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Recovery of phenolic compounds from bergamot juice by nanofiltration membranes

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ABSTRACT

In this work, nanofiltration (NF) membranes were used to produce purified and concentrated fractions of phenolic compounds from the bergamot juice that was previously clarified by ultrafiltration. In particular, the performance of three commercial NF membranes (NFPES 10, N30F, and NF270) with different molecular weight cut-off and polymeric material was evaluated in terms of productivity and selectivity towards compounds of interest (flavonoids and total phenolic compounds), total antioxidant activity, and sugars. Experimental results indicated that the NFPES10 membrane showed the largest gap between the rejection coefficients towards sugar and phenolic compounds. Therefore, the produced retentate fractions, characterized by high antioxidant activity, can be considered of interest for nutraceutical applications. The hydrophobic character of NF PES10 and N30F membranes accounted for their higher fouling index when compared to the NF270 membrane.

Keywords: Bergamot juice; Nanofiltration; Flavonoids; Phenolic compounds

1. Introduction

Bergamot (*Citrus bergamia* Risso) is a small tree belonging to the family Rutaceae. Ninety percent of bergamot's worldwide production is produced in the Southern coasts of Calabria region (Italy), where the microclimatic characteristics are particularly suitable for its cultivation. Bergamot fruit is used mostly for the extraction of its essential oil from the peel, a basic component of fragrances.

Bergamot juice, which is obtained from the endocarp after essential oils extraction, is considered just as a secondary and discarded product with related disposal costs. Over the past few years, bergamot juice attracted large attention as a result of its remarkable content of polyphenols and flavonoids [1]. Particularly, among flavonoids, bergamot juice is rich in neohesperidosides of hesperetin and naringenin, such as melitidine and brutieridine. These flavonoids, possess 3-hydroxy-3-methylglutaryl moiety with a structural similarity to the natural substrate of the 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, which catalyzes the rate-limiting step in mevalonate biosynthesis, a key intermediate in cholesterol metabolism, and are likely to exhibit statin-like proprieties [2,3].

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Experimental evidence obtained in animal models of diet induced hypercholesterolemia and renal damage, supports the hypolipemic and vasoprotective effects of bergamot constituents [4]. The anti-inflammatory potential of the bergamot juice has been also studied [5,6].

The pharmacological properties of flavonoids (polyphenols) and their health benefits have intensified research efforts to discover and utilize methods for the extraction, separation, and purification of these compounds from natural sources.

Conventional extraction techniques for the purification of natural products include solvent extraction, ultrasound-assisted extraction, pressurized-liquid extraction, enzyme-aided extraction, supercritical fluid extraction, resin-based extraction, and alkaline extraction. These extraction methods are characterized by some drawbacks, including the degradation of the target compounds due to high temperatures and long extraction times (as in solvent extractions) and health-related risks. Extraction methodologies are mainly focused on the application of environmentally friendly solvents while novel separation technologies, including membrane separation techniques, have been developed and studied [7].

With a number of advantages, such as high separation efficiency, low energy requirements, mild operating conditions, no additives, simple equipment, and easy scale-up, membrane-based technologies can provide interesting alternatives to conventional systems. In particular, ultrafiltration (UF) and nanofiltration (NF) technologies are interesting approaches for the recovery of bioactive compounds from vegetable sources and by-products of their industrial transformation [8]. Within this context, several research activities related to the concentration of phenolic compounds extracted from grape seeds [9], aqueous extracts of *Castanea sativa* leaves [10], grape pomace [11], aqueous and ethanolic propolis extracts [12,13], aqueous mate (*Ilex paraguariensis* St. Hil.) extracts [14], and roselle extract (*Hibiscus sabdariffa* L.) [15] have been performed recently.

The aim of this work was to compare the efficiency and performance of different NF membranes in the treatment of clarified bergamot juice aimed to the separation and concentration of phenolic compounds (mainly flavonoids) from sugar compounds. For this purpose, the clarified bergamot juice, coming from a preliminary UF treatment, was processed with three different NF spiral-wound membranes characterized by different molecular weight cut-off (MWCO) (from 150 to 1,000 Da) and different polymeric material (polyethersulphone (PES) and polyamide).

The performance of selected membranes was evaluated in terms of productivity and selectivity towards compounds of interest (sugars, flavonoids, total phenolic compounds) and total antioxidant activity (TAA). An analysis of the fouling index (FI) of selected NF membranes was also performed on the basis of their hydraulic permeabilities measured before and after the treatment of the clarified juice.

