Research Article

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Tartaric acid and polyphenols recovery from winery waste lees using membrane separation processes

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Abstract

BACKGROUND: Wine waste lees are currently partly exploited for tartaric acid (TA) production, through an environment-offensive process, while concurrently occurring bio-active polyphenolic compounds are wasted. This paper, deals with the development of an integrated, environment friendly process, using mild conditions, for recovering TA with simultaneous exploitation of total polyphenols (TPP) from wine lees.

RESULTS: A first process step, described in a previous publication, yields a liquid stream containing approx. 44.2 g L^{-1} TA and 323.3 mg GAE L^{-1} TPP. In the present study, various ultrafiltration and nanofiltration membranes are assessed, in bench-scale filtration tests, for their efficiency in separating the two main products (i.e. TA and TPP) from this stream. The most promising process configurations are also tested in a laboratory-scale cross-flow membrane filtration pilot plant and assessed concerning the separation efficiency and the membrane filtration performance, to determine process feasibility at industrial scale.

CONCLUSIONS: The results show that a nanofiltration membrane with typical pore size approx. 1 kDa exhibits satisfactory separation and low-fouling filtration performance. The permeate, containing the bulk of TA (approx. 42.6 g L^{-1}) could be used for TA recovery, whereas the concentrate, with antioxidant activity (EC_{50} = 10.0 mg sample mg^{-1} $DPPH^{\cdot}$), could be further purified to obtain polyphenolic-rich formulations.

Keywords: wine by-products; membrane technology; ultrafiltration; nanofiltration; antioxidant activity

ABBREVIATIONS

CWF	Clean water flux
GAE	Gallic acid equivalent
NF	Nanofiltration
PES	Polyethersulfone
TA	Tartaric acid
TMP	Transmembrane pressure
TPP	Total polyphenols
TPS	Total polysaccharides
UF	Ultrafiltration
VCF	Volumentric concentration factor

INTRODUCTION

The winemaking process generates a significant amount of wastes such as grape pomace, wine lees, and winery wastewater from cleaning, washing and other winemaking processes. Quantitative and qualitative characteristics of winery wastes vary widely according to the type of wine produced, the winemaking technologies employed, the size of winemaking facilities and the season. The wine industry operates seasonally and generates a large volume of wastes during the grape harvest and racking periods over approx. 3–4 months each year.[1,2] The treatment and disposal of winery wastes are critical issues in wine-producing regions with large-scale production. In a more environmentally holistic approach, these effluents are a potential source for the recovery of added-value compounds; in an extensive review, Galanakis[3] presented an appropriate methodology (the so-called '5-Stages Universal Recovery Process') dealing with recovery of high-added value compounds from food wastes (including winery wastes) through established or emerging technologies.

Grape pomace consists mainly of peels, seeds and stems, and accounts for about 20–25% of the weight of the grape crushed for wine production; it is estimated that for each 6 L of wine, 1 kg of grape pomace is produced which is mainly directed for rather low-value animal feed and compost.[4,5] Valorization of grape pomace through extraction of bioactive compounds has been extensively studied in recent years.[6–11] Grape seeds are rich in extractable phenolic antioxidants such as phenolic acids, flavonoids, procyanidins and resveratrol, while grape skins contain anthocyanins.

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Polyphenols are secondary metabolites typically found in plants. They are characterized by one or more phenolic groups in their structure[12] and can be classified into different groups, namely phenolic acids, flavonoids, stilbenes and lignans.[13] These phenolic compounds possess well-known antioxidant properties, acting as free-radical scavengers, electron or hydrogen donors and strong metal chelators, with significant potential human-health benefits.[14] Furthermore, according to Ky *et al.*,[15] they can prevent the oxidation of nucleic acids, proteins and lipids, which may initiate degenerative diseases. The health benefits of recovered polyphenols have attracted the interest of researchers, food industry and nutraceutical industry.[5]

