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SEPARATION OF POLYPHENOLIC COMPOUNDS BY ULTRAFILTRATION OF BORDO GRAPE (*Vitis labrusca* var. Bordo) SKIN EXTRACT

SEPARACIÓN DE LOS COMPUESTOS FENÓLICOS PROVENIENTES DEL EXTRACTO DE LA CÁSCARA DE LA UVA BORDO (*Vitis labrusca* var. Bordo) POR ULTRAFILTRACIÓN

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Abstract
Separation of the phenolic compounds from the acidified aqueous extract with 2% (w/v) citric acid from Bordo grape skin was carried out through ultrafiltration using polyestersulfone membranes with molecular weight cutoff 10 and 30 kDa. The performance of the membranes at different transmembrane pressures of 1.0 to 3.5 bar and temperatures of 25, 35 and 45 °C showed better separation conditions at 25 °C and 3.5 bar of transmembrane pressure. The results showed hydraulic permeability for the new membranes of 10 and 30 kDa of 10.96 and 20.52 $L.m^{-2}.h^{-1}.bar^{-1}$, respectively. The highest membrane resistance was the polarization by concentration, compared with the total resistance, from 80 to 90% and from 60 to 90% for the 10 kDa and 30 kDa membranes, while the fouling resistance was the lowest, from 1 to 3% and 1 and 16% in membranes of 10 and 30 kDa.
Keywords: ultrafiltration, membranes, fouling, polypheno.

Resumen
Fue estudiado la separación de los compuestos fenólicos a partir del extracto, previamente acidificado con ácido cítrico, de la cáscara de la uva Bordo por ultrafiltración, utilizando membranas de poliestersulfona con peso molecular de corte de 10 y 30 kDa. A través de los rendimientos obtenidos de las membranas, a las presiones de transmembrana de 1,0 a 3,5 bar y temperaturas de 25, 35 y 45 °C, se observó que la mejor condición de separación correspondió a 3.5 bar y 25 °C. Los resultados de permeabilidad hidráulica para las membranas nuevas de 10 y 30 kDa fueron de 10.96 y 20.52 $L.m^{-2}.h^{-1}.bar^{-1}$, respectivamente. La mayor resistencia fue debida a la polarización por concentración, que cuando comparado con la resistencia total, fue de 80 a 90% y de 60 a 90% para las membranas de 10 kDa y 30 kDa; mientras que la resistencia por *fouling* fue la menor con valores de 1 a 3% y de 1 a 16% para las membranas de 10 y 30 kDa, respectivamente.
Palabras clave: ultrafiltración, membranas, fouling, polifenol.

1 Introduction

Grape is a fruit rich in phenolic compounds that are mainly distributed in skin, stems, leaves and seeds (Makris *et al*., 2008). Grapes and their industrialized products are widely consumed and present high concentrations of phenolic compounds, which are mainly responsible for the antioxidant activity of fruits (Fu *et al*., 2011; Katalinić *et al*., 2010; Kuck and Noreña, 2016).

From pomace, one of the main by-products of winemaking and juice can be obtained bioactive compounds because it retains significant quantities of flavonoids, stilbenes, phenolic acids and a large variety of tannins and proanthocyanidins (Cirqueira *et al*., 2017).

Considering the importance of phenolic compounds as potential antioxidants and their presence in the by-products of the juice and grape wine industry, it turns out important to separate them through the Ultrafiltration process (UF).

Between the advantages of using UF we can state the possibility of elimination of the conventional clarification step, since it does not require the use of heat, there is no change of phase or pH,

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and avoid the use of chemical agents (Rezzadori *et al*., 2014). Therefore, the separation involving thermolabile compounds are favored, preserving the organoleptic and nutritional properties of the fresh product, significant for the final quality of the product (Carneiro *et al*., 2002; Cassano *et al*., 2007). Besides, the membrane separation processes are compact and easy-to-scale systems, offering the possibility of use in continuous systems and can be combined with other separation processes (Kelly *et al*., 2000). In the juice and wine clarification industry the UF method is studied in the removal of proteins and phenols responsible for the turbidity and instability of juices and wines, because it allows to work at room temperature, preserving in the final product the thermolabile compounds (Wilson *et al*., 1983; Vaillant *et al*. 1999). Phenomena derived from the process that causes a reduction of the permeate flux and deterioration in the membranes such as polarization by concentration, polarized gel layer and *fouling* have been found during those studies. Those phenomena were found in UF studies with grape juice and wine by (Rektor *et al*., 2004; Cassano *et al*., 2008; Fernández *et al*., 2015).