2. Materials and methods

2.1. Bergamot juice

Bergamot juice was supplied by Gioia Succhi Srl (Rosarno, Itay). Before use, the juice was filtered through a 200 micron cotton fabric filter. It was stored at −17˚C and defrosted to room temperature before use.

2.2. Bergamot juice clarification

Before NF experiments, the bergamot juice was pre-treated by UF in order to remove suspended solids and reduce fouling phenomena in the subsequent NF experiments. Cross-flow UF experimental runs were performed in a pilot unit (Verind S.p.A., Milan, Italy) equipped with a polysulphone hollow fiber membrane module supplied by China Blue Star Membrane Technologies, Co. Ltd (Beijing, China). Characteristics of the membrane module are reported in Table 1.

UF experiments were performed by using a juice volume of 14 L according to a batch concentration configuration (with simultaneous recycling of the retentate in the feed tank and removal of the permeate). The UF system was operated at a transmembrane pressure (TMP) of 0.38 bar, a temperature (*T*) of 20˚C, and a cross-flow velocity (CFV) of 0.12 m/s until it reaches a volume reduction factor (VRF) of 8.12 corresponding to a recovery factor of 87.8%. Each

Table 1
Characteristics of the UF membrane module

Type	DCQ III-006
Configuration	Hollow fiber
Membrane material	Polysulphone
Dimension (mm)	90 × 522
Typical operating pressure (bar)	1–1.5
Operating temperature (˚C)	0–40
Operating pH	2–13
Inner fiber diameter (mm)	2.1
Membrane surface area (m^2)	1.2
Nominal molecular weight cut-off (kDa)	100

experiment was repeated three times. Permeate fluxes were expressed as average values ± standard deviation (SD).

A cleaning-in-place procedure was used in order to recover the original water permeability after each experiment. The pilot unit was rinsed with tap water. Then a 1% NaOH solution was recirculated through the membrane module at a TMP of 0.15 bar, a CFV of 0.076 m/s, and an average temperature of 40˚C for 60 min. Finally, the circuit was rinsed with distilled water until pH 7.

2.3. NF experimental set-up and membranes

The clarified bergamot juice was used as feed solution of the NF process. NF experimental runs were performed by using a laboratory plant supplied by Matrix Desalination Inc. (Florida, USA). The equipment consists of a feed tank with a capacity of 10 L, a stainless steel housing for 2.4 × 21 inches spiral-wound membrane module, a high pressure pump, a back-pressure valve, two pressure gauges, a permeate flowmeter, and a permeate tank. A cooling coil, fed with tap water, was used in the feed tank to control the feed temperature.

Three different spiral-wound NF membrane modules were selected on the basis of their particular MWCO and materials: N30F and NFPES10, supplied by Mycrodin Nadir, and NF270 supplied by Filmtec/Dow. Properties of these membranes, obtained from manufacturers' data sheet, are summarized in Table 2.

Experimental trials, devoted to the investigation of the effect of TMP on the productivity of the selected membranes, were performed according to the total recycle mode in which both permeate and retentate were recycled back to the feed tank at a constant juice volume of 4.5 L. The permeate flux was measured every 2 min for 120 min in fixed conditions of T (25˚C) and TMP. Experiments were performed at 4, 8, 12, and 16 bar.

Batch concentration experiments in selected operating conditions (TMP, 6 bar and T, 20˚C) were also performed by using 7.8 L of clarified juice up to a final VRF of 3.5.

Each experiment was repeated three times. All the experiments showed repeatability to within ± 3% in terms of flux measurements.

Before the juice treatment, NF membranes were conditioned with water for 1 h at a TMP of 20 bar and the water permeability measured. The water permeability (L_w^0) of each membrane was determined by the slope of the straight line obtained by plotting the water flux (J_w) values, measured in fixed conditions of T (25˚C) against the TMP in the range 2–16 bar.

