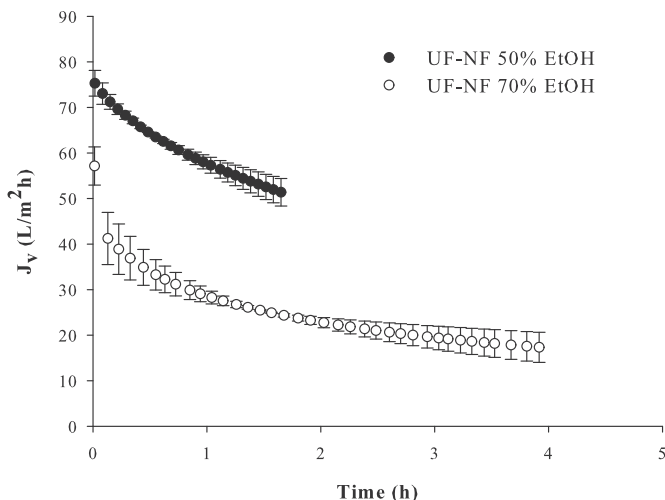
### 3.3. Combined process (UF-NF) of membrane concentration

Considering the possibility of obtaining a faster and more economic filtration process in this step of the work, the permeate stream of UF was concentrated by NF (UF-NF process). At first, differences in permeate fluxes and in compound retention related to the ethanol concentration in the extracts were evaluated. Then, different values of working pressure were evaluated from this result.

#### 3.3.1. Permeate flux and retention coefficients

The permeate fluxes of NF after UF were much higher than those observed in the direct NF processes (Fig. 5). When compared to direct filtrations, the average flux increased almost seven times for extracts with 50% EtOH, from 7.09 to 48.79  $\text{L h}^{-1}.\text{m}^{-2}$ , and almost three times for extracts with 70% EtOH, from 5.38 to 15.78  $\text{L h}^{-1}.\text{m}^{-2}$ .

The best fluxes and retentions were obtained for the extracts



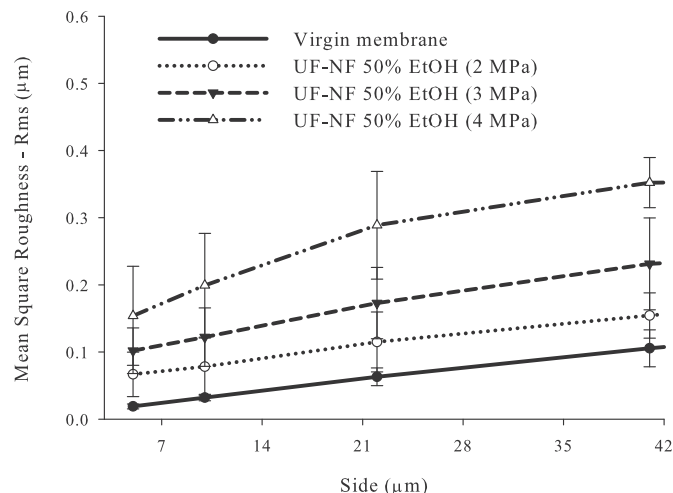
**Fig. 5.** Permeate fluxes of NF performed after UF (VCF = 2.3, 2 MPa) of the extracts with 50% and 70% ethanol as a function of time.

with 50% EtOH (Table 5). The average flux for extracts with 50% EtOH was more than 3 times higher than those with 70% EtOH. As stated earlier, the highest alcohol concentration in the extracts negatively affected the membrane filtration performance, increasing filtration time.

Regarding the retention coefficients (Table 5), firstly is important to highlight that the retention of bioactive compounds in the direct NF processes was significantly higher ( $p \leq 0.05$ ) than in the combined processes. Since the extracts feed into the system were previously ultrafiltered, there was not very much organic material being deposited on the membrane surface as was observed in the case of direct processes, in which cake formation contributed to a greater retention of the active compounds present in the matrix.