In this work, the influence of temperature and transmembrane pressure parameters was studied using polyestersulfone of 10 and 30 kDa flat membranes with the aim of separating phenolic compounds from Bordo grape skin extract.

2 Materials and methods

2.1 *Extract of Bordo grape skin*

Bordo Grape, from the municipality of Cotiporã, RS, Brazil, was used for the preparation of grape skin extract. The bunches were washed with drinking water, selected, packed in polyethylene bags and stored at -18 ± 2 ºC until use. For the preparation of the extract, after unfreezing the grapes were subjected to blanching in a thermostatic bath for five minutes at 80 °C and immediately cooled for 3 minutes in an ice bath (Agüero *et al*., 2008). The grape skin was then separated and subjected to leaching in aqueous solution acidified 2% citric acid in a ratio of 1:4 (m/v) for 20 hours at 20 °C. After that period, the solution was vacuum filtered through Whatman No.1 filter paper, and the extracts were packed in hermetically sealed amber glass bottles and stored at -18 ± 2 °C.

2.2 *Separation by ultrafiltration*

Ultrafiltration (UF) polymeric membranes were used with a molecular weight cut off of 10 and 30 kDa, made of polyethersulfone (Synder Filtration Headquarters, Vacaville, USA). The ultrafiltration equipment implemented has as main components a pre-filter with 5 μm pore size membrane, pneumatic pump (Ingersoll-Rand, model PD05P-ARS-PAA-B) with the nominal flow of 54.5 L.min^{-1} and pressure Maximum of 6.9 bar, with a module for flat membrane manufactured in stainless steel 316 and membrane surface area of 0.008903 m^2.

2.3 *Experimental procedure*

For the separation by UF, the new membranes were initially compacted by recirculating distilled water through the imposing of a transmembrane pressure (TMP) of 4 bar at 25 °C, to achieve the microstructure (Rai *et al*., 2007). The compression process was carried out by the permeate flow of water became constant eventually. Flow of water was calculated per the methodology described by (Cheryan, 1986; Mulder, 1996; Jiraratananon and Chanachai, 1996). After membrane compaction, the permeability of the new membrane (L.m^{-2}.h^{-1}.bar^{-1}) was determined by measuring permeate flux at different TMP (4.0, 3.0, 2.5, 2.0, 1.5 and 1.0 bar). The permeability was calculated from the angular coefficient of the linear equation obtained from the permeate flux data as a function of TPM (Czekaj *et al*., 2001). The same procedure was used to determine the hydraulic permeability with water, after the ultrafiltration of the extract (L.m^{-2}.h^{-1}.bar^{-1}) and cleaning of the system (L.m^{-2}.h^{-1}.bar^{-1}).

Prior to the membranes separation process, the extracts were unfrozen under refrigeration at a temperature of 4 ± 1 °C. The experiments of separation were carried out according to the methodology proposed by Cassano *et al*. (2008) in batch mode, with total recirculation of the retentate flow and separation of the permeate, with a flow rate of 125 L.h^{-1} at temperatures of 25, 35 and 45 ºC. The collected permeate volumes were stored at -18 ± 2 °C for further analysis. After separation, the volume corresponding to the retentate was withdrawn and replaced with distilled water to begin the cleaning of the system. After that, a chemical cleaning of the membrane was performed and finally rinsed with water, to make again the compaction with water.

2.4 *Determination of fouling, and recovery of the membrane flow*

With the results obtained in the experiments of permeability of the water of the new membrane, before and after each experiment of UF, it was possible to calculate the fouling Equation (1):

$$Fouling(\%) = \left(\frac{L_{PN} - L_{PE}}{L_{PN}}\right) \times 100 \quad (1)$$

Where L_{PN} is a hydraulic permeability of the new membrane and L_{PE} is the hydraulic permeability of the membrane after the process of UF of the extract before making the chemical cleaning (Saha *et al*., 2007).

