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The use of nanofiltration membranes for the fractionation of polyphenols from grape pomace extracts

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ABSTRACT

Filtration experiments in batch concentration mode (with recycling of the retentate stream) of grape pomace extract were performed in laboratory filtration membrane equipment by using nine commercial nanofiltration (NF) membranes with an approximate molecular weight cut-off (MWCO) of 1000-150 Da. The filtration experiments of the selected pomace extract were performed by modifying the most important operating variables : transmembrane pressure, tangential velocity, temperature, and the nature and MWCO of the membranes. The evolution of the cumulative permeate volumes and permeate fluxes with processing time was analyzed till a volume reduction factor (VRF) of 10 was reached. The effect of the mentioned operating conditions was discussed. The effectiveness of the filtration treatments was determined by the evaluation of the rejection coefficients for several families of polyphenols. Membranes possessing MWCO between 1000 and 500 Da were able to quantitatively recover polymeric proanthocyanidins in the concentrate stream and separate them from phenols that passed through the membrane into the permeate stream. On the other hand, the 600 to 300 Da membranes could also be used for the fractionation of monomeric phenolic families. The membranes were able to partially remove the anthocyanin fragments of phenolic acid derivatives and flavonols in the concentrate stream and at the same time.

KEYWORDS

nanofiltration, grape pomace extract, anthocyanins, proanthocyanidins, phenolic compounds

Abbreviations : AUC area under the curve - HPLC high-performance liquid chromatography - J_v permeate flux - J_w pure water flux - L_P hydraulic permeability - MF microfiltration - MWCO molecular weight cut-off - NF nanofiltration - ORAC oxygen radical antioxidant capacity - R_a fouling resistance - R_m membrane resistance - RO reverse osmosis - R_t total resistance TMP transmembrane pressure - UF ultrafiltration - UPLC ultra high-pressure liquid chromatography - v velocity - V_0 initial feed volume - V_P permeate volume - V_R retention volume - VRF volume reduction factor - μ_w dynamic viscosity of water

INTRODUCTION

Grape pomace is a by-product in wine production, representing around 30 % of the original fruit, consisting of skin, seed, and stem tissue. A large quantity of grape pomace is produced worldwide every year, and its disposal is a serious environmental issue. Researchers have proposed the use of grape pomace for the production of different value-added products including enzymes, organic acids, ethanol, aroma compounds, and natural antioxidants (Arvanitoyannis *et al.*, 2006).

As is well known, grapes represent an important source of bioavailable polyphenolic compounds such as flavonols, monomeric and oligomeric flavanols, and anthocyanidins (Spigno *et al.*, 2007 ; Yammine *et al.*, 2018). Conventional wine production results in a wine rich in phenolic compounds but only 10–40 % of the phenolic compounds of the fruit are transferred to the wine (Fragoso *et al.*, 2011), most of the compounds remaining in the grape pomace. Due to its abundance and the increasing interest in new natural sources of antioxidant products, grape pomace has been investigated as a potential source of bioactive polyphenols (Chidambara Murthy *et al.*, 2002 ; Kammerer *et al.*, 2005 ; Vergara-Salinas *et al.*, 2013, 2015), which can be used for various purposes in the food, pharmaceutical and cosmetic industry for their effective antioxidant and free radical scavenger activities.

In recent years, more environmentally friendly techniques have been investigated and used for the separation, purification and concentration of bioactive compounds, allowing to reduce extraction time and solvent consumption as well as to increase bioactive compounds yield (Galanakis, 2012).

Membrane operations are recognized as powerful tools for the purification and concentration of various solutions (e.g., juices, extracts, whey) and the separation of valuable compounds from by-products of the agro-food industry (Li & Chase, 2010). The basic properties of membrane operations make them competitive with conventional methodologies : they do not involve phase changes, chemical additives and heat treatment, they are modular and easy to scale-up, and they are characterized by unlimited selectivity of separation, thereby enabling a more rational utilization of raw materials and recovery and reuse of by-products. In addition, they respond efficiently to “process intensification”, allowing drastic improvements in manufacturing and processing, substantially decreasing the equipment-size/production-capacity ratio, energy consumption, and/or waste production (Akin *et al.*, 2012 ; Drioli & Romano, 2001).

Pressure-driven membrane operations such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) are based on the principle of selective permeation of solutes through polymeric or inorganic semi-permeable membranes : the driving force for mass transfer of solutes across the membrane is the transmembrane pressure (TMP). NF is a unit operation whose separation characteristics fall between UF and RO and whose molecular weight cut-off (MWCO) ranges from 100 to 1000 Da ($g.mol^{-1}$). The complex separation mechanisms that occur in NF (physical, chemical and electrical interactions between the solvent, solutes and membrane) make the number of the operating parameters that control separation efficiency high and give different results for the same feed and the same membrane. The specific performance of NF membranes and the large selection of membranes should facilitate their application (Massot *et al.*, 2008). NF appears to have great potential in the production of high-quality food, including water softening, wastewater treatment, beverage industry, dairy industry and sugar industry (Salehi, 2014). The recovery of biologically active compounds from agro-food by-products, also in combination with other membrane operations (i.e., UF and RO), is another research area of growing interest. For example, a composite fluoropolymer membrane (1 kDa) was able to separate hydroxycinnamic acids from anthocyanins and flavonols in winery sludge extracts and diluted wine samples (Galanakis *et al.*, 2013, 2015). The same membrane has been reported to recapture low-MW polyphenols (i.e., hydroxytyrosol, protocatechuic acid, catechol, tyrosol, caffeic acid, p-coumaric acid, and rutin) from pretreated olive mill wastewater (Cassano *et al.*, 2013) before the permeate stream is processed with NF to concentrate the valuable compounds (17 % polyphenol rejection). In another application reported by Díaz-Reinoso *et al.* (2009), two different membranes (1-kDa cut-off Inside Céram and GE2540) were used to recover total phenols from fermented grape pomace, and showed at least 80 % rejection of these

components. In addition, Díaz-Reinoso *et al.* (2010) recovered antioxidant and phenolic compounds from liquors obtained by pressing distilled grape pomace using a 1-kDa cut-off membrane (Inside Céram). This application showed a higher rejection of total phenolics (up to 72 %). Finally, the separation and concentration of phenolic compounds from press liquors obtained from pigmented orange peels was carried out by Conidi *et al.* (2012). High rejection of anthocyanins (89 %) and flavonoids (70 %) was observed using a 1-kDa membrane (NP010).