Wine lees is another wine-making waste stream comprising deposits of dead yeasts, particulates and other precipitates at the bottom of wine vats after fermentation, stabilization or racking (transfer of wine from one container to another), representing approx. 2–6% of the total volume of wine produced.[16] Wine lees have attracted less interest for valorization, despite their content of polyphenolic substances, in concentrations ranging between 1.9 and 16.3 g kg^{-1} on a dry basis[17] and 1.90 ± 0.24 g L^{-1} on a wet basis,[18] tartrates in concentration between 100 and 150 kg ton^{-1},[19] and other yeast deriving bio-active substances, like b-glucan[20] and squalene.[21,22] Currently, wine lees are the main source of commercial tartaric acid production, an important organic acid, which is widely applied in the food, pharmaceutical, and chemical industry. The traditional processes of tartaric acid production, including precipitation, acidification, extraction, crystallization, distillation, ion-exchange, and adsorption, are complicated, costly, labor intensive, and environmentally-offensive, given the significant quantities of obnoxious calcium sulfate waste sludge.[23,24] Moreover, the valuable polyphenolic substances are destroyed during the harsh chemical and heating treatment steps of conventional TA production.

Novel technologies, including membrane processes, hold promise to overcome problems and barriers of traditional technologies, such as the one described in the previous paragraph, and are currently assessed by researchers for commercial implementation.[25,26] Salgado *et al.*[27] presented a process scheme for recovering tartaric acid or calcium tartrate and fermentation nutrients from wine lees comprising an initial acidification, centrifugation for separating liquid from solid fractions, drying of solid residues and calcium tartrate precipitation using $CaCO_3$ and $CaCl_2$ salts. Their method was mainly based on the conventional tartaric acid production process and did not include separation and recovery of polyphenolic substances. Perez-Serradilla and de Castro,[28] and Tao *et al.*[29] proposed advanced extraction techniques for polyphenols recovery from wine lees, using microwave-assisted and ultrasound-assisted extraction techniques, respectively. In these studies, both microwave and ultrasound extraction with various ethanol/water mixtures exhibited improved results in terms of processing time and extraction efficiency, compared with conventional techniques; tartaric acid recovery was not attempted in either of the aforementioned studies.

Efficient, cost-effective and environmentally benign utilization of wine lees is important for increased profitability and minimal environmental impact of the entire wine-making process. Giacobbo *et al.*[30,31] have described membrane-based processes for recovery of antioxidant compounds and polysaccharides. Membrane processes were proposed due to their inherent advantages, compared with conventional technologies, such as low energy requirements, gentle product treatment at low to moderate temperatures, good separation efficiency and high selectivity.[26] Ultrafiltration and nanofiltration membranes were tested for separation of polyphenols and polysaccharides, and a membrane process scheme was developed that achieved efficient separation of these two classes of substances. However, tartaric acid, which can also be recovered from the wine lees was not considered in these studies, and probably was removed as a waste stream during the pre-treatment step of wine lees sedimentation.[30,31] Galanakis *et al.*[32] used membrane technology to separate and fractionate phenolic compounds from hydro-ethanolic extracts from wine lees, collected after the first decanting stage. 100 KDa and 20 KDa UF membranes were not capable of fractionating phenolic classes; however, they could separate them from pectins and their derivatives. Moreover, a 1 KDa NF membrane provided a successful technique to separate different phenolic classes such as hydroxycinnamic acids, flavonols and anthocyanins based on their polarity.

In a previous study, Kontogiannopoulos *et al.*[33] described a first process step for the development of an effective and environment-friendly process for recovering tartaric acid and bioactive polyphenols from winery waste lees. This process does not include wine lees drying (thus avoiding the corresponding high-energy cost); it is performed under mild conditions (ambient pressure and temperature) and does not produce calcium sulfate waste sludge as the conventional process does. The first step described, which is focused on tartrates dissolution, comprises the addition of cation exchange resin and acidified water. At the identified optimum conditions of 3.5 g resin and 10 mL of acidified water (pH = 3.0) per g of dry lees, sampled after the stabilization process of red wine (Syrah), a liquid stream is obtained after resin removal by centrifugation. The latter contains approx. 44.2 g L^{-1} of tartaric acid and 323.3 mg GAE L^{-1} of polyphenolic substances, which favors subsequent recovery of both tartaric acid and polyphenolic substances.