The flow recovery was calculated from the Equation (2) (Liikanen *et al*., 2002):

$$Rec.\ of\ flux(\%) = \left(\frac{J_{PCh}}{J_{PN}}\right) \times 100 \quad (2)$$

Where J_{PCh} (L.m^{-2}.h^{-1}) is the permeate flow of water after chemical cleaning of the membrane and J_{PN} (L.m^{-2}.h^{-1}) the permeate flow of water from the new membrane.

2.5 *Analysis of the resistances*

The resistances corresponding to each one of the phases of the separation cycle: new membrane, after separation of the extract, and after chemical cleaning were determined according to the serial resistance model (Jiraratananon and Chanachai, 1996; Cheryan, 1998; Bruijn *et al*., 2002), whose equations to calculate the total resistance (R_T, m^{-1}), the resistance of the new membrane with water (R_M, m^{-1}), resistance to polarization by concentration (R_C, m^{-1}) and resistance to *fouling* (R_F, m^{-1}) were described by Cassano *et al*. (2007).

Equation (3) represents the total resistance that considers all resistive effects as a sum of components (Jiraratananon and Chanachai, 1996; Cassano *et al*., 2007):

$$R_T = R_M + R_C + R_F \quad (3)$$

In order to determine those resistances, the following equations were implemented (Cassano *et al*., 2007):

$$R_M = \frac{1}{\mu_W L_{PN}} \quad (4)$$

Where μ_W is the viscosity of the water (kg.m^{-1}.s^{-1}) and L_{PN} (L.m^{-2}.h^{-1}.bar^{-1}) is the hydraulic permeability of the new membrane.

$$R_T = \frac{1}{\mu_W L_{PE}} \quad (5)$$

Where L_{PE} is the hydraulic permeability of the membrane (L.m^{-2}.h^{-1}.bar^{-1}) after the process of UF of the extract and μ_W is the viscosity of the water (kg.m^{-1}.s^{-1}).

$$R_M + R_F = \frac{1}{\mu_W L_{PCh}} \quad (6)$$

Where L_{PCh} is the hydraulic permeability of the membrane after the chemical cleaning (L.m^{-2}.h^{-1}.bar^{-1}).

The resistance by concentration R_C was calculated by difference of the total resistance from the others through the equation (3).

2.6 *Viscosity*

Viscosity was measured with a rotational viscometer (Brookfield, DV2T), provided of a small sample adapter, that consists of a precision cylindrical sample chamber and spindle rotating at 60 rpm, and with circulating water bath for temperature control at 20 ºC (Aguilo-Aguayo *et al*., 2010).

2.7 *Total polyphenol content*

It was determined according to the colorimetric method of Folin-Ciocalteau (Singleton and Rossi, 1965). The calibration curve was elaborated using a standard solution of gallic acid (0 to 40 mg.mL^{-1}). The readings were performed in a spectrophotometer (Genesys S10, Thermo Scientific) at an absorbance of 765 nm. The results were expressed as mg of gallic acid equivalent (GA).g^{-1} of the sample.

2.8 *Flavonoid content*

It was determined according to the methodology proposed by Dewanto *et al*. (2002). The calibration curve was elaborated using a standard solution of catechin (0 to 4 mg.mL^{-1}). The readings of the absorbance were performed in the spectrophotometer (Genesys S10, Thermo Scientific) at an absorbance to 510 nm. The results were expressed as mg of catechins equivalent (CEQ). g^{-1} of the sample.

2.9 *Statistical analysis*

For data analysis was used a factorial experiment, where two factors: membrane molecular weight cut off (10 and 30 kDa) and temperature (25, 35 and 45 ºC) were evaluated. Analysis of variance (ANOVA) was performed with the Program SAS 9.3.

For comparison of the difference between treatment means, the test of Tukey was used.