Still, it is possible to observe that there were not significant differences in total phenolic or antioxidant capacity measured by ORAC for the different extracts according to Student's t-test at  $p \leq 0.05$ . However, the retention observed for extracts containing 50% EtOH for antioxidant capacity measured by ABTS was significantly higher, considering the same significance level (Table 5).

A higher retention of phenolic compounds in more less



**Fig. 6.** Mean square roughness (Rms) as a function of the scale length L (side of the scanned area in  $\mu\text{m}$ ) of the NF membranes before and after the UF-NF process of extracts with 50% EtOH at 2, 3 and 4 MPa.

concentrations of ethanol was also observed in others studies (Pinto et al., 2014; Rabelo et al., 2016; Machado et al., 2013; Geens et al., 2005) which highlight the lower membrane solvation by EtOH when compared to water, causing a greater opening of pores, and resulting in lower retention of the compounds when this solvent is used in filtration with polymeric membranes of a hydrophilic nature. However, in this study, this evidence cannot be verified because only one of the three evaluated responses presented a significant difference between hydroalcoholic extracts with different concentrations of EtOH.

Other important information we can observe during filtration process was that EtOH rejection was not observed in any of evaluated experiment, which is in agreement with the results observed by Weinwurm et al. (2016).

### 3.3.2. Pressure variation

Trials were performed under 2, 3 and 4 MPa in order to establish the best operating pressure in NF step of combined processes. The permeate fraction from the UF process with 50% EtOH (extract that shows highest fluxes) were used as feed.

**Table 5**  
Retention coefficients of bioactive compounds and measured permeate fluxes of combined membrane processes (UF-NF) of hydroalcoholic extracts from graviola leaves with 50% and 70% EtOH performed at 2 MPa and 35 °C.

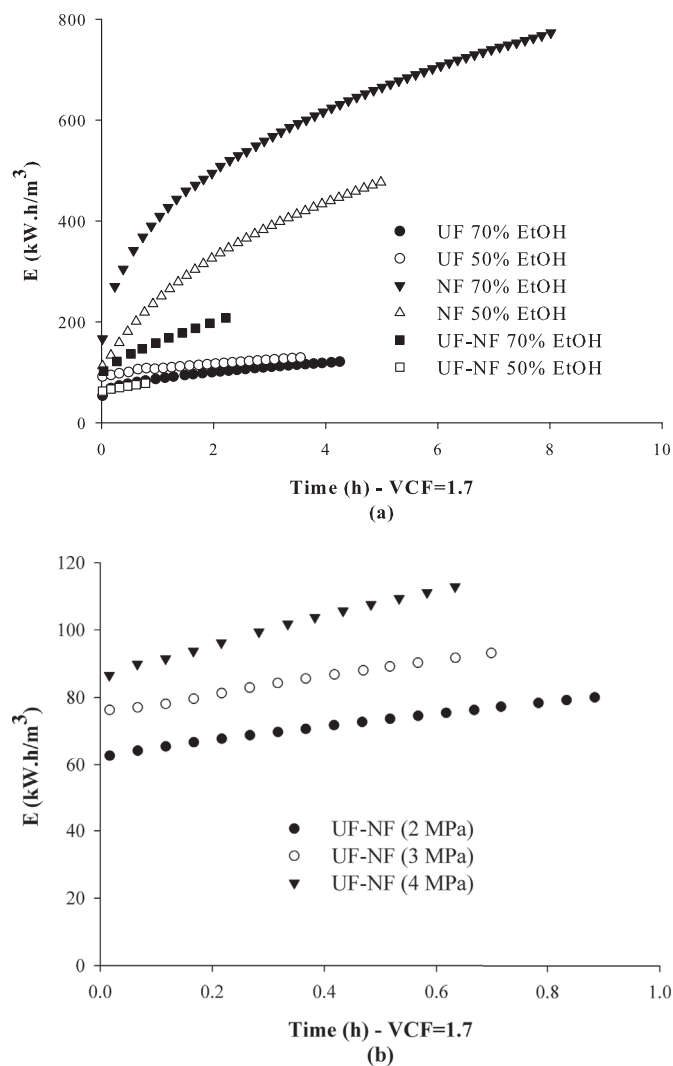
Combined process UF-NF	Permeate fluxes			Retention coefficients		
	Initial (L/h.m <sup>2</sup> )	Average (L/h.m <sup>2</sup> )	Decline (%)	TP (%)	ABTS (%)	ORAC (%)
50% EtOH	75.3 ± 2.8	48.8 ± 5.2	35.0 ± 9.4	77.6 ± 1.0	87.0 ± 0.6	74.9 ± 10.6
70% EtOH	57.1 ± 9.2	15.8 ± 4.1	64.6 ± 3.6	76.3 ± 4.6	59.8 ± 4.2	70.4 ± 2.8