In the light of the aforementioned work, the objective of this study is to further develop the processes for recovering tartaric acid in a cost-effective and environment-friendly manner, with simultaneous exploitation of the polyphenolic bioactive compounds of wine lees. Specifically, determination of membrane-process conditions is pursued that will allow separation of tartaric acid and polyphenolic substances, will not tend to degrade the sensitive polyphenols, and will allow fouling-free membrane operation. In the following, the steps are described toward development of a realistic process, which involve UF and NF membranes of various pore sizes and materials. The membranes are assessed for their efficiency in fractionating the two main products (i.e. tartaric acid and polyphenols) through bench scale dead-end filtration tests, followed by laboratory-scale pilot-plant testing of the most promising process configurations. The resulting process streams are analyzed concerning the concentration of TA and TPP, as well as concentration of K^+ and of polysaccharides (TPS). Potassium interferes with tartaric acid recovery due to precipitation of the sparingly soluble potassium bitartrate, whereas polysaccharides are substances of low value – compared with polyphenols – thus reducing the purity and value of recovered bioactive substances. Taking into account that polyphenolic substances are very sensitive, the anti-oxidant properties of the streams produced are also evaluated. Finally, membrane filtration performance concerning membrane fouling, is assessed to determine the feasibility of employing the developed process at industrial scale.

Table 1. Characterization of the wine lees used in this study

Parameter	Value
pH	3.57
Electrical conductivity (mS cm^{-1})	0.87
Water content (%)	58.2
Total solids (g L^{-1})	499.4
Tartaric acid content (mg g^{-1} dry lees)	575.8
Total polyphenolics (mg GAE g^{-1} dry lees)	11.25

MATERIALS AND METHODS

Wine lees

Red wine lees (Syrah variety) samples were provided by a 600 ha winery (Ktima Gerovasileiou, Epanomi, Greece) during the 2013–2014 season. The samples were collected from the bottom of a stainless steel wine stabilization tank. The collected wine lees were immediately transferred to the laboratory and stored in a freezer (~20 °C); a representative sample of the wine lees was immediately analyzed to specify basic physicochemical parameters as summarized in Table 1.

Wine lees dissolution

In this work, the liquid fraction from wine lees dissolution obtained in a previous study[33] has been used as feed to the various membrane filtration experiments. Briefly, the wine lees samples were diluted with DI water at a water dosage 10 mL g^{-1} dry lees and acidified with H_2SO_4 solution (18 N) at pH = 3.0. A commercial strongly acidic, gelular cation exchange resin (Lewatit® MonoPlus S 108 H) was added (3.5 g resin g^{-1} dry lees) for the removal of potassium cations; before its use it was prepared according to the manufacturer's protocol.[33] The mixture was stirred at 300 rpm with a magnetic stirrer for 4 h at ambient temperature (i.e. 20–25 °C). Samples were obtained and centrifuged at 8000 × g for 15 min at 4 °C to remove particulate matter and cation exchange resin, and the resulting liquid fraction was subsequently used for the membrane filtration experiments. Deionized water and chemical reagents (Sigma–Aldrich) of analytical grade were used in all experiments.

Membranes

The main characteristics of the various ultrafiltration and nanofiltration membranes employed in this study are summarized in Table 2. Before filtration, the membranes were thoroughly rinsed with water to remove the conservation chemicals (sodium meta-bisulfite and/or glycerin). Selected membranes included ultrafiltration and nanofiltration polymeric membranes with a wide range of typical pore sizes between approx. 250–300 Da (NF270 and NF90) and 20.000 Da (M-U2540). It should be noted that the pH value of the feed was close to the lower end of the operating pH range of all the membranes. Flat-sheet membranes were used in dead-end filtration tests for screening of favorable filtration properties, whereas in laboratory cross-flow filtration tests both tubular and hollow-fiber membranes were used due to the potential for higher fouling resistance.

Bench-scale dead-end filtration test cells

Experiments were conducted in dead-end filtration mode with agitation using an experimental set-up comprising a high-pressure stirred cell, presented in more detail elsewhere.[34,35] The experimental set-up included a SEPA-ST model cylindrical test cell (Osmonics Inc., Minnetonka, MN) accommodating a membrane disk of diameter 4.0 cm (resting on a porous support), which corresponds to an active filtration area of 12.6 cm^2. All tests were performed with constant fluid agitation (250 rpm). The filtration cell was filled with test liquid (200 mL). High-pressure nitrogen was used to control the applied pressure. The prevailing trans-membrane pressure (TMP) was monitored by a manometer (Cole-Palmer Instr. Co., USA) connected at the cell inlet. The permeate flux was determined by measuring the permeate mass through an electronic balance (PL602-S Mettler-Toledo AG, Switzerland), which was connected to a computer for data acquisition. The temperature was maintained constant (25 °C) in all tests.