3 Results and discussion

3.1 Compaction and permeability of the UF membranes

It was found that under compaction conditions of 4.0 bar at 25 °C, the initial permeate fluxes were 75.5 and 58.5 $L.m^{-2}.h^{-1}$, with a reduction in the permeate flux 6.3 and 40.8% until reaching the conditions of Steady-state flux for the membrane of 30 and 10 kDa, respectively. That observed behavior is typical in membrane compaction, and the greater independence of the permeate flux with the membrane time of 30 kDa might indicate a greater mechanical stability of the membrane when comparing to the 10 kDa membrane (Cheryan, 1998). The hydraulic permeability of 10.96 and 20.52 $Lm^{-2}.h^{-1}.bar^{-1}$ were determined for the membranes 10 and 30 kDa, and the higher permeability is a consequence of the larger pore size. Hydraulic permeability, according to the Hagen-Poiseuille equation depends on various parameters, between them porosity, membrane thickness, tortuosity and pore size, and therefore their value is characteristic of each membrane (Cheryan, 1986). However, Mulder (1996) states that permeability depends not only on membrane characteristics, such as material and morphology but also on temperature.

3.2 Study of the flux behavior of the grape skin extract at different pressures and temperatures

The permeate flux increased with the pressure, and for pressures greater than 2.5 bar that increase was higher than the lower pressures. According to Cheryan (1998), the increase in the transmembrane pressure tends to increase the flow up to the consolidation of the gel layer, after the flow becomes independent of the pressure, just increasing the thickness or the density of the layer. For fluids that do not contain components that cause clogging in the membranes, high pressures may increase the permeate flow value. Although, in cases which the polarized layer and clogging are easily formed, the increase in pressure results in the compaction of the particles on the surface of the membrane. This leads to a steady flow or even the reduction of the flow with increasing pressure, as higher pressures can lead to compaction and therewith reducing the permeate flows. According to Cheryan and Alvarez (1995), this behavior is due to the consolidation of the polarized gel layer, which in the case of grape juice, is formed due to the pectin present in it, once the pectin has a great potential to form gels. Then, in the membrane separation process, the gel layer is formed on the membrane, which is compressed by the pressure resulting in a decrease of the permeate flux values. In addition, the membrane is exposed to macromolecules that may be present in the grape extract such as proteins, carbohydrates and lipids, molecules that can be quickly adsorbed in the surface of the membranes or inside of the pores (Carneiro *et al*., 2002). The accumulation of those molecules on the surface of the membrane generates a gradient of concentration that can initially encourage the polarization by concentration and cause the back diffusion of the molecules towards the circulating fluid until reaching the quasi-stationary state (Marshall and Daufin, 1995). Thus, permeate flux reduction is due to polarization by concentration, *fouling* and/or blocking of membrane pores (Carneiro *et al*., 2002).

In general, an increase in permeate flux was achieved with an increase in transmembrane pressure. In this model, the flux is directly proportional to the transmembrane pressure, considering that the characteristics of the membrane are not altered, a change in the permeate flux at a certain pressure occurs due to the change in viscosity, which is influenced by the accumulation of solids and by the temperature where a higher concentration of the retained species occurs near the surface of the membrane until a decrease in the flux reaches its limit value (Campos *et al*., 2013). However, as the pressure increases the permeate flux ceases to be linear and tends to decrease its rise until it stabilizes. The consequence is that the permeate flux remains unchanged with increasing pressure. This phenomenon occurs due to the polarization by concentration that is formed on the surface of the membrane (Campos *et al*., 2013). In the 10 kDa membrane, for greater pressures than 2.5 bar no more linearity between the permeate flux and the applied pressure, but it was not possible to verify the existence of limit flux, now that it was not possible to verify the stationary state for pressures greater than 3.5 bar, due to the equipment's operational limitations. Accordingly, it was defined to conduct the UF experiments on the 10 kDa membrane with operating conditions of 3.5 bar. In the 30 kDa membrane, the increase in the

permeate flux was evidenced, and under the pressure conditions employed, there was no tendency for the flux independence with respect to the transmembrane pressure, thus demonstrating that the limit flux was not reached, being also chosen the pressure of 3.5 bar as operating pressure, protecting the membrane from the factors that affect its performance as *fouling* and polarization by concentration.

