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Cross-flow Filtration of Wines with Polymeric Membranes: The Influence of Wine Making Technology and Backpulse on the Fouling Process

»Cross-flow« filtracija vina s pomoću polimernih membrana: utjecaj tehnologije proizvodnje vina i povratnog pranja na proces onečišćenja membrana

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Summary

To study the influence of wine making technology on the fouling process, five microfiltration experiments were performed without backpulse, using different wines and a polymeric membrane. To evaluate the influence of backpulse on fouling, these experiments were repeated with bakcpulse every 2.5 minutes keeping constant all other parameters.

Results have shown that fouling caused by wine constituents is a complex phenomenon and its different components vary with grape cultivar and pomace contact. Adsorption of polyphenols to membrane matrix reduced membrane permeability, indicating that other constituents than wine macromolecules, may be involved in fouling.

Sažetak

Ispitivan je utjecaj tehnologije proizvodnje vina na proces onečišćenja membrana: provedeno je pet pokusa mikrofiltracije na polimernoj membrani bez povratnog pranja s različitim vinima. Da bi se provjerio utjecaj povratnog pranja na onečišćenja membrane, pokusi s povratnim pranjem ponavljani su svake 2,5 minute, a pritom su svi ostali parametri ostali nepromijenjeni.

Rezultati su pokazali da je onečišćenje membrana, uzrokovano sastojcima vina, složeni fenomen, a razni sastojci vina, mijenjaju se ovisno o vrsti grožđa i dodiru s tropom. Adsorpcija polifenola u strukturu membrane smanjuje njezinu propusnost pokazujući da i drugi sastojci, osim makromolekula vina, mogu biti uključeni u proces onečišćenja membrana.

Introduction

Cross-flow microfiltration is an efficient process for one step clarification and stabilization of wines. Nevertheless its use in enology is limited by the low average permeation fluxes obtained, which renders the process less interesting economically.

Flux decline during cross-flow microfiltration cycles is caused by phenomena like concentration polarization, gel layer formation, pore blocking, adsorption, etc. and it is one of the most limiting factors in the acceptance of membrane separation processes like ultrafiltration, reverse osmosis and microfiltration. It especially occurs for complex fluids that are used in biotechnology and food industries (e.g. fruit juices, wine, beer, milk). It is generally recognized that the adsorption of constituents of fluids like proteins, lipids, minerals, on membrane surfaces and matrix is a critical element in membrane fouling. Pro-

tein adsorption to polymeric membrane material is nearly an universal phenomenon. Although some research has been done into adsorption of polyphenols in polymeric membranes in static conditions *(1)*, few authors have reported the adsorption of polyphenols on polymeric membrane material in cross-flow operational conditions.

The aim of this paper is to contribute to demonstrating that:

(i) Polysaccharide content of wines before filtration is an insufficient criterion to explain the differences observed in performances of wine during microfiltration.

(ii) Phenolic constituents of wines adsorb to the membrane polymer during microfiltration, and contribute to the reduction of membrane permeability.

(iii) The amount of phenolic constituents adsorbed (estimated by the increase of water flux after washing with acidified methanol), increases with the increase of total phenolics that were in contact with membrane polymer.

Materials and Methods

Wine samples

Five different wines (three red and two white) were prepared from fully matured grapes. Red wines (called R_1, R_2, R_3) were prepared from grapes (cv. Carignan Noir) harvested in 1993 from a vineyard of the INRA (Pech Rouge-Narbonne) Experimental Station (Gruissan, France). A batch of Carignan Noir grapes was divided in three identical portions and then destemmed, crushed and fermented separately. The first wine (R_1) was obtained without pomace contact, i.e. the grapes being immediately pressed after crushing and the juice fermented separately for five days. The second wine (R_2) was prepared with eight days of pomace contact and the third (R_3) with thirty days of pomace contact. White wines (called W_1 and W_2) were prepared from grapes (cv. Maccabeu) harvested in 1993 from another vineyard of the same Experimental Station. A batch of Maccabeu grapes was divided in two identical portions, destemmed, crushed and fermented separately. The first wine (W_1) was obtained without pomace contact, i.e. the grapes being immediately pressed after crushing and the juice fermented separately for five days. The second wine (W_2) was prepared with eight days of pomace contact.