Laboratory-scale cross-flow filtration pilot unit

All cross-flow filtration experiments were conducted in a custom-made laboratory-scale cross-flow filtration pilot unit, schematically presented in Fig. 1.

The pilot plant comprised a feed tank, the feed pump, the membrane module, a balance for permeate flow measurement, the necessary measuring devices, as well as recording and control equipment. A medium pressure (max 12 bar) feed diaphragm-pump (HydraCell, Wanner Engineering, Inc.) with chemically resistant wetting materials (Kynar) was used to feed the membrane module; the flow of the feed pump was controlled through adjustment of the rotating speed through an AC inverter. The membrane pressure vessel was custom-made from 316SS and could accommodate various commercial tubular membranes; alternatively, other membrane modules and/or pressure vessels were also employed through fittings adjustments. Both in the inlet and outlet of the membrane there were pressure transducers to measure the relative pressure, whereas the trans-membrane pressure was regulated through a valve. Both flow and temperature of the feed stream were also recorded through electronic measuring devices. The concentrate of the membrane filtration was recycled to the feed tank and the membrane permeate either recycled to the feed tank (recirculation mode) or removed and collected in the permeate tank (concentration mode) through a manual three-way valve. The volumetric flow rate of the membrane permeate was calculated by measuring the permeate mass flow with an electronic balance, connected to a PC, and the density of the permeate.

The experimental procedure comprised the following steps. Initially, the membranes were rinsed with clean DI water and the pilot plant was operated in concentration mode using DI water for at least 4 h to remove chemical preservatives. The conductivity and the total organic carbon (TOC) concentration of both the feed and the permeate were monitored. The initial cleaning step was paused when both the conductivity and the TOC concentration of the feed and the permeate differed by no more than 5%. Clean water flux (CWF) tests were also performed at various operating trans-membrane pressures (TMP) to calculate the initial clean membrane permeability. The temperature of the membrane feed was not regulated, and thus it fluctuated between 20 and 30 °C, according to the ambient conditions and the pump operating parameters. However, feed temperature was constantly recorded and all measurements were corrected to a reference temperature of 25 °C, according to the following equation.

$$L_{25^\circ C} = \frac{L_T \cdot \eta_T}{\eta_{25^\circ C}} \quad (1)$$

Table 2. Basic membrane characteristics

Model (Company)	Type	Material	pH operating range	Max operating pressure (bar)	Max operating temperature (°C)	MWCO/ rejection
Bench-scale dead-end filtration						
M-U2540 (AMI Membranes)	UF flat sheet	Polyacrylonitrile	3–9	14	50	20 000 Da
HYDRACoRe 70pHT (Hydranautics)	NF flat sheet	Sulfonated Polyethersulfone	1.0–13.5	41	70	720 Da
NF270 (Dow Filmtec)	NF flat sheet	Polypiperazine	3–10	41	45	97.0 % $MgSO_4$
NF90 (Dow Filmtec)	NF flat sheet	Polyamide	2–11	41	45	97.0 % $MgSO_4$
Laboratory-scale cross-flow filtration						
ESP04 (PCI Membranes)	UF tubular	Modified Polyethersulfone	1–14	30	65	4 000 Da
HFW1000 (Pentair)	NF hollow fiber	Modified Polyethersulfone	3–11	6	40	1 000 Da

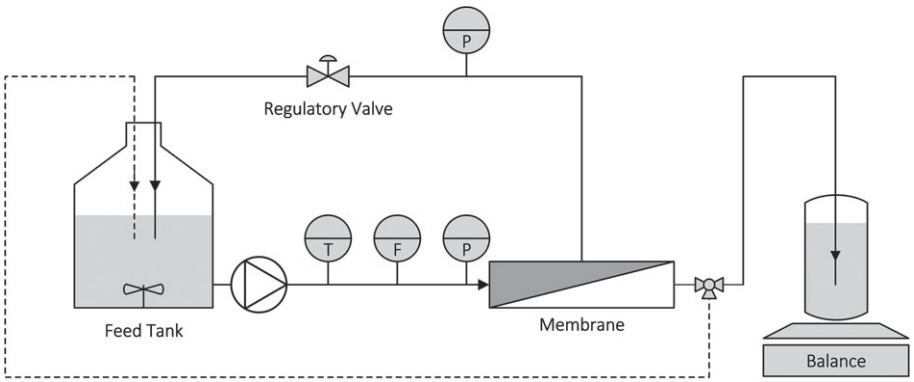

Figure 1. Custom made laboratory-scale cross-flow filtration pilot unit.