TP = Total phenolics. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and ORAC (Oxygen Radical Absorbance Capacity) = antioxidant capacity measure methods.

**Table 6**  
Retention coefficients of bioactive compounds and permeate fluxes of hydroalcoholic extracts from graviola leaves with 50% EtOH performed at 2, 3 and 4 MPa, at 35 °C.

50% EtOH	Retention coefficients			Permeate fluxes		
	TP (%)	ABTS (%)	ORAC (%)	Initial (L/h.m <sup>2</sup> )	Average (L/h.m <sup>2</sup> )	Decline (%)
2 MPa	77.6 ± 1.0 <sup>A</sup>	87.0 ± 0.6 <sup>A</sup>	74.9 ± 10.6 <sup>A</sup>	75.3 ± 2.8 <sup>B</sup>	48.8 ± 5.2 <sup>B</sup>	35.0 ± 9.4 <sup>A</sup>
3 MPa	78.4 ± 1.2 <sup>A</sup>	86.3 ± 0.0 <sup>A</sup>	78.5 ± 0.1 <sup>A</sup>	92.8 ± 4.2 <sup>AB</sup>	66.4 ± 0.4 <sup>A</sup>	28.4 ± 3.7 <sup>A</sup>
4 MPa	79.8 ± 0.5 <sup>A</sup>	87.1 ± 0.2 <sup>A</sup>	77.8 ± 0.2 <sup>A</sup>	109.0 ± 0.9 <sup>A</sup>	70.0 ± 1.5 <sup>A</sup>	35.8 ± 0.9 <sup>A</sup>

Values followed by the same letter in the same column are not significantly different at  $p \leq 0.05$ . TP = Total phenolics. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and ORAC (Oxygen Radical Absorbance Capacity) = antioxidant capacity measure methods.



**Fig. 7.** Prediction of energy consumption of UF, NF and UF-NF membrane processes of hydroalcoholic extracts from graviola leaves with 50% and 70% EtOH as a function of time.

No significant differences in retention coefficients with pressure variation ( $p \leq 0.05$ ) were observed, as shown in Table 6. Combined processes exhibited high retention coefficients, but smaller than those obtained in direct filtrations. This is probably due to cake formation, which was considerably higher in the direct processes. Once the cake is formed, it acts as a selective layer, causing a greater retention of the compounds.

The increment in pressure led to a decrease in the process time as a consequence of an increase in the permeate flux, as expected in NF processes (Table 6). Average fluxes of processes carried out at 3 and 4 MPa were significantly higher ( $p \leq 0.05$ ) than those performed at 2 MPa, and the flux decline for the three evaluated pressures was moderate, with no significant differences between