Microfiltration experiments

The microfiltration experiments were performed with a pilot plant equipped with a module of 96 capillaries (0.4 m^2) of modified polyethersulfone *(2)* provided by X-Flow (Almelo, The Netherlands). The maximum pore size was 0.2 μm und the average pore size was 0.08 μm. The experimental conditions were: tangential velocity 2 m/s; transmembrane pressure 1.2 bar; temperature 18 °C.

Each wine was submitted to two different microfiltration experiments (except wine W_2, which was submitted only without backpulse): first without backpulse, using volumes of filtered wine of 180 L and volumetric concentration factors of ≈12; second with backpulse every 2.5 minutes, using volumes of filtered wine of 170 L and volumetric concentration factors of ≈13.

Sequential recovery of fouling constituents

After each microfiltration experiment, the membrane module was submitted to the following treatments:

(I) *Extensive washing with water.* This treatment was intended to eliminate the surface colloidal deposit and fouling constituents other than adsorbed. At the end of each microfiltration experiment, the residual wine was pumped out of the pilot plant, and hot (60 °C) ultrafiltered water was circulated through the module. This operation was performed without recirculation of the ultrafiltered water, during 20 min with backpulse every 2.5 min. Previously, we checked that this period was long enough to measure zero absorbance at 280 nm at the exit of permeate tubing.

(II) *Washing with acidified methanol.* This treatment was intended to recover constituents (namely polyphenols) reversibly adsorbed. After the treatment described in (I) the membrane module was removed from the pilot plant and filled with 2.5 L of methanol acidified with 1 % hydrochloric acid. After 30 min contact, the acidified methanol was removed and replaced with 2.5 L of fresh acidified methanol and left in contact for additional 30 min. In this paper, we will call »methanolic extract« the first 2.5 L methanolic effluent of this washing process.

(III) *Regeneration.* After treatment (II), the membrane was regenerated by a cleaning procedure using detergents. The regeneration procedure was repeated until the inital membrane permeability was fully recovered.

Measurements of membrane permeability

After each washing step the water flux of the membrane was measured with ultrafiltered water at 20 °C. Water flux (expressed in L/h m^2 bar) was used as estimation of membrane permeability.

Results are presented in Table 3 and expressed as percent recovery of initial water flux obtained after each washing step. In Table 3, washing steps were noted with the symbols I, II, III signifying the corresponding increases after the washing steps, referred in precedent paragraph. For instance for wine R_2 without backpulse, we can know the exact value of water flux after washing step II by the following calculation: we first multiply 1350 L/h m^2 by 0.14 to obtain 189 L/h m^2, corresponding to the water flux recovery obtained with washing step I; then we multiply 1350 L/h m^2 by 0.19 to obtain 257 L/h m^2, corresponding to the water flux recovery going from washing step I to washing step II. The water flux after washing step II is (189 + 257) L/h m^2 = 446 L/h m^2.

Analysis

Total polysaccharides

Total polysaccharides were determined by precipitation in 80 % ethanolic acidified medium. The precipitate was washed twice with distilled water, centrifuged and dissolved in distilled water. The polysaccharide content of the precipitate was determined by the phenol sulphuric colorimetric method *(3)*. To the water-solubilised precipitate an equal volume of solution of phenol in water (φ = 5 %), together with a 5-fold volume of pure sulphuric

Table 1. Polysaccharide content of wines compared with their average permeation flux
Tablica 1. Maseni udjel polisaharida u vinima uspoređen s njihovim prosječnim postotcima