The FI of the investigated NF membranes, expressed as a percentage drop in water permeability, was estimated by measuring the water permeability before and after the treatment of bergamot juice, according the following equation:

$$\mathrm{FI} = \left(1 - \frac{L_w{}^1}{L_w{}^0}\right) \cdot 100 \quad (1)$$

Table 2
Characteristics of NF membrane modules

Membrane type	NF-270	N30 F	NF PES 10
Manufacturer	Dow/Filmtec	Microdyn Nadir	Microdyn Nadir
Material	Semi-aromatic piperazine-based polyamide layer on top of a polysulphone microporous support	Polyethersulfone	Polyethersulfone
NMWCO (Da)	150–250	400	1,000
Maximum operating pressure (bar)	41	40	40
Maximum operating temperature (˚C)	45	50	50
NaCl retention (%)	–	25–35	5–15
Na_2SO_4 retention (%)	–	85–95	25–40
$CaCl_2$ retention (%)	35–50	–	–
$MgSO_4$ retention (%)	>97	–	–
pH range	3–10	2–11	2–11
Membrane charge (neutral pH)	Negative	Negative	Negative
Membrane surface area (m^2)	2.6	1.6	1.6

where L_w^0 and L_w^1 are the pure water permeability before and after the bergamot juice filtration.

After the juice treatment, all selected membranes were previously cleaned with tap water for 20 min in order to remove the reversible polarized layer. Then the membranes were cleaned with a 0.2% NaOH solution for 45 min at 40˚C and a TMP of 2 bar. A final rinse with tap water for 20 min was carried out before to measure the water permeability again.

2.4. Analytical evaluations

Sugars concentration, in terms of ˚Brix, was measured by using a hand refractometer (Atago Co., Tokyo, Japan) with a scale range of 0–32 ˚Brix. The suspended solids content was determined in relation to the total juice (%, w/w) by centrifuging 15 mL of a pre-weighted sample at 2000 rpm for 20 min; the weight of settled solids was determined after removing the supernatant.

Total phenolics were estimated colorimetrically by using the Folin–Ciocalteau method [16] and results were expressed as gallic acid equivalents.

Flavonoids, such as hesperidin (MW, 610.57 g/mol), naringin (MW, 580.53 g/mol), and neohesperidin (MW, 612.58 g/mol), were determined by using a HPLC system (Agilent 1100 Series, USA) equipped with a pump, an UV–vis detector and a data acquisition system. Chromatographic separation was performed by using a Luna C 18(2) column (250 × 4.6 mm, 5 m, Phenomenex, Torrance, CA, USA); the following conditions were used: flux, 1 mL/min; T, 5˚C; pressure, 100 bars; λ, 284 nm. The mobile phase was a mixture of 80:20 water/KH_2PO_4 0.25 M (solvent A) and a mixture of 46:4:50 water/KH_2PO_4 0.25 M/acetonitrile (solvent B). A six-step linear gradient analysis for a total run time of 40 min was used.

Prior to HPLC analysis all samples were filtered by using 0.45 micron cellulose acetate filters. All flavonoids were identified by matching the retention time and their spectral characteristics against those of standards. Quantization was made according to the linear calibration curves of standard compounds.

TAA was determined according to an improved version of the ABTS radical cation decolourisation assay in which the radical monocation of the 2,2-azino-bis- (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS+) is generated by oxidation of ABTS (Sigma Aldrich, Milano, Italy) with potassium persulphate (Sigma Aldrich, Milano, Italy) before the addition of the antioxidant [17,18]. Results were expressed as Trolox Equivalents Antioxidant Capacity.

The rejection (R, %) of UF and NF membranes towards specific compounds was calculated as follows:

$$R = \left(1 - \frac{C_p}{C_r}\right) \cdot 100 \quad (2)$$

where C_p and C_r are the permeate and the retentate solute concentrations, respectively.

The analyses were performed in triplicate; all data given represented mean values ± SD.

3. Results and discussion

3.1. Clarification of bergamot juice

Fig. 1 shows the time evolution of permeate flux and VRF when the raw juice is clarified in the selected operating conditions. The initial permeate flux was of 11 L/m^2 h; it decreased rapidly within the first 20 min and continued to decrease until to reach a steady-state value higher than 3 L/m^2 h corresponding at a VRF of 8.1. Experimental data are referred to the treatment of 13.6 L of bergamot juice with the production of about 11.9 L of clarified juice.

The decline of permeate flux can be attributed to the accumulation of bergamot juice components in the membrane pores and on the membrane surface which are responsible of membrane fouling and gel formation phenomena, respectively. These fouling materials are mainly composed of cell walls, polysaccharides such as pectin, cellulose, lignin, and hemicelluloses [19].