In previous works, a conceptual process design for recovering and concentrating phenolic compounds from grape pomace was proposed on the basis of a NF treatment of multiple grape extracts with different NF membranes in selected operating conditions (Díaz-Reinoso *et al.*, 2009; Galanakis *et al.*, 2013; Santamaría *et al.*, 2002; Zagklis & Paraskeva, 2015). In a different context, NF fractionation experiments (with recycling of the retentate stream) of grape pomace extracts were performed in the present work with several objectives: study the evolution of permeate flux with filtration time and volume reduction factor (VRF); establish the effect of operating parameters [TMP, crossflow velocity (v), temperature and MWCO] of the membranes used on the permeate flux; and characterize fractionated streams in terms of total antioxidant activity (TAA), sugars and phenolic compounds. Membrane performance in terms of productivity and selectivity towards compounds of interest is evaluated and discussed.

MATERIALS AND METHODS

1. Experimental equipment and membranes

NF experiments were conducted in a laboratory cross-flow mode filtration apparatus, Sepa® CF II Membrane Cell System (GE Osmonics, Minnetonka, MN, USA). The equipment was composed of a 2-L pressurized and inert storage vessel and an M-03S Hydra-Cell feed flow pump that fed the solution to the flat-sheet membrane module at the desired flow rates. The whole equipment was temperature controlled by means of a water stream that circulated through an external jacket that surrounded the storage vessel. A pressure control valve controlled the TMP in the experiments after the filtration apparatus. The cumulative permeate volume (V_P) was measured with a Mettler precision balance linked to a computer.

The nine flat-sheet membranes used were provided by GE Osmonics and Alfa Laval. The NF membranes were made of polyamide, with the exception of ETNA01PP and BQ01 which were made of fluoropolymer and polysulfone, respectively; MWCO ranged from 1000 to 125 Da. Table 1 lists their characteristics.

All the membranes had an effective area of 0.014 m^2 and an experimentally measured flow section of 14.9 mm^2 (4.5 mm × 3.3 mm). A new membrane was used in each experiment, rinsed with ultrapure water, and compacted by filtering ultrapure water for 1 h before starting the next filtration experiment. The water contact angles of the membranes were measured by the sessile drop technique.

TABLE 1. Characteristics of tested nanofiltration membranes (manufacturer's data).

Designation	manufacturer	Polymer type	MWCO	%NaCL rejection	Recommended and max pressure (Mpa)		pH range	Max temp (°)
MS19	GE Osmonics	Polyamide	125-200	≥ 99	1.4	6.8	2-12	80
	GE Osmonics	TH (thin fim)	150-300	≥ 96	0.70	4.10	2-10	50
	GE Osmonics	TH (thin fim)	150-300	≥ 98	0.70	4.10	2-10	50
	Alfa Laval	Polyamide	200-400	≥ 98	2.2	5.5	3-10	50
	GE Osmonics	Polyamide	300-600	50-70	0.7	6.8	2-12	80
	GE Osmonics	Polyamide	500-1000	20-30	0.7	6.8	0.5-11	100
	GE Osmonics	Polyamide	1000	1K-PEG	2.76	6.8	1-11	50
	Alfa Laval	Fluoropolymer	1000		1	5.5	1-11	60

MWCO, molecular weight cut-off; PEG, polyethylene glycol

2. Subcritical water extraction

In the extraction system, a high-performance liquid chromatography (HPLC) pump (Shimadzu LC-10AC) was used for deionized water delivery, pressurization and controlling the pressure of the system. A pressure transducer (Davidson, Druck) and a thermocouple (Cleveland Electric Lab) were installed in the custom-made high-pressure vessel to monitor both the pressure and temperature of the system. Extract was collected in an inerted vessel after passing in an ice bath.

In each run, red Dunkelfelder pomace (70 g) was loaded into the high-pressure vessel, which can contain 325 cm^3 of material. The vessel was placed in an oven at 150 °C. The outlet valve of the extraction vessel was then closed and the system was pressurized to 25 ´ 10^5 Pa at a constant flow rate. The water flow rate was adjusted to 20 $mL.min^{-1}$ using a metering valve on the HPLC pump. After 3 L of extraction, the solution collected in a sampling vessel and pomace were then stored at 4 °C for further analysis and membrane separation.

3. Filtration experiments

The filtration experiments were conducted at the natural pH of the extract (3.7) in cross-flow mode, with the feed stream flowing tangential to the membrane surface. The extract was prefiltered at 0,3 µm (GE Osmonics JX) for microbial stabilization. The operating method was batch concentration mode : that is, the retentate or concentrate stream was flowed back to the feed tank, while the permeate stream was collected separately and not recirculated to the storage vessel. The initial volume of extract treated was 2 L in all cases, and the flow rate was dependent on the tangential velocity selected (v = 1, 2 or 3 $m.s^{-1}$). Temperature, tangential velocity and TMP remained constant during each experiment but varied between experiments. The duration of each experiment varied according to the desired VRF to be reached.

The standard protocol for NF experiments included three steps. First, the new membrane was rinsed with ultrapure water (Milli-Q system), and membrane hydraulic permeability (L_p) was determined by measuring the water permeate flux (J_w) at different TMP. Second, the storage vessel was emptied and filled with grape pomace extract to perform the filtration experiment. During these experiments, V_p was measured at regular time intervals. Finally, several parameters frequently used to evaluate the content of grape pomace extract were analyzed in the permeate stream : sugar content, absorbance at 280 nm, total polyphenol content, and antioxidant activity by ORAC (oxygen radical antioxidant capacity). The concentration of different families of phenolic acids was also measured : these compounds were specifically selected for their high added value, and the interest in their purification. With the values obtained for these parameters, their respective rejection coefficients were determined.

4. Analytical methods

4.1 Contact angle

The water contact angles of the membranes were measured by the sessile drop technique. Prior to the experiments, the membranes were fixed to a smooth support surface by using a double side sticky tape. Ultrapure water droplets (5 µL) were automatically deposited on the membrane surface by using a Digidrop (GBX, France) equipped with needles of 0.5 mm of external diameter. Once the drop was placed on the surface, the Drop Shape Analysis System of the GBX software (Windrop, GBX, France) allowed the direct measurement of the water contact angle by averaging the water contact angles measured on the left and right sides of the sessile drop. At least 10 drops were deposited on different zones of the membrane at room temperature. The mean water contact angle and

Table 2. Contact angles of tested nanofiltration membranes.

Designation	Contact angles
MS19	37.3
DL	27
HL	32,7
DK	45,1
NF	48,7
MX07	33,2
BQ01	57,1
GE	51,2
ETNA01PP	65,3

These values indicate that the ETNA01PP, GE and BQ01 membranes present a hydrophobic surface, while the MX07, NF, DK, HL, DL and MS19 membranes are relatively hydrophilic.

its standard deviation were calculated for each sample (Table 2).

4.2 pH and total sugars

pH values of feed samples were measured with a digital pH-meter (Thermo Scientific™ Orion™ Star A324). The total sugar content was determined by the anthrone method (Trevelyan *et al.*, 1952) and expressed as glucose equivalents (GE).