Wine		Pomace contact	Average permeation flux (without backpulse)	Average permeation flux (with backpulse)	Total polysaccharides (glucose equivalent)
		t/day	L/h m^2	L/h m^2	mg/L
White wines	W_1	0	62	133	209
	W_2	8	24	–	301
Red wines	R_1	0	91	198	188
	R_2	8	28	99	298
	R_3	30	35	90	293

acid was added, heated during six minutes at 100 °C and then the 490 nm absorbance was measured. A calibration curve was established in the same conditions with solutions of glucose in water of increasing concentration ranging from 100 to 1000 mg/L. The results were expressed in milligrams of polysaccharides (glucose equivalent) per liter of wine.

Total phenolic compounds

Total phenolic compounds were determined by measuring the absorbance of the wine at 280 nm (after 10-fold dilution with aqueous 2 % HCl). A calibration curve was established with standard solutions of (+)–catechin in methanol. The results were expressed in milligrams of catechin equivalent per liter of wine.

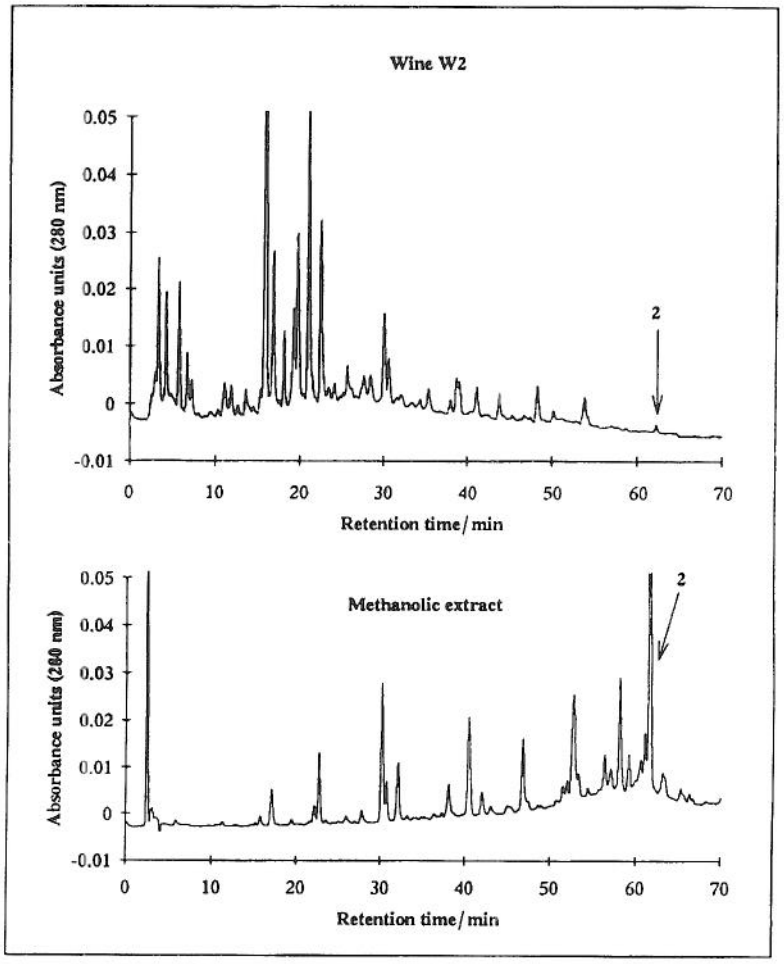

Fig. 1. Chromatographic profiles of the wine W_2 (top) and corresponding methanolic extract (bottom), recorded at 280 nm.
(2) Ethyl-p-coumarate
Slika 1. Kromatografski profil vina W_2 (gore) i odgovarajući metanolni ekstrakt (dolje) snimljeni pri 280 nm.
(2) etil-p-kumarat