where:

$L_{25°C}$ = the membrane permeability at the reference temperature of 25 °C (L m^{-2} h^{-1} bar^{-1})

L_T = the membrane permeability at the given temperature T (L m^{-2} h^{-1} bar^{-1})

η_T = the permeate viscosity at the given temperature T (Pa s)

$\eta_{25°C}$ = the permeate viscosity at the reference temperature of 25 °C (Pa s)

Next, the membranes were compacted through operation in recirculation mode for 2 h, using DI water, and at TMP at least 20% higher than that of experiments. This step was employed to minimize errors in the permeate flux calculations due to membrane compaction and alteration of the initial clean membrane filtration resistance. The cross-flow velocity used in all the compaction steps and the cross-flow filtration experiments was 0.34 m s^{-1}, which corresponds to a feed volumetric rate of approx. 2 L min^{-1}. The liquid fraction that originated from the dissolution of the wine lees was tested in concentration mode to determine the variation of permeate flux as a function of the operation time and volumetric concentration factor (VCF). The VCF was defined as:

$$VCF = \frac{V_{Feed}}{V_{Feed} - V_{Permeate}} \quad (2)$$

The targeted VCF was around 4, which was considered a typical operating value of future industrial scale processes. Samples from the feed, the permeate and the concentrate were collected and analyzed for pH, conductivity, tartaric acid concentration, total polyphenols, total polysaccharides, and K^+ concentration. The rejection rates of the various dissolved substances were calculated based on their initial respective concentrations in the feed. Furthermore, the separation factor of the tartaric acid (compound A) and the polyphenols (compound B) was calculated based on the following equation:

$$S_FAB = \frac{\left[\frac{C_A}{C_B}\right]_{Permeate}}{\left[\frac{C_A}{C_B}\right]_{Concentrate}} \quad (3)$$

After the filtration test, the membranes were thoroughly rinsed with DI water and subsequently chemically cleaned by rinsing the membrane in recirculation mode with DI water that contained 0.5% v/v Mucasol® (BrandTech Scientific Inc.), as a universal detergent, and 0.15% v/v NaOCl solution (14% w/v available Cl·), which resulted in approx. 200 mg L^{-1} Cl·. The pH of the cleaning solution was approx. 10.5. The chemical cleaning lasted for approximately 8 h. Next, the CWF was calculated in order to assess the chemical cleaning efficiency.

Analytical methods

The different parameters analyzed included pH and conductivity, using a WTW inoLab multiparameter analyzer (pH/ION/Cond 750) coupled with a pH Electrode (SenTix81) and a conductivity electrode (TetraCon 325). These analyses were carried out according to the standard methods for examination of water and wastewater.[36] Tartaric acid concentration was determined by reversed-phase HPLC using a Shimadzu (LC-10 AD VP) liquid chromatograph fitted with a AQUASIL C18 (Thermo Scientific) column,

Table 3. Physico-chemical characteristics of the feed, permeate and concentrate obtained in dead-end bench-scale filtration experiments

Membrane type		Volume (mL)	VFC	Conductivity (mS cm^{-1})	TA (mg L^{-1})	K^+ (mg L^{-1})	Total polyphenols (mg GAE L^{-1})	Total polysaccharides (mg L^{-1} glucose)	Anti-oxidant activity (mg sample mg^{-1} DPPH·)
M-U2540	**Feed**	200	4.4	3.27	10,929	299.8	157.6	252.2	-
	Permeate	155		2.95	10,553	257.8	83.6	96.3	-
	Concentrate	45		3.02	11,813	264.5	479.3	452.5	13.4
70pHT	**Feed**	140	1.3	2.35	18,721	565.7	293.2	309.4	-
	Permeate	30		1.59	16,922	289.2	23.7	42.3	-
	Concentrate	110		2.52	18,753	469.0	332.1	398.5	27.5
NF270	**Feed**	200	2.5	2.35	18,721	565.7	293.2	309.4	-
	Permeate	119		1.78	6,053	152.7	14.8	7.3	-
	Concentrate	81		3.05	30,583	707.6	535.7	572.8	21.3
NF90	**Feed**	200	1.8	2.35	18,721	565.7	293.2	309.4	-
	Permeate	30		0.67	814	31.3	5.8	6.4	-
	Concentrate	170		2.51	19,938	509.5	327.7	402.7	28.2