With respect to effect of the temperature, in the 10 kDa membrane, the permeate flux at steady state increased from 1.75 to 2.6 $L.m^{-2}.h^{-1}$ with temperature, and this phenomenon can be attributed to that constant pressure, with the increasing of the temperature to the density and viscosity of the fluid decrease, with the consequent increase of the flux (Cheryan, 1998). The viscosity values in membrane retention of 10 kDa were from 1.35 to 25 ºC, 1.30 to 35 °C, and from 1.20 mPa.s to 45 ºC. Cassano *et al* (2008) in the UF study of 100 kDa membrane grape pomace extract also observed a stationary-state increase of 52% in flux when the temperature increased from 15 to 39 °C.

In the 30 kDa membrane, permeate flux values under stationary conditions were 2.7, 3.8 and 3.7 $L.m^{-2}.h^{-1}$ to 25, 35 and 45 °C, respectively. The permeate flux increased as the temperature increased from 25 to 35 ºC, but when the temperature changed from 35 to 45ºC the permeate flux value had a slight decrease with respect to the flux to 35°C. That decrease in the permeate flux by increasing the temperature at 45°C, may be because in less viscous media the particles are loaded more easily by the juice (the values of viscosity in the retained were 1.36, 1.34 e 1.27 mPa.s a 25, 35 e 45ºC, respectively), and with the increase in temperature the viscosity of the medium decreased, and with the increase of the pore size, it facilitates the access of these particles through the pores, resulting in the closure of these and consequent reduction of flux. According to the Hagen-Poiseuille model, increasing the temperature results in an increase in flux, but in some suspensions, it could result in a decrease in flux due to the decrease in the solubility of some components and the precipitation of the insoluble salts and, in that way, aggravate the *fouling* and the deterioration of the membrane, with the consequent reduction of permeate flux (Cheryan, 1986). In some biological systems, it might happen that because of the increase in temperature there may be protein denaturing and other damage, such as changes in membrane structure that may result in *fouling* (Cheryan, 1986). Maubois (1980) also informed that in the UF of whey, that with the increasing of temperature the permeate flux decreased due to the decrease of the solubility of feed components at high temperatures. Goosen *et al*. (2002) informed that the porosity of the polysulfone membranes may be very sensitive to changes in the temperature of the feed solution. Campos *et al*. (2013) and Watkinson and Wilson (1997) mention that temperature is a very important variable that influences the appearance of *fouling* of the membrane, because depending on the components of the feed solution, could change the characteristics of the fluid getting to produce deposits. Jiraratananon and Chanachai (1996) also, reported that in passion fruit juice, the results showed that the flow increased with the temperature from 30 to 40 °C, and decreased to 50 °C.

Analyzing those results and considering the degradation of the phenolic compounds due to the increase in temperature and the energy costs to operate at higher temperatures, it was defined as separation parameters by UF 25 °C and 3.5 bar.

3.3 Determination of fouling and efficiency of the chemical cleaning

Table 1 shows the recovery and fouling percentages in the membranes for the three temperatures studied. The results indicate that the 30 kDa membrane was the one that has the highest significant recovery (89.4%) at 35ºC, after chemical cleaning (p <0.05). Nevertheless, at 25 ºC it was possible to verify a good recovery in the two membranes, showing that effects such as concentration polarization or reversible fouling were possible to be removed by chemical cleaning. According to Wiley (2007), the polarization by concentration causes the accumulation of particles or solutes, forming a boundary layer to the transfer of mass adjacent to the surface of the membrane, being that phenomenon unavoidable, but can be reversible by chemical cleaning of the membrane. Rodrigues *et al*. (2003) evaluated the ultrafiltration parameters of banana juice in 10 and 30 kDa polyestersulfone membranes and found that the chemical cleaning of the membrane allowed the recovery of the initial permeate flux in a 75%, being an indicative of the possibility of reuse of the membrane.

As far as *fouling* had values higher than 80%, for both membranes and for all conditions. The recovery of the permeate flux value that has been lost because of *fouling* and polarization by concentration only occurs with chemical cleaning (Cheryan, 1998) or membrane exchange.

Table 1: Calculated values of fouling (%) and recovery of the flow (%) in the membrane of 10 and 30 kDa at different temperatures.