4.3 Total polyphenol content

The total phenolic content was spectrophotometrically measured according to a modified Folin Ciocalteu method to be applied in 96-well microplates. Stock solutions (10 $mg.mL^{-1}$) of the grape pomace extracts were prepared in $EtOH/H_2O$ (25 : 75, v/v), and a microplate spectrophotometer (MultiSkan Spectrum, Thermo Scientific) was used for the incubation and measurement. Briefly, each well was filled with 184 µL of distilled water and 24 µL of the sample solution, followed by 12 µL of the Folin Ciocalteu reagent and 30 µL of 20 % (w/v) Na_2CO_3 solution. Prior to the measurement of the absorbance at 765 nm, the mixture was incubated for 1 h under dark conditions at 25 °C. The total polyphenolic concentration was calculated from a calibration curve using gallic acid as a standard (50-500 $mg.L^{-1}$; Sigma Chemicals). The result of total phenolic content was calculated from the regression equation of the standard plot ($y = 0.0013x + 0.0318$, $r^2 = 0.998$). Results, expressed as milligrams of gallic acid per 100 g of grape pomace sample (on a dry matter basis), were a mean of six determinations.

4.4 Antioxidant activity – ORAC

ORAC analysis was applied by using 96-well fluorescence microplates. The reaction was carried out in phosphate buffer (75 mM, pH 7.4). In this order, 30 µL of the pomace extract solution, 180 µL of fluorescein (117 nM final concentration), and 90 µL of AAPH (40 mM) were added to each well. The mixture was shaken and allowed to stand for 1.5 h at 37 °C. Fluorescence was recorded every minute during this period at excitation and emission wavelengths of 485 and 530 nm, respectively. In parallel, a blank sample (phosphate buffer) and Trolox calibration solutions (1–40 µM) were also analyzed ($R^2 = 0.983$). The area under the curve (AUC) was calculated for each extract sample by integrating their relative fluorescence curves. The net AUC of the pomace extracts was calculated by subtracting the blank, and correlated with Trolox concentrations.

4.5 Phenolic classes

Ultra high-pressure liquid chromatography (UPLC) analyses were performed in an Agilent 1260 apparatus (G1377A) consisting of an autosampler module, a degasser, a binary pump, a column heater/selector and a UV–visible DAD detector (G1314F, Agilent Technologies, USA). Chromatographic separation was performed on an Agilent C18 (2.1 mm x 100 mm, 1.8 µm). Anthocyanins were eluted at a flow rate of 0.4 $mL.min^{-1}$ with a gradient of water/acetonitrile/formic acid (87/3/10 ; solvent A) and water/acetonitrile/formic acid (40/50/10 ; solvent B) according to the following gradient program (v/v) : 0 min 94 % A 6 % B, 15 70 % A 30 % B, 30 min 50 % A 50 % B, 35 min 40 % A 60 % B, 40 min 35 % A 65 % B, 41 min 100 % B isocratic for 5 min. Detection was performed at 518 nm. Other polyphenols were eluted at a flow rate of 0.4 $mL.min^{-1}$ with a gradient of water/formic acid (99.9/0.1 ; solvent A) and acetonitrile/formic acid (99.9/0.1 ; solvent B) according to the following gradient program (v/v) : 0 min 93 % A 7 % B, 15 min 86 % A 14 % B, 40 min 65 % A 35 % B, 44 min 50 % A 50 % B, 54 min 30 % A 70 % B, 55 min 100 % B isocratic for 5 min. Detection was performed at 280 nm for flavanols, 306 nm for stilbenes, 310 nm for coumaric acid derivatives and 370 nm for flavonols (Chira, 2009).

Phenolic compounds were eluted at a flow rate of 1 $mL.min^{-1}$ with a gradient of water/formic acid (99.9/0.1 ; solvent A) and acetonitrile/formic acid (99.9/0.1 ; solvent B) according to the following gradient program (v/v) : 0 min 70 % A 30 % B, 18 min 65 % A 35 % B, 46 min 20 % A 80 % B, 47 min 100 % B isocratic for 5 min. This HPLC was coupled to an Esquire 3000+ ion trap mass spectrometer using an ESI source from Bruker Daltonics (USA). Nitrogen was used as drying gas. ESI-MS parameters were the following : positive mode, nitrogen flow rate 10 $L.min^{-1}$, nebulizer pressure 0.275 ´ 10^5 Pa, drying gas temperature 365°C, HV capillary −3700 V, end plate offset −500 V, capillary exit 111.2 V, skimmer 40 V and trap drive 45.9 ; negative mode, nitrogen flow rate 10 $L.min^{-1}$, nebulizer pressure 0.172 ´ 10^5 Pa, drying gas temperature 350 °C, HV

capillary +3400 V, end plate offset −500 V, capillary exit −115.3 V, skimmer −40 V and trap drive 42.9.

Identification of phenolic compounds was achieved using their UV/vis spectra, ion mass and MS/MS fragments using available standards. The results were expressed as mg of specific compound per L of extract, and the data represent the means of three replicates ± SE.

RESULTS AND DISCUSSION

1. Water permeability determination

Hydraulic permeability (L_p) is an intrinsic feature of a non-fouled membrane that must be determined. Therefore, prior to the general filtration experiments of the grape pomace extracts, several filtration experiments of pure water were carried out with each of the filtration membranes selected, with the aim to measure the evolution of J_w with the variation of TMP. The applied pressure during this process ranged from 10 to 30 ´ 10^5 Pa for the NF membranes. The results obtained showed that J_w increased linearly with TMP for the nine types of membranes tested (T = 20 °C; Figure 1).

L_p was obtained from the slopes of the straight lines. Thus, after regression analysis, the following values were deduced, with correlation coefficients higher than 0.99 : from 1.35 to 8.4 $L.h^{-1}.m^{-2}.10^5$ Pa^{-1} for the MS19 (125–200 Da) to GE membranes (1000 Da), respectively. In the previous work, UF membranes showed that the increase in L_p occurred as expected : among membranes of the same nature, larger pore sizes or MWCO led to higher J_w. The different L_p in the NF membranes can be attributed to their internal structure and not only to the MWCO. The L_p value is also an inherent characteristic related to the composition, morphology, porosity and hydrophobicity/hydrophilicity of the membranes, and is not indicative of process flux. In the present case, the ETNA01PP membrane (1000 Da) in fluoropolymer exhibited relatively hydrophobic surface and lesser pure water permeability compared to the GE membrane (1000 Da) in polyamide.

Since the temperature effect on the filtration process was investigated with the GE membrane, its hydraulic permeability was also measured at several temperatures. The L_p values obtained at 30 and 40 °C were 5.7 and 6.5 $L.h^{-1}.m^{-2}.10^5$ Pa^{-1}, respectively. Therefore, a temperature increase leads to higher pure water permeate flux due to a decrease of the viscosity.