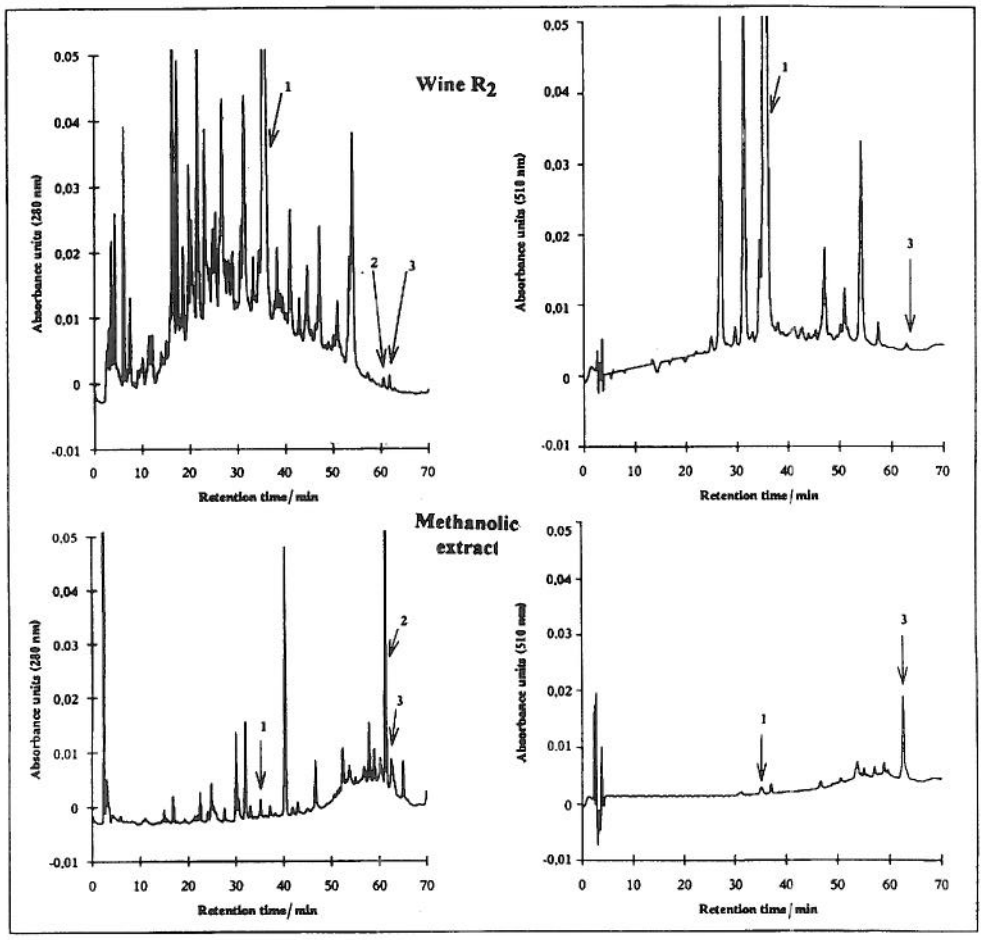

Fig. 2. Chromatographic profiles of the wine R_2 (top) and corresponding methanolic extract (bottom), recorded at 280 nm (left), and 510 nm (right). (1) Malvidin-3-glucoside; (2) Ethyl-p-coumarate; (3) Pigment X

Slika 2. Kromatografski profili vina R_2 (gore) i odgovarajućeg metanolnog ekstrakta (dolje) snimljeni pri 280 nm (lijevo) i pri 510 nm (desno). (1) malvidin-3-glukozid; (2) etil-p-kumarat; (3) pigment X

Total red pigments

Total red pigments were determined by measuring the absorbance of the wine at 510 nm (after 10-fold dilution with aqueous 2 % HCl). A calibration curve was established with standard solutions of malvidin-3-glucoside in aqueous 2 % HCl. The results were expressed in milligrams of malvidin-3-glucoside equivalent per liter of wine.