	10 kDa		30 kDa	
Temperature (°C)	% Fouling	% Recovering	% Fouling	% Recovering
25	$85.9^{A,B}$	63.1^{c}	89.6^{A}	80.9^{b}
35	82.3^{B}	19.6^{e}	82.3^{B}	89.4^{a}
45	80.3^{B}	20.6^{e}	88.6^{A}	54.5^{d}

Same letters indicate no significant difference (p >0.05).
Capital letters and lowercase letters for % Fouling and % Recovering, respectively.

Table 2: Contribution of each resistance with regard to the total resistance in the membranes of 10 and 30 kDa at 25 ºC (tangential velocity of 0.54 m.s^{-1}, and flow rate of 125 L.h^{-1})

	10 kDa			30 kDa		
Pressure (bar)	R_M/R_T (%)	R_F/R_T (%)	R_C/R_T (%)	R_M/R_T (%)	R_F/R_T (%)	R_C/R_T (%)
1	9.2	1.1	89.6	37.6	9.7	62.4
2	11.1	2.1	87	16.3	7.2	83.9
2.5	10.1	2.4	87.6	15.3	1.6	85
3	15.9	2.8	80.7	16.7	1.9	83.1
3.5	16.5	2.9	80.5	14	15.2	86

R_M: New membrane resistance; R_T: Total resistance; R_F: Resistance to fouling; R_C: Resistance to polarization and concentration.

Borsi *et al*. (2012) also mention that organic *fouling* is related to the reduction of permeability caused by the accumulation of dissolved or colloidal organic compounds on the surface of the membrane, and that the high *fouling* percentages, even at low pressures, may be due to membrane hydrophobicity, which has a significant tendency to adsorption on the surface, provoking the cake layer formation.

According to Cheryan (1986), if the formation of solids on the membrane is significant, it can act as a secondary membrane and change the permeation and transport properties of the system. *Fouling*, in the case of fruit juice, can be caused by pectin, tannins, proteins, starch, hemicellulose and cellulose (Carneiro *et al*., 2002).

The factors that affect permeate flux in UF are strongly influenced by the chemical nature of the membrane and its membrane-solute, chemical-solute surface, solute-solute interactions are as much as the operating conditions of the equipment (Cheryan, 1986). In the case of this study, in terms of the conditions of operation, the limits of the trans-membrane pressure of 3.5 bar, the low tangential velocity of 0.54 m.s^{-1} and the small membrane area were limited. The tangential velocity to the module promotes the near-membrane turbulence required to entrain the solid particles that would tend to settle on the membrane surface and reduce the polarization effects by concentration. Cheryan (1986) illustrates that the most effective way of minimizing *fouling* and the factors that affect the permeate flux depends on the nature of the feed solution and the treatment practiced before the separation membrane process, the equipment used in the experiments of this study has a size pre-filter of 5 μm filter pore, which helps to protect the membrane by reducing the amount of suspended solids. Cheryan (1986) also tells that proper handling of operating conditions such as temperature, feed rate and pressure is important, and controlling such variables can minimize effects that affect membrane performance such as *fouling* and polarization by concentration.

3.4 Analysis of the resistance to flux during the UF

Table 2 shows the contribution of each of the resistances in the total, considering the serial resistances model. It is observed in it that the greatest contribution was given by the resistance to polarization by concentration (from 60 to 90%), while the resistance to *fouling* was the lowest (from 1 to

16%).

The predominance of concentration polarization is characteristic in fruit juices, which consist mainly of polysaccharide compounds of the cell wall, such as lignin, pectin, cellulose and hemicellulose (Ryu *et al*., 1986). Turbidity in grape extract occurs due to the presence of suspended substances contained therein, such as lipids, starch, cellulose, tannins and especially pectins (Campos *et al*., 2016). In the experiments of this work, preliminary filters of 11 μm (Whatman nº1 filter paper) and the 5 μm micro filter installed in the equipment) were used as preliminary stages to the ultrafiltration, approximately separating particles larger than 500 kD, in order to preserve the efficiency of the membrane, minimizing cake formation and polarization by concentration, so that, taking into account the particle sizes of the substances contained in the extract and the sizes of the pre-filters, it is possible to separate the pre-filters with starches and cellulose (Machado *et al*., 1999).