2. Influence of operating conditions on the permeate flux

Filtration experiments of the grape pomace extract were performed with the nine selected membranes in batch concentration mode by

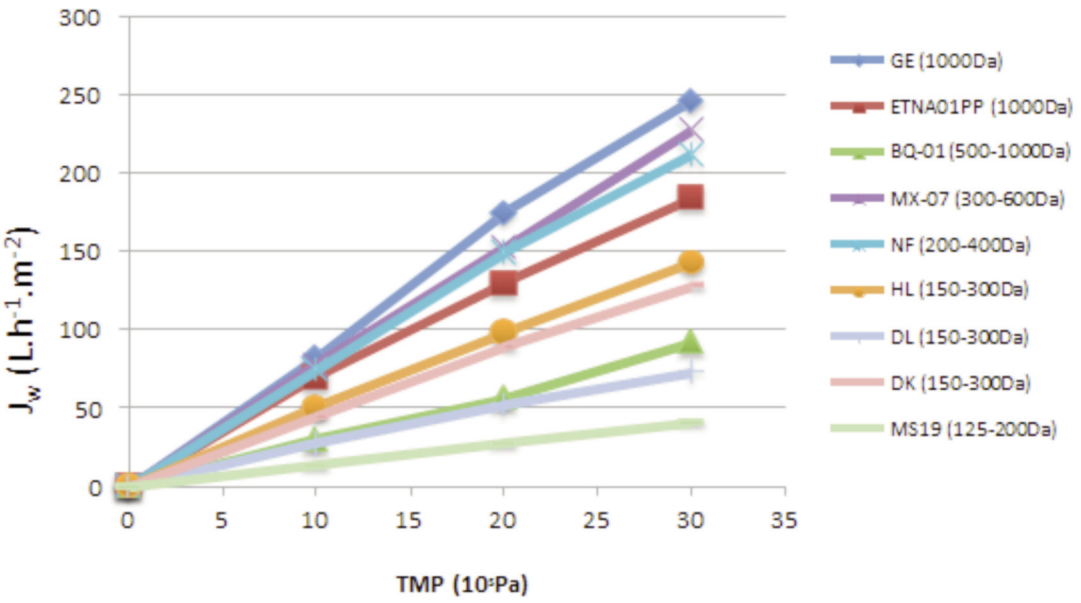


FIGURE 1. Hydraulic permeability (L_p) for the nanofiltration membranes (T = 20 °C). J_w, pure water flux ; TMP, transmembrane pressure.

TABLE 3. Experimental conditions applied in the nanofiltration experiments and results obtained at VRF = 10.

Experiment	TMP (10^5 Pa)	v (m.s^{-1})	T (°C)	Jv (L.h^{-1}.m^{-2})
ETNA01PP-1	10	2	20	34
ETNA01PP-2	20	2	20	53,4
ETNA01PP-3	30	2	20	67,5
GE-1	10	2	20	15,4
GE-2	20	2	20	25,8
GE-3	30	2	20	29,7
GE-4	30	1	20	23,1
GE-6	30	3	20	35,8
GE-7	30	2	30	34,2
GE-8	30	2	40	37,4
BQ01-1	10	1	20	7,8
BQ01-2	20	2	20	12,8
BQ01-3	30	3	20	17,1
MX-07-1	10	2	20	14,7
MX-07-2	20	2	20	29
MX-07-3	30	2	20	37,5
NF-1	10	2	20	18,2
NF-2	20	2	20	21,8
NF-3	30	2	20	25
HL-1	10	2	20	10,8
HL-2	20	2	20	23,6
HL-3	30	2	20	36,2

VRF, volume reduction factor; TMP, transmembrane pressure; v, crossflow velocity; Jv, permeate flux

modifying the most important operating variables: TMP, crossflow velocity, temperature, and the nature and MWCO of the membranes (Table 3).

The cumulative V_p obtained as a function of time for the GE membrane (1000 Da) at different TMP and temperatures, and with a constant velocity (v = 2 m.s^{-1}), is represented in Figure 2. As it can be observed, these volumes increased with processing time, but simultaneously, a decrease occurred in the permeate rate. Additionally, for a given time, the volumes increased with increasing TMP, and increased with increasing temperature, in the investigated range of operating conditions. Similar effect of the TMP was obtained for all of the membranes.

Figure 2 also includes the cumulative permeate volume obtained in the previous experiments for the filtration of pure water with the new membrane. The lower values of V_p obtained for the grape pomace extract in comparison to those of pure water were due to the fouling of the membrane (Cissé *et al.*, 2011). Membrane resistance is defined by the following equation for the water permeate flux (J_w):

$$R_m = \frac{\Delta P}{J_w \mu_w} \qquad (1)$$

where P is the transmembrane pressure, μ_w is the viscosity of the pure water permeate and R_m is the hydraulic resistance to pure water.

The decline of the grape pomace extract J_w with filtration time is represented in Figure 3 for some experiments performed with the GE membrane, at varying TMP (v = 2 m.s^{-1} and T = 20 °C). The results show that J_w decreased gradually with the operating time, which is due to fouling caused by the compounds found in grape pomace extract (Díaz-Reinoso *et al.*, 2009; Zagklis & Paraskeva, 2015).

At the same time, Figure 3 also depicts the evolution of VRF with filtration time with the GE membrane, which is defined by:

$$VRF = \frac{V_0}{V_R} \qquad (2)$$

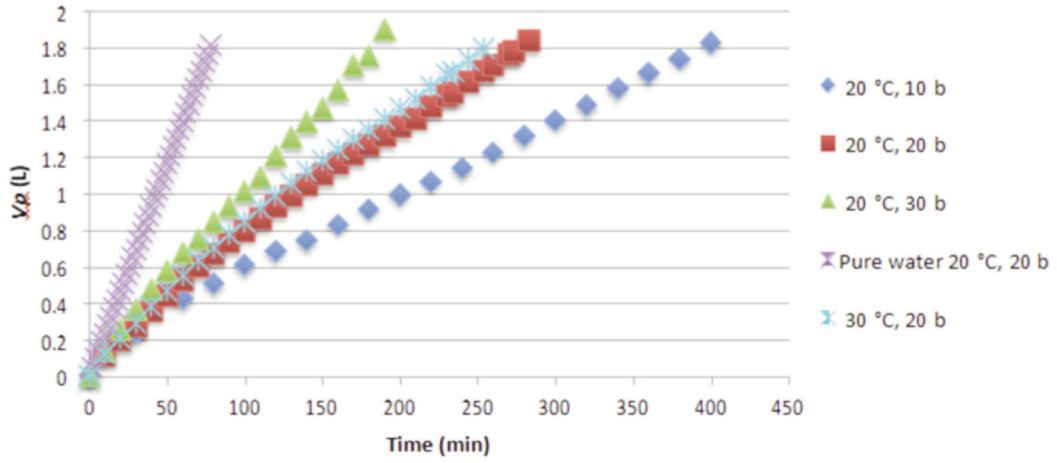


FIGURE 2. Evolution of the cumulative permeate volume (V_P) with processing time for the grape pomace filtration experiments performed with the GE membrane at a tangential velocity v = 2 m.s^{-1}.