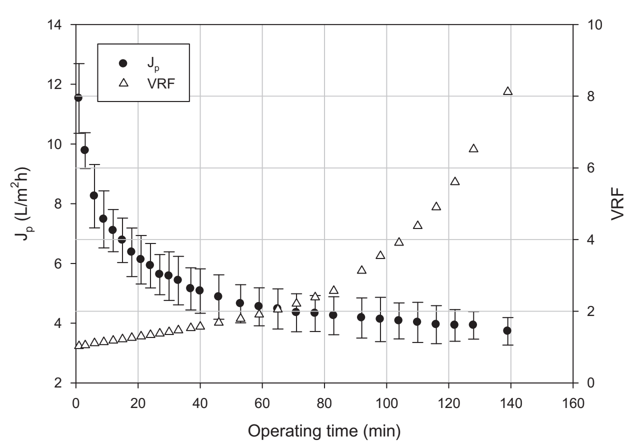

Fig. 1. UF of bergamot juice. Time course of permeate flux and VRF (TMP, 0.38 bar; CFV, 0.12 m/s; T, 20˚C).

The initial water permeability of the membrane was completely restored after the chemical cleaning.

The physico-chemical characterization of the bergamot juice before and after the UF process is reported in Table 3. Suspended solids were completely removed by the UF membrane while sugar compounds were recovered in the permeate stream as expected. The UF membrane retained only 4.9% of the total polyphenols and 19.2% of TAA. This was in agreement also with the low rejection observed for flavonoids (naringin, hesperidin, and neohesperidin) which was in the range of 7.2–14.8%.

Compared with the sum of the individual flavonoids quantified by HPLC analysis, spectrophotometric results from the total phenolic assay revealed an overestimation. This well-known phenomenon is caused by the interference of other reducing compounds with the unspecific Folin Ciocalteau reagent [20].

3.2. NF of clarified bergamot juice

The selected NF membranes, prior to their use in the filtration experiments with the clarified juice, were characterized with pure water in order to establish the dependence of the water flux on the TMP.

The obtained results showed that there is a linear increase of the pure water flux by increasing the pressure in the range of the TMP investigated (2–16 bar) for all tested membranes (Fig. 2). The NF PES10 membrane showed higher water permeability in comparison with the other two NF membranes in agreement with its highest MWCO; in general, higher permeability implies the membrane having larger pore size that favors water molecules to transport through. However, other factors, such as hydrophobicity/hydrophilicity, pore density, active layer thickness, and morphology of the membranes, in addition to MWCO, also affect this value. These factors can explain the lower permeability obtained with the N30F membrane, in comparison with the NF 270 membrane, although its MWCO is higher.

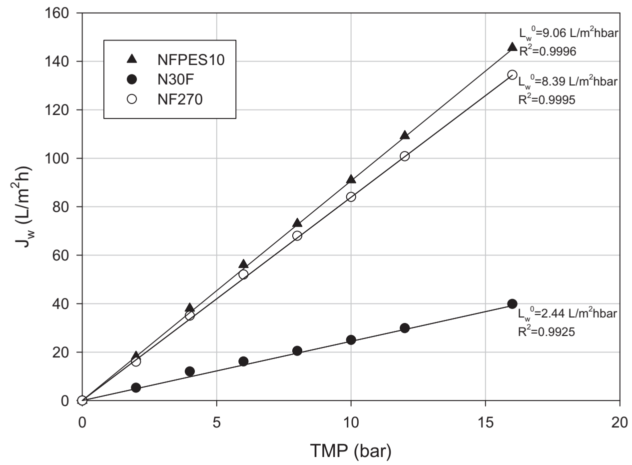

Fig. 2. Permeate flux variation with TMP for NF membranes using pure water (*T*, 20˚C).

Fig. 3(a)–(c) show the time course of the permeate flux at different TMP values for the investigated membranes in the treatment of the clarified bergamot juice according to a total recycle configuration. For each membrane, steady-state permeate flux values increased by increasing the applied pressure. It is also clear that the flux decay is more pronounced at higher TMP values.

The effect of TMP on the steady-state permeate fluxes is reported in Fig. 4. As can be seen the steady-state permeate flux increased linearly by increasing the operating pressure in the range of the TMP used. In addition, contrary to the water permeability measurements, the NF 270 membrane showed the highest values of permeate fluxes when compared with the PES membranes. This behavior can be explained assuming that in the treatment of the bergamot juice permeate flux is also affected by interactions between juice compounds and membrane polymer materials.