In ultrafiltration, it is possible to separate present macromolecules with molar masses greater than 10 or 30 kDa depending on the used membrane, such as pectic substances and proteins of the grape. However, particles of phenolic compounds such as tannins (0.5 to 3 kDa), catechins (0.29 kDa), resveratrol (0.22 kDa), gallic acid (0.30 kDa), ellagic acid (0.30 kDa) and quercetin (0.30 kDa) (whose molar mass values, on average), were obtained from (Pubchem, 2017), they would all pass through the pores of the two membranes.

Depending on the particle size distribution of the product to be ultrafiltered and the pores of the membrane, a complete or partial blocking thereof may occur, with consequent formation of a secondary layer, which then becomes the filter medium itself, then limiting the step of the particles of smaller compounds. Those mechanisms directly influence the permeate flux of the membrane (Campos *et al*., 2013). In the case of grape skin extract, the presence of pectins encourage the formation of a polarized gel layer, since pectin has a great potential for gel formation (Campos *et al*., 2013), and at pHs lower than 3.5 the tendency to form gels is greater. In our experiments, the extract presented pH values lower than 2.8.

Reversible *fouling* is characterized by the recovery of the membrane permeate after the chemical cleaning operation, while the irreversible is permanently presented because it causes changes in the membrane structure and does not allow the recovery of water flow (Maartens *et al*., 2002). In this way, the fouling of 1-16% may correspond only to the irreversible fouling component, which was not possible to recover by chemical cleaning of the membrane. In the ultrafiltration of enzymatically hydrolyzed kiwi juice, Cassano *et al*. (2007) found that the reversible fraction of *fouling* resistance was 29.4% of the total resistance, while the irreversible fouling fraction was 2.75%.

Habert *et al*. (2006), mention that concentration polarization is a reversible phenomenon that occurs in the first few minutes of filtration, where a concentration profile will occur perpendicular to the membrane surface, resulting in an increase in the concentration of retained species near the surface of the membrane. The establishment of a gradient of concentration causes additional resistance to mass transfer, leading to a decrease in permeate flux.

3.5 Content of total polyphenols and flavonoids

The concentration of polyphenols in the extract was 10.89 (mg GA/g d.b.), lower values than the reported for the extract of Bordo grape skin by Kuck and Noreña (2016) of 26.26 (mg GA/g d.b.). Pereira *et al*. (2011) reported levels of 2.46, 2.22, 1.43 and 1.59 mg gallic acid/g) in grape skins of the Isabel, Niagara, Benitaka and Brazil varieties, respectively. Soares *et al*. (2008) reported values close to 1.93 mg GA/g using acetone as solvent at different concentrations of Isabel and Niagara grape skins extracts. These contents can be compared to the other fruit such as strawberry, which contains 3.2 (mg GA)/g (Luna-Ramírez *et al*., 2017). For polyphenols and flavonoids, the type of membrane did not affect significantly the separation of these compounds, but the temperature was significant in the retentate (p <0.05). In the case of membrane used, the results indicated that is recommendable to use membranes with lower molecular weight cutoff, in order to get the higher amount of polyphenol compounds.

In Figures 1A it is observed in the membrane of 10 KDa, that in the retentate at temperatures higher than 25 °C the concentration of polyphenols decreased significantly, and in the permeate, the concentrations were significantly lower than in the retentate (p <0.05). In the meantime, in the 30 kDa membrane (Figure 1B), it can be observed the retention with the increase in temperature of the concentration of polyphenols increased significantly (p <0.05), but in the permeate, the temperature did not influence the concentration of the polyphenols (p >0.05).