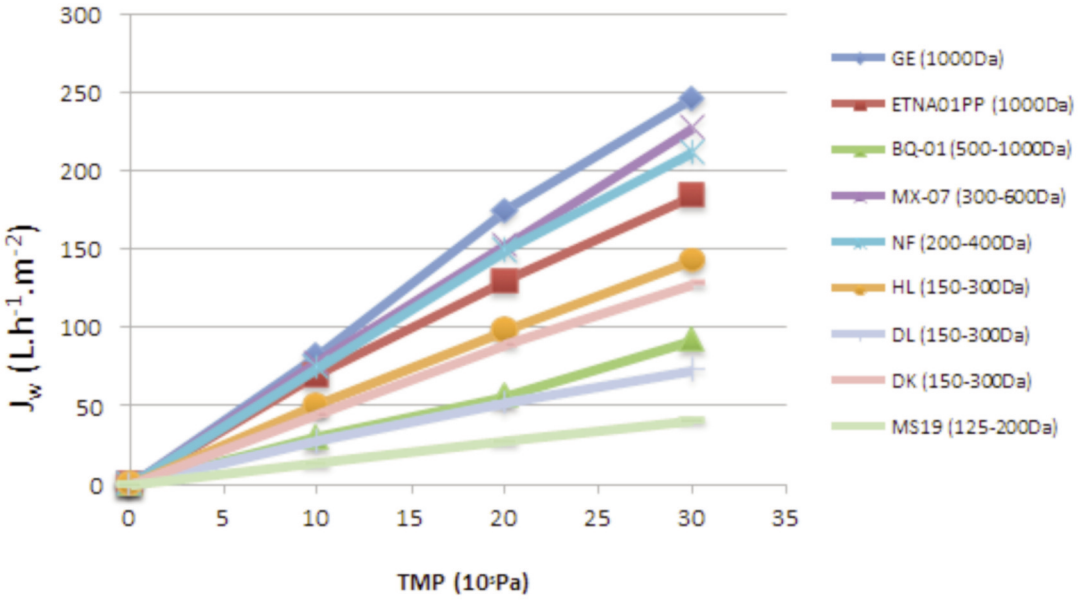


FIGURE 3. Evolution of the permeate flux (Jv) and volume reduction factor (VRF) with processing time for the grape pomace extract filtration experiments performed with the GE membrane at v = 2 m.s^{-1} and T = 20 °C.

where V_0 is the initial feed volume and V_R is the retention volume, that is the extract volume remaining in the storage vessel ($V_R = V_0 - V_P$). For the experiment with GE at TMP = 3 ´ 10^5 Pa, the initial permeate flux was 60.4 L.h^{-1}.m^{-2} and decreased up to 30 L.h^{-1}.m^{-2} after 2.25 h of operation, which corresponded to a final VRF = 10.

Figure 4 depicts the evolution of J_v with VRF for the selected experiment GE-2 taken as example: a clear decline of J_v occurs with the increase in VRF, due to the increase of material concentration in the retentate, thereby increasing membrane fouling. Moreover, this curve could be divided into three periods: an initial stage with a rapid decrease of the permeate flux; a second stage with a smaller decrease of the permeate flux that takes place around VRF = 1.25; and a final stage with a very slight decrease of J_v up to near steady-state conditions, which occurred after VRF = 4. Similar trends have been observed in previous studies for the filtration of grape juice (Cancino-Madariaga *et*

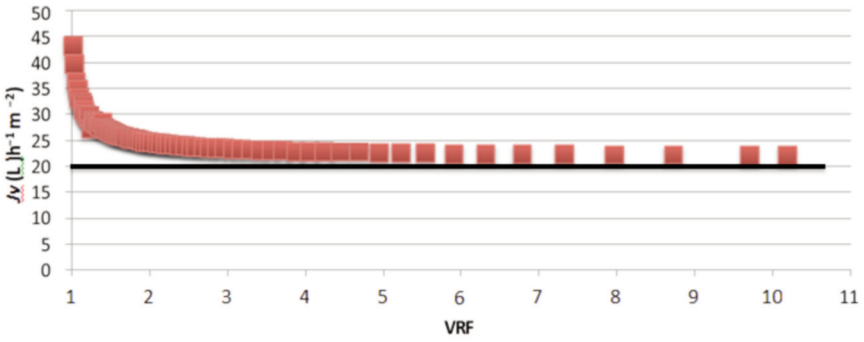


FIGURE 4. Evolution of the permeate flux (J_v) with the volume reduction factor (VRF) for the experiment GE-2 at 20 × 10^5 Pa.

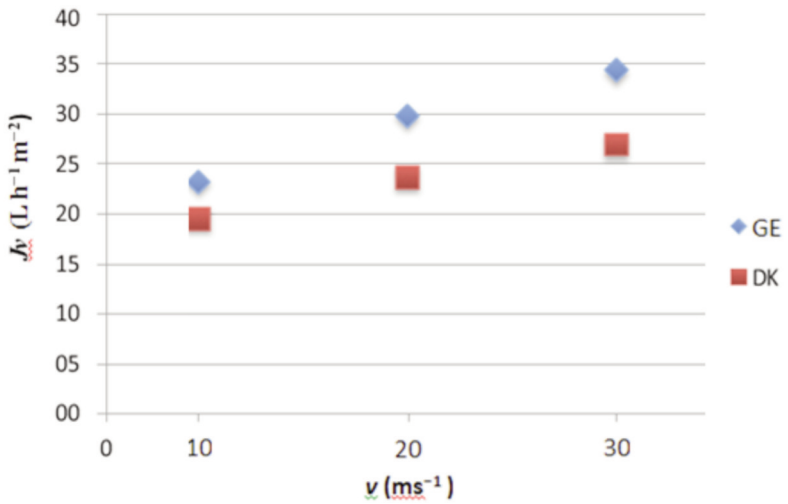


FIGURE 5. Effect of the crossflow velocity (v) on the permeate flux (J_v) for VRF = 10 with the GE and DK membranes at 20 °C and 3 × 10^5 Pa.

al., 2012) and kiwifruit juice (Cassano *et al.*, 2008). As similar curves were obtained for the remaining experiments, VRF = 10 was adopted as the standard value for steady-state conditions in the filtration of grape pomace extract; Table 3 only depicts the J_v specifically obtained at VRF = 10.