Table 3
Physico-chemical composition of bergamot juice before and after the UF process

Parameters	Feed	Permeate	Retentate
Suspended solids	15.1 ± 1.2	–	16.2 ± 0.8
Sugars (˚Brix)	6.0 ± 0.02	4.8 ± 0.05	5.8 ± 1.2
TAA (mM Trolox)	12.2 ± 1.7	9.2 ± 0.8	11.4 ± 0.6
Total polyphenols (mg/L gallic acid)	723.7 ± 19.1	627.9 ± 7.0	660.8 ± 9.7
Naringin (mg/L)	28.0 ± 2.2	27.0 ± 5.4	29.1 ± 4.5
Hesperidin (mg/L)	10.5 ± 0.2	9.8 ± 1.4	8.2 ± 2.8
Neohesperidin (mg/L)	23.2 ± 2.6	20 ± 1.8	23.5 ± 2.3

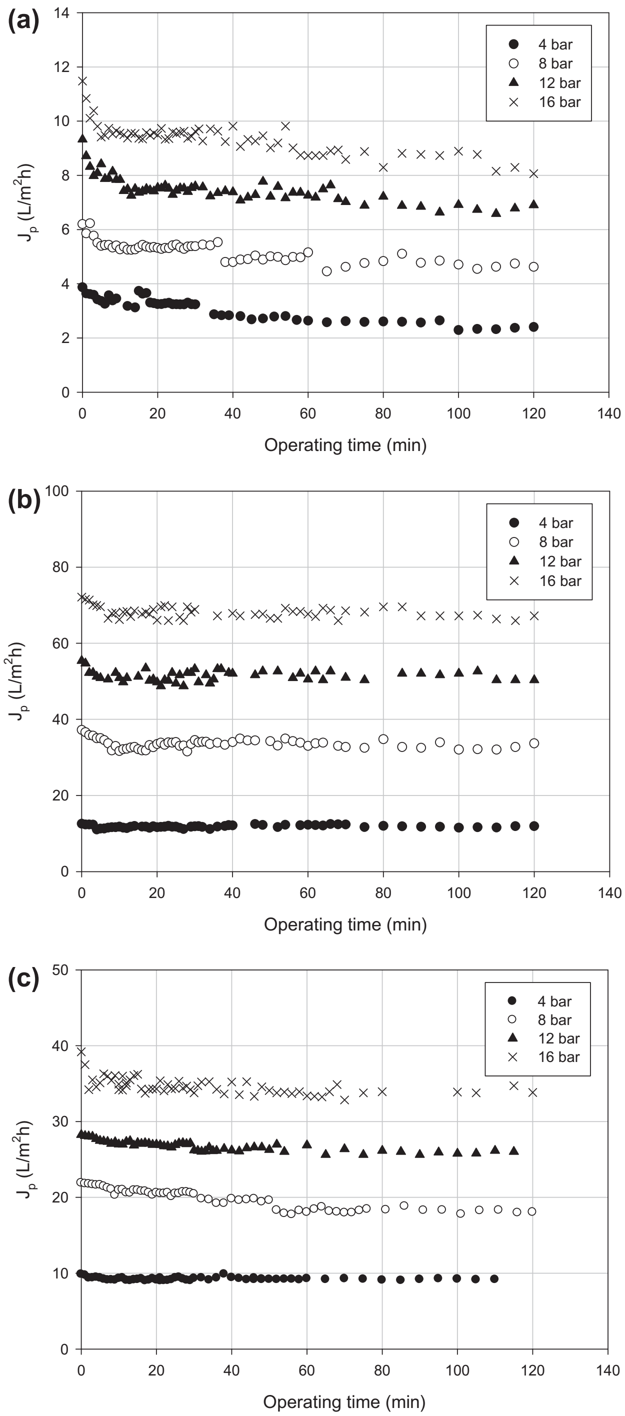

Fig. 3. NF of clarified bergamot juice. Time course of permeate flux at different TMP values (Total recycle configuration; T, 20˚C). (a) N30F membrane; (b) NF270 membrane; and (c) NF PES10 membrane.