High performance liquid chromatography

Reverse phase high performance liquid chromatography (HPLC) of the wines and methanolic extracts was performed with a Waters (Millipore Corp., Milford, MA) system equipped with a Waters 990 Photodiode Array Detector. UV-visible spectra were recorded from 250 to 600 nm. The column was a Merck RP-18. Solvents: A, acetonitrile: water: formic acid (80:18:2); B, water: formic acid

Table 2. Polyphenol content of wines compared with their average permeation flux
Tablica 2. Maseni udjel polifenola u vinima uspoređen s prosječnim protocima vina

Wine		Pomace contact	Average permeation flux (without backpulse)	Average permeation flux (with backpulse)	Total phenolic compounds (catechin equivalent)	Total red pigments (malvidin-3-glucoside equivalent)
		t/day	L/h m^2	L/h m^2	mg/L	mg/L
White wines	W_1	0	62	133	427	0
	W_2	8	24	–	1314	0
Red wines	R_1	0	91	198	457	12
	R_2	8	28	99	3323	441
	R_3	30	35	90	2990	354

Table 3. Performances of wines during cross-flow microfiltration and recovery of membrane permeability after each washing step
Tablica 3. Karakteristike vina tijekom »cross-flow« mikrofiltracije i obnovljivost propusnosti membrana nakon svakog stupnja pranja

		Without backpulse					With backpulse every 2.5 minutes				
		White wines		Red wines			White wines		Red wines		
		W_1	W_2	R1	R_2	R_3	W_1	W_2	R_1	R_2	R_3
	Initial water flux / L/h m^2 bar	1350	1350	1350	1350	1350	1350	–	1350	1350	1350
	Average permeation flux / L/h m^2	62	24	91	28	35	133	–	198	99	90
I	Water flux recovery after washing with hot water / %	39	9	32	14	22	42	–	45	28	26
II	Water flux recovery after washing with acidified methanol / %	13	7	35	19	25	43	–	47	57	39
III	Water flux recovery after regeneration / %	48	84	33	67	53	15	–	8	15	35

(98:2). Gradient: 0 to 40 min: 5 to 30 % A; 40 to 60 min: 30 to 50 % A; 60 to 70 min: 50 to 80 % A, followed by washing and reconditioning of the column. Flow rate: 1 mL/min. Column temperature: 30 °C. Calibration curves were established by injecting increasing amounts of standards [malvidin-3-glucoside (Extrasynthèse, France), p-coumaric acid (Sigma, USA) as an equivalent ot ethyl-p-coumarate, and compound X (purified in the laboratory)], and measuring the corresponding areas of peaks at 310 nm (ethyl-p-coumarate) or 510 nm (malvidin-3-glucoside, compound X).

Results and Discussion

Chromatographic profiles of wines by HPLC compared with the corresponding methanolic extracts.

HPLC analysis was performed on each wine before microfiltration and on the corresponding methanolic extract (Figures 1 and 2 show two examples of chromatograms obtained). These analyses were intended to demonstrate the presence of adsorbed polyphenols in fouled polymeric membranes, and if possible, to identify them. Since methanolic extracts correspond to the release of adsorbed constituents from membranes previously washed with hot water, we admit that all non-adsorbed material was washed out from the membrane polymer with this operation. It is probably that, one part of adsorbed material was washed out too, but we can be quite sure that non-adsorbed molecules were not included in the analyses.

Chromatograms obtained were similar for all comparative cases studied of wines and methanolic extracts. They have shown in all cases complex profiles with a large diversity of constituents. Nevertheless, significant differences could be seen between the profiles of a wine and of the corresponding methanolic extract. Methanolic extracts have shown a predominance of weakly polar constituents (which elute late in the HPLC gradient), whereas wines contained mostly polar constituents (which elute early in the HPLC gradient). Although the phenolic composition of the wines, determined from the chromatographic profiles and from the UV-visible spectra (recorded for each compound by means of the photodiode array detector), were similar to those reported in literature *(4,5)*, the corresponding methanolic extracts contained mostly unknown compounds.