Those calculated J_v values are affected by the main operating parameters already mentioned: tangential velocity, TMP, temperature, and MWCO and nature of the membranes. Thus, the effect of the tangential velocity on the steady-state permeate flux can be observed for the GE (1000 Da) and DK (150-300 Da) membranes (Figure 5). As it is seen for TMP = 30 ´ 10^5 Pa, J_v increases when the tangential velocity is increased, due to an increase of the shear stress at the membrane surface, which prevents the accumulation of the components in the laminar sublayer and decreases the thickness of the concentration polarization layer (Wei *et al.*, 2007).

In a similar way, Figure 6 shows the evolution of the steady-state permeate flux with the TMP in the experiments carried out with NF membranes and with a crossflow velocity of 2 $m.s^{-1}$. It is observed that J_v increased linearly with increasing pressure in the range of TMP used, as it has been reported by other authors in similar studies performed with different extracts (Díaz-Reinoso *et al.*, 2009; Santamaría *et al.*, 2002).

Figure 6 also provides the influence of the MWCO of the membranes on the steady-state J_v. For instance, at 30 bar, the higher J_v value obtained with the ETNA01PP membrane (67.4 $L.h^{-1}.m^{-2}$) in comparison to the GE membrane (29.6 $L.h^{-1}.m^{-2}$) can only be attributed to the different nature of the membranes. However, the

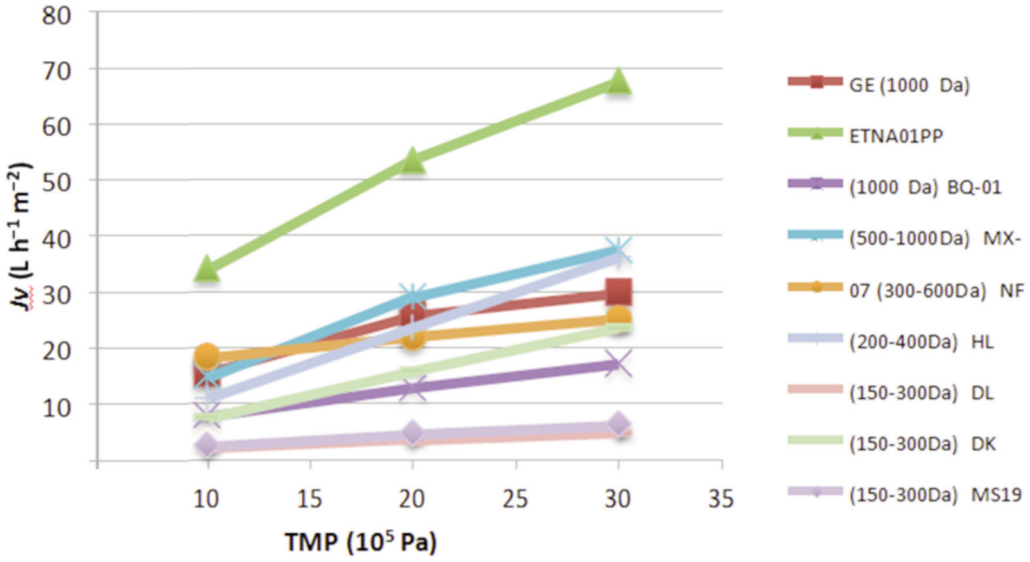


FIGURE 6. Effect of the transmembrane pressure (TMP) and molecular weight cut-off (MWCO) on the steady-state permeate flux for experiments performed at v = 2 m.s^{-1} and T = 20 °C.

hydraulic permeability with pure water of the GE membrane (L_p = 184 L.h^{-1}.m^{-2}.10^5 Pa^{-1}) was lower than that of the ETNA01PP membrane (L_p = 245 L.h^{-1}.m^{-2}.10^5 Pa^{-1}). The higher reduction in the permeate flux from pure water to grape pomace extract filtration with the GE membrane is an indication of a greater fouling effect with this membrane, made of polyamide, than with the fluoropolymer ETNA01PP membrane. This behavior could be explained by the greater hydrophobicity in terms of contact angle (65.3°) of the ETNA01PP membrane.

With respect to the influence of the operating temperature, there is a decrease of J_v from 29.95 to 27,04 l.h^{-1}.m^{-2} when the temperature is increased from 20 to 40°C (see experiments GE-6, GE-7 and GE-8 in Table 3). The negative effect leads to lower mass-transfer coefficients at higher temperature, contrary to the film model (Hoek *et al.*, 2004). These results can be explained by a greater fouling of the membrane at higher temperatures, which can be due to the formation of a gel layer of pomace extracts (essentially pectins, glucans) at the membrane surface. Similar negative temperature effects were observed by Jiraratananon & Chanachai (1996) for passion fruit juice ultrafiltration.