A similar behavior was observed in the concentration of anthocyanins in acai juice and roselle extract, respectively, by using NF membranes [15,21]. In particular, the permeate flux increased linearly by increasing the operating pressure for both acai juice and roselle extract and among different tested membranes, the NF 270 exhibited the highest flux. These results confirm that the permeate flux is not strictly correlated with the MWCO of the membrane, suggesting that the chemical nature of the membrane plays a key role in membrane performance.

In Fig. 5, the effect of TMP on the rejection of total polyphenols for different NF membranes is shown. The increase of operating pressure in the range between 4 and 16 bar results in an increased rejection of phenolic compounds. Diaz-Reinoso et al. [10],

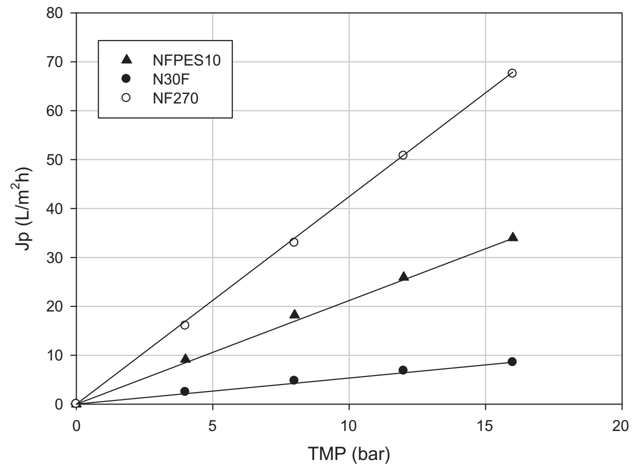

Fig. 4. NF of clarified bergamot juice. Effect of TMP on steady-state permeate flux (Total recycle configuration; T, 20˚C).

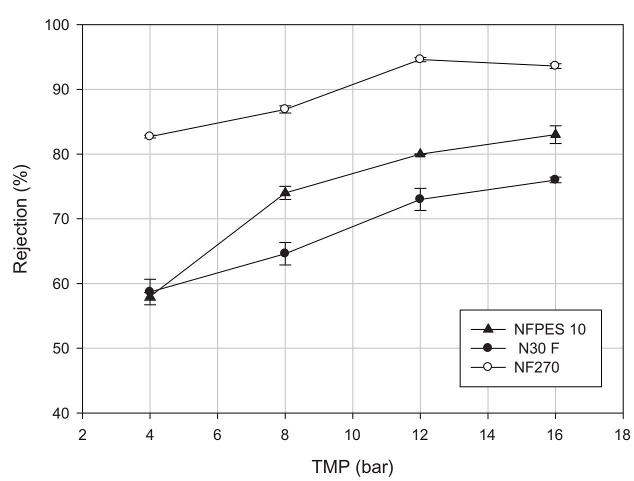

Fig. 5. NF of clarified bergamot juice. Polyphenols rejection at different TMP values (Total recycle configuration; T, 20˚C).

reported the same pressure-rejection polyphenols profile, during the treatment of aqueous extracts from distilled fermented grape pomace with NF membranes.

In Fig. 6, the permeate flux as a function of VRF related to the treatment of the clarified juice according to the batch concentration configuration is shown. Results confirmed highest permeate fluxes for the NF270 membrane.

In order to identify the degree of membrane fouling, the hydraulic permeability of the NF membranes was determined before and after the treatment of the clarified bergamot juice. In Fig. 7, the decline of water permeability of each membrane and the relative FI are reported. The NF PES10 and N30F membranes showed higher FI (with values of 75.4 and 76.3%, respectively) when compared with the NF 270 membrane (FI of 21.4%). This phenomenon can be attributed to the higher hydrophobicity of the PES membranes (contact angle values of 72˚ and 88˚ for NF PES10 and N30F membranes, respectively) in comparison with the NF270 membrane (contact angle of 27˚) [22,23].

Susanto et al. [24] and Cartalade and Vernhet [25] examined the interaction of polyphenols compounds on the surface and inside PES membranes. They showed that polyphenols can be adsorbed on membrane surface by hydrophobic interactions, polar interactions (dipole forces), and hydrogen bonds towards the additive polyvinilpyrrolidone used in the manufacture of the membranes.