**Table 7**  
Energy consumption of direct and combined membrane process to achieve a VCF = 1.7.

Direct process (2.0 MPa)	E (kWh/m <sup>3</sup> )	Time <sup>a</sup> (h)	Combined process	E (kWh/m <sup>3</sup> )	Time <sup>a</sup> (h)
UF 50% EtOH	129.3 ± 3.8	3.6	UF-NF 50% EtOH	80.4 ± 1.9	0.9
UF 70% EtOH	121.8 ± 6.2	4.3	UF-NF 3.0 MPa	93.1 ± 0.3	0.7
NF 50% EtOH	481.1 ± 2.2	5.1	UF-NF 4.0 MPa	113.4 ± 0.7	0.6
NF 70% EtOH	773.2 ± 5.3	8.0	UF-NF 70% EtOH	210.7 ± 5.4	2.3

<sup>a</sup> Process time.

processes.

### 3.3.3. Topography of membranes - AFM

The roughness of NF membranes after UF at 2, 3 and 4 MPa is shown in Fig. 6. In comparison with the result shown in Fig. 4, the roughness of these membranes was much lower than those observed in the direct NF. These results suggest that the use of ultrafiltration as a previous step to the concentration process of the extracts can increase the useful life of nanofiltration membranes.

In Fig. 6 we can observe also an increase in roughness values with the increase of pressure. This behavior is consistent with the mass transfer phenomena and is indicative of the non-occurrence of compaction on the membrane surface in the range of tested pressures.

### 3.4. Energy consumption

A prediction of the energy consumption of direct and combined systems was made according to Equation (6) in order to select the more productive filtration strategy for the hydroalcoholic extracts from graviola leaves in terms of yield and energy. Results for energy consumption are shown in Fig. 7(a) and (b).

As expected, UF processes conducted at 0.7 MPa had a significantly lower energy consumption compared to the NF processes. For both NF direct and combined processes, extracts with 70% EtOH spent much more energy than those with 50% EtOH. There were not significant differences for UF processes. Extracts with 70% EtOH spent about 62% more energy than extracts with 50% EtOH in the direct processes, and 1.6 times more time to achieve the same VCF (1.7). Such difference was about 38% and 2.6 times more time for UF-NF processes (Table 7). With the use of combined processes (UF-NF done sequentially) there was energy saving of approximately 43% compared to the application of direct NF, 209.7 kWh/m<sup>3</sup> versus 481.1 kWh/m<sup>3</sup> for extracts with 50% EtOH, and 332.5 kWh/m<sup>3</sup> versus 773.2 kWh/m<sup>3</sup> for extracts with 70% EtOH.

The low permeate fluxes obtained in the filtrations of extracts containing 70% EtOH resulted in an increased energy consumption. In UF-NF processes with 50% EtOH, the energy consumption was 17% and 40% higher for processes performed at 3 and 4 MPa, respectively, than for the ones carried out at 2 MPa (Table 7).

Energy consumption increased with pressure (Fig. 7b). The average energy consumption was 80.41 kWh/m<sup>3</sup> for processes performed at 2 MPa, 93.1 kWh/m<sup>3</sup> at 3 MPa and 113.4 kWh/m<sup>3</sup> at 4 MPa. These results are important when considering the scale-up to commercial units. Other costs inherent to the process should also be considered, such as the energy needed to reduce the particle size of the leaves prior to extraction and solvent recovery.

## 4. Conclusion

Combined UF-NF processes proved to be effective in separating phenolics from hydroalcoholic extracts from graviola leaves to obtain enriched fractions. This sequential design resulted in higher fluxes of NF, the fluxes increased almost seven times for extracts with 50% EtOH, and almost three times for extracts with 70% EtOH when compared to direct processes. The retention of the permeated



compounds on the UF membrane which were concentrated by nanofiltration was higher than 75% for extracts with 50 or 70% ethanol indicating the potential of this strategy, in detriment of the direct NF process.

The highest ethanol concentration in the extracts negatively affected the flux permeation performance, but had little influence on rejecting the compounds of interest. The pressure variation in the NF step conducted with extracts containing 50% EtOH also did not result in significant differences in the retention of bioactive compounds.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jclepro.2017.11.062>.

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