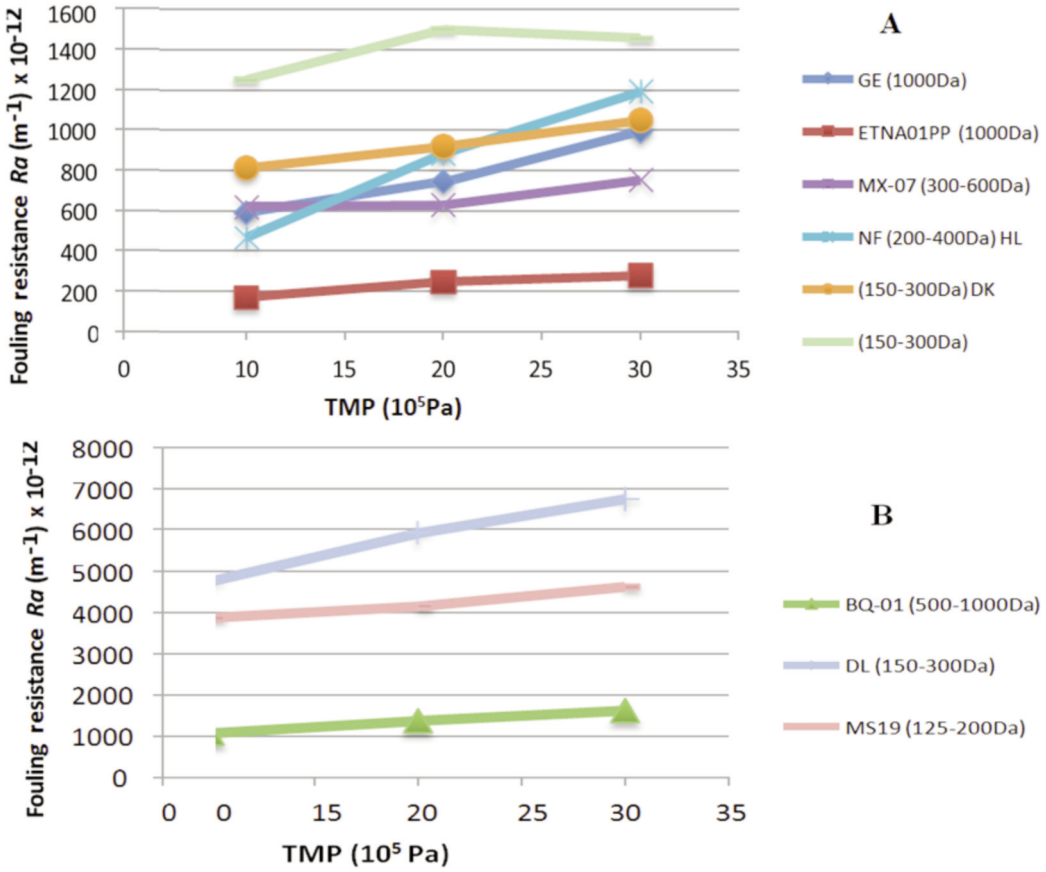
3. Fouling resistance

The total resistance (R_t) could be defined with the results of the filtrate flux for a given pressure. Then, resistance created by fouling and/or concentration polarization (R_a) during grape pomace extract filtration was calculated as the difference between total resistance (R_t) obtained during the filtration experiment and membrane resistance (R_m):

$$R_a = R_t - R_m \qquad (3)$$

Fouling resistance (R_a) includes intrinsic membrane fouling resistance, fouling layer resistance and resistance due to concentration polarization phenomena and/or gel layer formation (Bernat *et al.*, 2009; Butylina *et al.*, 2006). As TMP increases, R_a increases for all NF membranes tested. When TMP varies from 10 to 30 × 10^5 Pa, R_a increases up to 3 times, depending on the membranes.

In most cases, R_a could be separated in two categories: low and high fouling membranes (Figure 7). The higher fouling resistance was observed with the more hydrophilic membrane (DL) having the lower contact angle (27°). Comparing GE and ETNA01PP membranes, the material could explain the more important fouling with the GE membrane made of polysulfone. The same observation was made on the tested UF membranes. For the same MWCO (150-300 Da), the DL and HL membranes have very different fouling resistances (6000 × 10^{12} and 900 × 10^{12}.m^{-1}, respectively) even with the same material (thin film) and similar contact angles (27 and 32.7°, respectively). HL is a typical composite membrane; it consists of three layers: a thin top selective polyamide layer of a



(High fouling).

FIGURE 7. Evolution of fouling resistance (Ra) for nanofiltration membranes tested on the steady-state permeate flux for experiments performed at v = 2 m.s-1 and T = 20 °C. A (Low fouling); B (High fouling).

few hundred nanometers thick poly(piperazine-amide), an asymmetric microporous polysulfone support layer, and a polyester non-woven fabric layer for mechanical strength. A mean pore radius of 0.46 ± 0.08 nm was obtained by Silva *et al.* (2016) supposing slit pores in retention measurement. The top active layer of DK and DL consists of three sublayers, as opposed to the HL top active layer composed of two sublayers. Furthermore, these thin film composite (TFC) membranes typically suffer from compaction effects under pressure. At the pH of the extracts tested (pH 3.9), the membranes are positively charged or close to neutral for the HL membrane (isoelectric points (pHi) such as 3.9, 4.8 and 4.0 for HL, DL and DK membranes, respectively; Chandrapala *et al.*, 2016). This pH of the extract near the isoelectric point for the HL membrane could explain the decreased interactions between the compounds of the extract and the surface of the membrane. All these parameters (contact angles, composition, pHi, etc.) could explain the differences observed with the various membranes tested. It is generally recognized that membrane hydrophobicity/hydrophilicity, pore size (and their distribution) and surface charge may be important factors determining separation performance and the fouling tendency of NF membranes (Al-Amoudi, 2013). For the other membranes, no correlations were observed between R_a and the known parameters: nominal MWCO and contact angle. The structure of the membrane, nature of materials and the different interactions most likely explain the differences observed.

4. Phenolic compound fractionation

Table 4 shows the composition of the subcritical extract used in the NF experiments with the main families of molecules. The extract was acidic in nature (pH 3.9 ± 0.1) probably due to the wine organic acids and phenolic compounds.

Furthermore the extract was rich in total phenols (3309 mg.L^{-1}) determined at 765 nm. Different phenolic classes like phenolic acids, flavonols and anthocyanins were found in the ranges of 243.7, 46.6 and 153.6 mg.L^{-1} in the extract, respectively. Finally, the majority of the detected flavan-3-ols were in the polymeric form (153 mg.L^{-1}), whereas the concentration of respective monomeric compounds was negligible (76.3 mg.L^{-1}).

Table 5 shows the retention percentage of the permeate flux in terms of sugars, flavonoids and anthocyanins for all the NF membranes investigated. The initial feed showed a content of anthocyanins similar to that reported by Díaz-Reinoso *et al.* (2010, 2009) and Santamaría *et al.* (2002) in grape pomace extract.

All the NF membranes investigated presented high average rejections towards polymeric flavan-3-ol (in the range 59.3-100 %), while for other families macromolecules such as catechin (in the range 23.0–99.4 %) the range was variable. Sugar compounds were weakly retained by the majority of the membranes (22.8 %), while the lowest MW membranes showed a high retention of the compounds.

In particular, the membranes with the lower MWCO range (150-400 Da) showed high average rejection towards flavonoids and anthocyanins (95.4 and 95.9 %, respectively) but not phenolic acids. The DL membrane retained all flavonoids and anthocyanins in the retentate side (rejection of 82.4 and 87.2 % towards flavonoids and anthocyanins, respectively); in contrast, about 64 % of sugars were measured in the permeate stream. The fluoropolymer membrane ETNA01PP showed low average rejections towards all the families of macromolecules in comparison to thin film membranes. This may be due to the hydrophobicity of the membranes and the lower fouling.

Sugars on the other hand showed lower rejection rates than the similar MW phenolic acids. The rejection of the MX-07 membrane towards sugar compounds was 32.7 %, while for phenolic acids it was 38.6 %. These results were different to those obtained with the HL membrane, with a higher rejection of sugars (99.6 %) in comparison to phenolic acids (74.7 %). Thus the use of the HL membrane for fractionation may lead to a certain recovery of phenolic acids in the permeate stream, indicating that this membrane offered the best separation of phenolic compounds from sugars.

TABLE 4. Characteristics of the grape pomace extracts used as feed liquids. Values represent mean ± standard deviation (n = 6).

pH	3.9	±0.1
Sugars (mg/L)	4096	± 217
Total polyphenols (mg/L)	3309	± 366
Phenolic acids (mg/L)	244	± 90
Polymeric flavan3-ol (mg/L)	153	± 8.5
Catechin (mg/L)	76.3	± 3.8
Quercetin (mg/L)	46.6	±5.9
Taxifolin (mg/L)	21.3	±2,3
Anthocyanin	153.6	±12,4

Basically, the rejection of NF membranes towards the analyzed compounds decreased by increasing the MWCO of the selected membranes. However, the rejection of all selected membranes towards anthocyanins was higher than 52 %. This behavior can be explained assuming that anthocyanins, unlike other subgroups of flavonoids with a similar C6-C3-C6 skeleton, have a positive charge in their structure at acidic pH (the pH of the pomace extract is 3.9). At this pH most of the membranes exhibit a positive charge (Boussu *et al.*, 2008). Consequently, the electrostatic repulsion, independent of the MWCO of the selected NF membranes, contributes to the high average rejection of the membranes towards anthocyanins.