Ethyl-p-coumarate (λmax = 310 nm) was tentatively identified as one of the major UV-absorbing phenolic constituent of wines selectively adsorbed onto the membrane and solubilised by the acidified methanol, whatever the type of wine. Indeed, it was present as trace amounts in all wines and accumulated dramatically as seen in Figures 1 and 2.

Besides phenolic acids, and unknown red pigment (λmax = 505 nm) eluting at the end of the gradient, accumulated too in the case of rosé and red wines (R_1, R_2, R_3), although present as trace amounts in these starting wines. Conversely, malvidin-3-glucoside (λmax = 529 nm), in spite of being the major anthocyanin of these wines, was hardly visible in chromatograms of methanolic extracts. Apart from these compounds visible in the chromatograms as sharp peaks, undifferentiated red constituents eluting late in the gradient as a massif were also strongly enriched by adsorption onto the membrane material. The hot water washing procedure was thus very efficient since it removed almost all water soluble phenolics present in the fouling material (considered roughly as non-adsorbed material, including malvidin-3-glucoside). So, the percent recovery of the initial permeability after washing with acidified methanol could be attributable to phenolics constituents, which were preferentially adsorbed onto the membrane constituting polymer, probably by hydrophobic interactions.

Wine filterability in realtion to polysaccharide content of wines

The filterability of wines was evaluated by the average permeation flux (L/h m^2) over the filtration period and compared with their polysaccharide content (Table 1). Two groups of wines are easily distinguished with regards to the above parameters (filterability, polysaccharides). The wines within these two groups had similar polysaccharide content and exhibited similar filterability. First group: wines obtained after preliminary separation of pomace (W_1, R_1), and second group: wines prepared with pomace contact (W_2, R_2, R_3). Whatever the duration of pomace contact and type of cultivar (white or red), pomace contact induced higher levels of polysaccharides and a significant decrease in filterability. These results agree with those reported in literature.

Nevertheless, polysaccharide content of wines before microfiltration is an insufficient criterion to completely explain the differences observed in wine performances during microfiltration. For instance, wines W_1 and R_1 had the same approximate level of total polysaccharides and wine R_1 filtered better than wine W_1. The same reasoning could be made comparing wines W_2 and R_2 or R_3.

Wine filterability in realtion to phenolic content of wines

The filterability of wines was also compared with their phenolic content (Table 2). The same two groups referred above were found. Wines obtained after preliminary separation of pomace (W_1, R_1) had the lowest and similar phenolic content and exhibited the highest filterability. Conversely, wines prepared by fermentation with the presence of pomace (W_2, R_2, R_3), whatever the duration of pomace contact and type of cultivar (white or red), were richer in phenolics and showed the lowest filterability.

So, the already known better filterability of rosé and white wines as compared to red wines *(6,7)*, could be partially related to their lower total phenolic content.

However, wine filterability was not only related to the level of total phenolics since wine W_2 exhibited the lowest filtration flux although it was devoid in anthocyanins and poorer in total phenolics than red wines (R_2, R_3). We need to analyze the results in more detail (by groups of phenolic compounds), to obtain more conclusions. So, for the same approximate level of total phenolic content (e.g. W_1 compared to R_1), the presence of red pigments seemed to improve wine filterability. Also, in spite of much larger amounts of total phenolics, wines R_2 and R_3 behave better in cross-flow microfiltration than wine W_2. Again, this indicates that the presence of red pigments seems to improve wine filterability.

According to literature, in red wines, condensed tannins complexed with anthocyanins give macromolecular structures with increased solubility and stability *(8,9)*. This is in agreement with our results, since we admit that condensed tannins adsorb to membrane polymer and have a role in fouling. According to this hypothesis, in rosé and red wines (R_1, R_2 and R_3), the presence of anthocyanins leads to the formation of complexes between these pigments and tannins that decrease the intensity of tannin adsorption onto the membrane polymer (and thus increase permeation flux). Conversely, in white wines being devoid of anthocyanins, condensed tannins are not complexed and may have an increased affinity to polymeric membrane, and thus adsorption is larger.