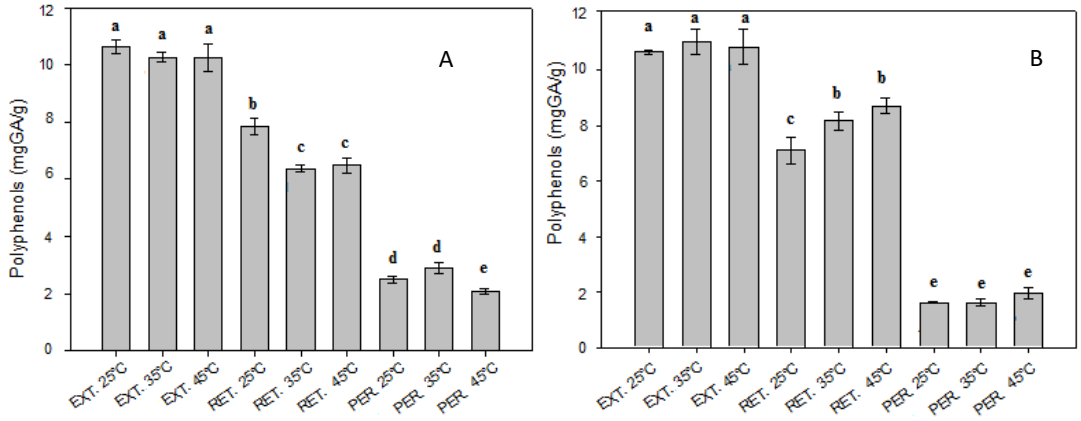

Fig. 1: Polyphenols concentration (mg gallic acid equivalent/g dry basis) in the extract (EXT), retentate (RET) and permeate (PER) in the UF to 25, 35 and 45 ºC in the membrane of 10 kDa (A) and of 30 kDa (B). Same lowercase letters indicate no significant difference (p >0.05).

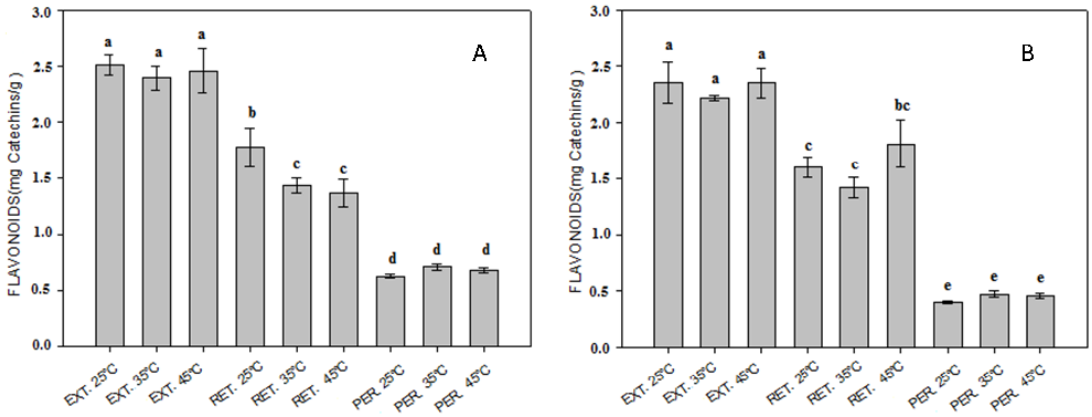

Fig. 2: Flavonoids (mg catechins/g dry basis) in the extract (EXT), retentate (RET) and permeate (PER) in the UF to 25, 35 and 45 ºC in the membrane of 10 kDa (A) and of 30 kDa (B). Same lowercase letters indicate no significant difference (p >0.05).

With respect to the flavonoids content, the extract had a concentration of 2.42 mg of catechins/g d.b. Katalinić *et al*. (2010) in the study of 14 species of Vitis vinifera varieties in Croatia, obtained flavonoids contents of 0.47 and 0.77 mg equivalents catechins (CE)/g for white and red grapes. Values of 0.98 to 3.02 mg catechin/g were found by Yang *et al*. (2009) in several varieties of grapes grown in New York (*Vitis vinífera*). These found differences in relation to other studies may be due to the extraction method implemented, the varieties, as well as the cultivation method.

As for the extractions (Figures 2A and 2B), the flavonoids contents were significantly higher in the retentate than in the permeate in all cases (p <0.05). It was also found that the temperature did not significantly influence the permeate separation (p >0.05).

Conclusions

The percentages of *fouling* were of 85.9 and 89.6% for the membranes of 10 and of 30 kDa, respectively, and after the chemical cleaning the membranes resulted in a flow recovery of 63.2 and 80.9%.

From the analysis of the flow resistance, it was verified that the greatest contribution of resistance was given by the resistance to the polarization by concentration (from 60 to 90% of the total resistance),

while the resistance caused by fouling was the lowest (from 1 to 16% of the total resistance).

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