In terms of retention, these results are very similar to those reported in the NF treatment of orange peel residues with the Osmonics DL membrane (Conidi *et al.*, 2014). Two different NF membranes have been used to recover flavonoids and anthocyanins from press liquor obtained from pigmented orange peels (Conidi *et al.*, 2012). The first (NF70, 180 Da) showed flavonoids and anthocyanins rejection values greater than 90 %, whereas the second (NF200, 300 Da) showed rejection values greater than 85 % for these components. The two membranes are made of semi-aromatic piperazine-based polyamide skin layer and have different MWCO (180 and 300 Da), which could explain the higher compound rejection with NF70. Also Díaz-Reinoso *et al.* (2009) recovered total phenols from fermented grape pomace using two

different NF membranes (Nanomax 95 - polyamide and Desal DL 2540 with MWCO of 250 and 150-300 Da, respectively). According to this study, the Desal DL 2540 was much more effective : 80 % rejection compared to 25 % for Nanomax. Díaz-Reinoso *et al.* (2010) also tested NF membranes (Nanomax 95 and Nanomax 50 with MWCO of 250 and 350 Da, respectively) to recover phenolic compounds from liquors obtained by pressing distilled grape pomace. The highest rejection was obtained using Nanomax 50 (97 % compared to 52 % for Nanomax 95). In addition, it is possible to recover more than 95 % of polyphenols from olive mill wastewater using a fine NF membrane. For instance, Coskun *et al.* (2010) used three different NF membranes (NP030, NP010, and NP270) to treat olive mill wastewater. According to the results, these membranes were able to remove chemical oxygen demand associated with polyphenol content in terms of retention efficiency and high permeate fluxes. Besides, low-MW polyphenols such as hydroxytyrosol, protocatechuic acid, catechol, tyrosol, caffeic acid, p-coumaric acid, and rutin were concentrated by Cassano *et al.* (2013), using an NP90 membrane (100 % polyphenol rejection).

The above information has shown that the array of membrane fractionation is large. Consequently, the process could be adapted to produce fractions with different phenolic content and purity and thus could be used in different applications. Depending on the targeted family of molecules, the separation of phenolics seems to be possible with the application of NF membranes. For instance the HL and NF membranes could be used to separate phenolic acids, since they passed into the permeate stream (≥ 57.5 % retention), while catechins and quercetins were partially retained in the concentrate stream by MX07 and BQ01. Likewise, the BQ01 permeate stream sustained the anthocyanins, as the retentions were at 52.5 %. The higher retention of anthocyanins in comparison to catechins and quercetins could be explained by the fact that the anthocyanin structure, with higher positive charges, interacts with membranes (Galanakis *et al.*, 2013). The GE and ETNA01PP membranes could be used to separate polymeric proanthocyanidins. Although the performance parameters of these membrane processes were very satisfying (permeate fluxes were relatively high, averaging 1.08 $L.h^{-1}.m^{-2}.10^5$ Pa^{-1}), significant attention should be given to fouling. Eventually, NF could be used to concentrate specific phenolic classes. In particular, the elimination of sugars and water at the same time as the retention of phenolic classes using the HL membrane with a permeate flux of 1.15 $L.h^{-1}.m^{-2}.10^5$ Pa^{-1}.

CONCLUSIONS

The current study suggests that the fractionation as well as the recovery of valuable compounds from grape pomace extracts is possible with the use of membrane technologies. The separation of these ingredients was mainly governed by the MWCO characteristics of the applied membranes. With regard to the grape pomace extract used, the membranes possessing MWCO between 1000 and 500 Da were able to quantitatively recover polymeric proanthocyanidins in the concentrate stream and separate them from phenols that passed into the permeate stream. On the other hand, the 600 to 300 Da membranes could also be used for the fractionation of monomeric phenolic families. The membranes were able to partially remove the anthocyanin fragments of phenolic acid derivatives and flavonols in the concentrate stream. This process would improve the value of the different families due to their purity. Finally, nanofiltration could be used to fractionate and concentrate the grape pomace extracts.

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TABLE 5. Retention coefficients (%) obtained for several parameters of subcritical grape pomace extracts as a function of different nanofiltration membranes.

Group of macromolecules	MW range g.mol^{-1}	ETNA01PP (1 kDa)	GE (1 kDa)	BQ-01 (500-1000 Da)	MX-07 (300-600 Da)	DK (150-300Da)	NF (200-400 Da)	HL (150-300 Da)	DL (125-300 Da)	MS-19 (125–200 Da)
Sugars	180	± 1,2	2 ± 2,0	47,0 ± 3,1	32, ± 2,3	64 ± 1,0	6 ± 5,7	9 ± 0,1	' ± 0,1	± 0,3
Total phenolic compounds		4 ± 5,9	4 ± 3,4	64,6 ± 6,0	74, ± 5,0	82 ± 2,5	9 ± 5,4	9 ± 0,5	1 ± 0,2	± 0,6
Phenolic acids	170-198	2 ± 3,9	1 ± 2,3	36,4 ± 3,1	38, ± 4,0	42 ± 5,9	5 ± 5,3	7 ± 3,3	' ± 0,5	± 0,4
Polymeric flavan3-ol	579-867	5 ± 4,0	7 ± 5,0	89,0 ± 5,0	95, ± 5,3	98 ± 2,2	1 ± 2,5	1 ± 0,7	1 ± 0,8	± 0,7
Catechin pK = 4.6	290	2 ± 3,0	3 ± 3,8	54,6 ± 3,6	42, ± 4,1	78 ± 4,7	9 ± 2,6	9 ± 0,4	1 ± 0,5	± 0,6
Quercetin pK > 7	302-508	6 ± 4,8	5 ± 3,9	70,3 ± 5,6	91, ± 2,8	99 ± 1,0	1 ± 1,0	1 ± 0,8	1 ± 0,6	± 0,7
Taxifolin pK = 7.4	450	3 ± 4,5	4 ± 4,1	61,1 ± 3,5	76, ± 4,6	73 ± 1,0	1 ± 1,3	1 ± 0,5	1 ± 0,5	± 0,2
Anthocyanins	287-639	6 ± 7,6	5 ± 5,4	52,5 ± 3,2	87, ± 7,7	87 ± 4,3	1 ± 1,0	1 ± 0,4	' ± 0,7	± 0,4

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