Wine filterability in realtion to backpulse

The contribution of each washing step to the recovery of membrane permeability is expressed in Table 3. Values represent the gain (in percent of the initial water flux) of membrane permeability obtained after each washing procedure.

Results expressed in Table 3 also show that, for the cases in study, backpulse every 2.5 minutes increased average permeation flux whatever the cultivar type and pomace contact.

Microfiltration experiments without backpulse

The contribution of water soluble constituents (washing step I), presumably mostly polysaccharides and other weakly adsorbed material, as percent recovery, ranged from 14 to 32 % (red wines) and from 9 to 39 % (white wines). It must be noted that W_1 and R_1 exhibited a similar recovery level, while other wines showed significantly lower figures (especially W_2). The washing procedure using acidified methanol (step II), regenerated 19 to 35 % (red wines), and 7 to 13 % (white wines) of the inital membrane permeability, indicating a noticeable contribution of strongly adsorbed fouling material (mostly polyphenols).

A good agreement was found between average permeation fluxes and the percent recovery after washing with hot water (step I). This observation could not be related to the concentration of wines in soluble polysaccharides since wines obtained without pomace contact (white wines, e.g. W_1) or after a short contact duration (rosé wines, e.g. R_1), contain less polysaccharides ($\approx$ 50 %) than wines prepared in the presence of pomace (e.g. W_2, R_2, R_3) *(10)*. This correlation might simply be due to the cohesiveness of the weakly adsorbed fouling material which must be looser in the case of W_1 and R_1, due to lower pressure drop through capillaries. No such correlation could be observed after washing with acidified methanol; however, since polysaccharides are strictly methanol insoluble, the percent recovery after washing step II, especially high in the case of R_1, was to be attributed to fouling phenolic compounds.

Microfiltration experiments with backpulse every 2.5 minutes

More or less the same comments made for the preceding case, can be made for microfiltration experiments with backpulse every 2.5 minutes.

Nevertheless, in this case higher recoveries of water flux after washing step II (acidified methanol), could be obtained. This corresponds to a higher mass of phenolic constituents strongly adsorbed.

Since backpulse reverses the product flow through the membrane pores every 2.5 minutes, this procedure

increases the total volume of wine that was in contact with membrane polymer during the microfiltration experiment, and thus the total amount of polyphenols in contact.

According to the Langmuir's adsorption law, if saturation is not reached, the amounts of polyphenols adsorbed should increase with the increase of amounts in contact with the membrane polymer. This can be at the origin of the higher recoveries of water flux observed after washing step II in microfiltration experiments with bakcpulse.

Conclussion

As expected, wine making technology influences wine performance in cross-flow microfiltration. Fermentation performed with the presence of pomace, whatever the cultivar type (red or white), yields strongly fouling wines, due to higher levels of polysaccharides and polyphenols. So, the role of wine polyphenols in fouling, as it can be influenced by wine making technology, could be partially demonstrated.

Weakly polar phenolics were preferentially adsorbed onto the membrane polymer, as evidenced by the comparison between chromatographic profiles of wines and methanolic extracts. Ethyl-p-coumarate and pigment X (an unknown red pigment the structure of which is being elucidated), were the major molecules adsorbed.

The presence of red pigments improved wine filterability, probably due to a phenomenon of complexation anthocyanins-tannins, which decrease the intesity of adsorption of these latter constituents.

Backpulse increased average permeation fluxes, as well as the amount of polyphenol adsorbed. This apparent contradiction is probably due to the complexity of fouling, which makes, in spite of the fact that backpulse increases adsorption of polyphenols, its contribution to removal of other causes of fouling (like pore blocking and concentration of polarization) greater, and the final result is an increase of permeation flux.

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