The active layer of the NF270 membrane is a very thin semiaromatic piperazine-based polyamide layer. Steric exclusion and hydrophobic attraction have been proposed as the main interactions in the membrane-

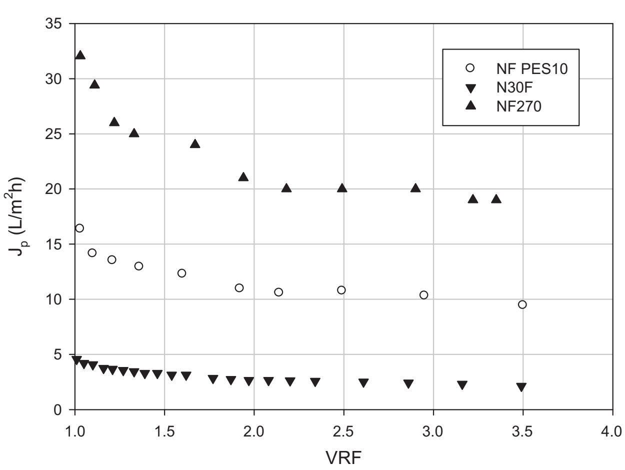

Fig. 6. NF of clarified bergamot juice. Permeate flux as a function of VRF. (Batch concentration configuration; TMP, 6 bar; T, 20˚C).

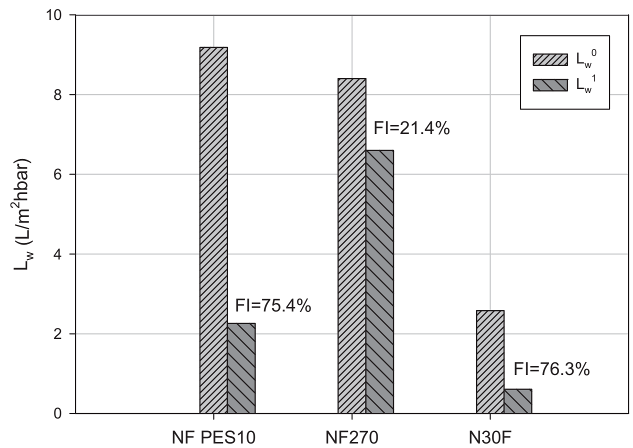

Fig. 7. Water permeability and FI of selected NF membranes (T, 20˚C).

solute interface for this type of polymeric material [26].

Analyses of total polyphenols, flavonoids, TAA, and sugars in samples coming from the NF treatment performed according to the batch concentration configuration are reported in Table 4. The NF 270 membrane showed a rejection towards phenolic compounds and TAA of about 82 and 88%, respectively; while the observed rejection towards flavonoids was in the range of 90–97.7%. A high rejection towards sugars (87.5%) was also measured. Therefore, this membrane showed a high rejection towards both sugar and phenolic compounds that can be concentrated in the retentate fraction. This is in agreement with the MWCO of this membrane (150–250 Da) and the molecular weight of the analyzed compounds which is in the range of 180 and 610 g/mol.

Results obtained with the N30F membrane showed that the content of flavonoids in the permeate fraction is very low due to the high rejection measured (85–90%) while the rejection towards TAA and polyphenols was of about 70–76%. The observed rejection towards sugar compounds was of 65%. This type of membrane allows only a partial purification of flavonoids from sugars. Different results were obtained with the NF PES10 membrane. The rejection of this membrane towards sugar compounds was of 35%, the lowest value measured if compared with the other two investigated membranes. Adversely, the rejection towards flavonoids was between 88.4 and 90.1%, while the rejection towards phenolic compounds and TAA was in the range of 71–80%, respectively. Therefore this membrane retains phenolic compounds having a lower molecular weight in comparison with its MWCO (1,000 Da). This behavior could be explained

Table 4
Analysis of TAA, sugars, flavonoids and total polyphenols in samples of bergamot juice treated by NF membranes (Batch concentration configuration; TMP, 6 bar; *T*, 20˚C; VRF, 3.5)

Membrane type	Sample	TAA (mM Trolox)	TSS (˚Brix)	Naringin (mg/L)	Neohesperidin (mg/L)	Hesperidin (mg/L)	Total polyphenols (mg/L)
NFPES10	Feed	8.6 ± 0.3	5.8 ± 0.2	28.0 ± 3.53	23.8 ± 5.09	8.0 ± 0.70	660.2 ± 9.6
	Permeate	3.3 ± 0.1	3.85 ± 0.4	7.0 ± 0.28	5.53 ± 0.82	2.5 ± 0.14	302.7 ± 5.4
	Retentate	16.7 ± 2.0	6 ± 0.8	65 ± 3.4	56.4 ± 8.76	22.0 ± 5.72	1,067 ± 10.1
N30F	Feed	7.2 ± 0.4	6.0 ± 1.2	14.36 ± 3.18	13.2 ± 2.19	5.24 ± 1.13	462.2 ± 9.35
	Permeate	3.7 ± 0.2	2.84 ± 0.5	5.0 ± 0.28	3.65 ± 0.73	1.64 ± 0.56	294.4 ± 8.34
	Retentate	16 ± 1.1	8.1 ± 0.45	34.0 ± 2.88	36.54 ± 2.68	11.0 ± 0.56	972.2 ± 17.34
NF 270	Feed	9.4 ± 0.1	4.06 ± 0.2	12.0 ± 1.97	12.7 ± 1.55	4.74 ± 0.93	602.7 ± 15.45
	Permeate	2.1 ± 0.3	1.0 ± 0.05	0.74 ± 0.13	0.76 ± 0.17	1.28 ± 0.19	207.2 ± 12.25
	Retentate	17.9 ± 1.3	7.8 ± 0.8	33.1 ± 1.34	33.54 ± 1.41	13.0 ± 0.56	1,148.1 ± 16.34

by steric effect, physicochemical interactions with the membrane or adsorption of phenolic compounds on membrane surface. These values also confirmed the high FI measured for this membrane. Similar results were also observed in the treatment of orange press liquor with PES membranes [27].

Rejections of NF membranes towards sugars and flavonoids (naringin, hesperidin, and neohesperidin) are summarized in Fig. 8. According to these data, the NF PES10 membrane showed the best performance in terms of purification of flavonoids from sugars due to the low retention of sugars and high rejection towards flavonoids. Similar results were reported by Cissè et al. [15] in the concentration of anthocyanins from roselle extract by using NP010 (former NFPES10) and N30F membranes.

According to the obtained results, the retentate fractions of the different investigated membranes show high antioxidant activity and appear as formulations of interest for nutraceutical applications (i.e. functionalized fruit juices) due to the presence of bioactive compounds (mainly flavonoids).

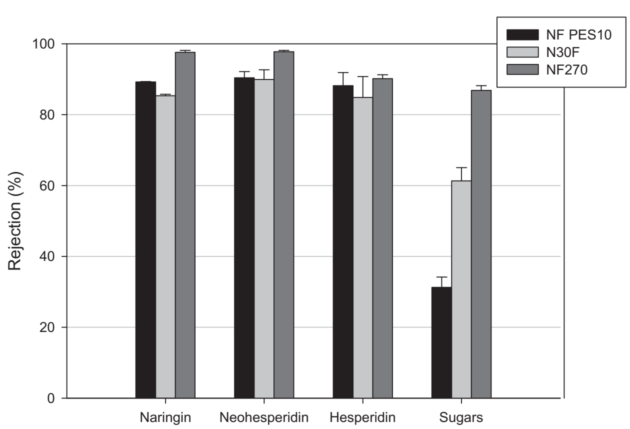

Fig. 8. Rejection of NF membranes towards flavonoids and sugars (Batch concentration configuration; TMP, 6 bar; *T*, 20˚C; VRF, 3.5).

4. Conclusions

The performance of different spiral-wound NF membranes in the treatment of ultrafiltered bergamot juice was evaluated in terms of productivity and selectivity towards phenolic compounds, TAA, and sugar compounds.

The NFPES10 membrane gave the lowest average rejection towards sugar compounds (35%) and high rejections towards both phenolic compounds (71%) and flavonoids (between 88.4 and 90.1%). Therefore, the use of NF PES10 membrane allows recovery of some sugars in the permeate stream, yielding the best separation of phenolic compounds from sugars.

The hydrophobic character of PES membranes, such as NF PES10 and N30F, accounts for the high FI measured for these membranes.

Retentate fractions produced in the NF process exhibit high antioxidant activity and can be considered of interest for nutraceutical applications.

The use of diafiltration to increase the removal of sugar compounds (i.e. in the treatment of the clarified juice with the NF PES10 membrane) and the combination of different NF membranes with different selectivity towards sugars and phenolic compounds represent useful approach to improve the purification of flavonoids in the retentate fraction and will be considered for further investigations.

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