5 µm, 250 mm × 4.6 mm (i.d.) at 45 °C, and coupled with a Diode Array Detector (SPD-M20A) (Shimadzu) at 210 nm. The mobile phase of the applied isocratic elution consisted of 0.05 mol L^{-1} KH_2PO_4 (pH 2.81) at a flow rate of 1.25 mL min^{-1}. The injection volume of the samples was 20 μL. Potassium concentration was measured using a Metrohm 690 Ion Chromatograph coupled with Metrohm 697 IC Pump and fitted with a Metrosep C4–150/4.0 (Metrohm) column, 150.0 mm × 4.0 mm (i.d.) at 25 °C. The mobile phase of the applied isocratic elution consisted of 1.7 mmol L^{-1} nitric acid + 0.7 mmol L^{-1} dipicolinic acid at a 0.9 mL min^{-1} flow rate. The injection volume (sample loop) of the samples was 10 µL.

The total polyphenols content was determined by the modified Folin–Ciocalteu (F-C) method of Singleton *et al.*[37] using as reference/comparative standard - gallic acid. 1 mL of diluted sample or reference standard or water was mixed with 50 μL F–C reagent, allowed to react for 30 s before adding 150 μL 20% (w/v) Na_2CO_3, mixed and allowed to stand 60 min at room temperature. Absorbance was measured at 750 nm.[38,39] The result was expressed as mg L^{-1} of gallic acid equivalent (GAE). The total polysaccharides (TPS) content was determined by the phenol-sulfuric acid method expressed as mg L^{-1} of equivalent glucose.[40] Radical scavenging activity of polyphenol solutions against stable 2,2-diphenyl-1-picrylhydrazyl (DPPH·) was determined by the method of Brand-Williams *et al.*[41] with some modifications. DPPH· reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH·. The color change (deep violet to light yellow) was monitored at 517 nm, where the DPPH· solution absorbance is maximum, on a UV visible light spectrophotometer. Fresh solution of 60 µmol L^{-1} DPPH· solution diluted in ethanol was prepared prior to UV measurements. 2.925 mL of this solution was mixed with 75 μL of properly diluted samples, kept in darkness for 60 min (where the reaction reaches a plateau) at room temperature and the absorbance decrease was measured. Radical scavenging activity was calculated by the following formula:

$$\%\ \text{DPPH Inhibition} = \frac{\text{Abs (blank)} - \text{Abs (sample)}}{\text{Abs (blank)}} \cdot 100 \quad (4)$$

where: blank: DPPH· solution + ethanol
sample: DPPH· solution + sample

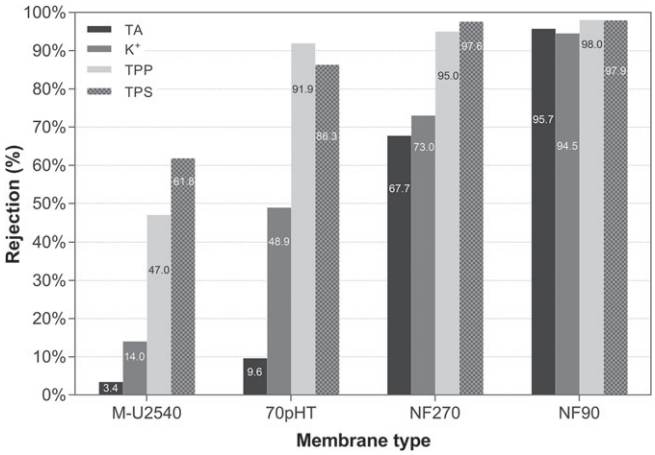

Figure 2. Membrane rejection of various components during bench-scale dead-end filtration tests.

Antiradical activity is defined as the amount of antioxidant needed to decrease the initial DPPH· concentration by 50% (Efficient Concentration = EC_{50}) and is expressed as mg sample mg^{-1} DPPH·.[41,42] Furthermore, EC_{50} was estimated for the standard antioxidants Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and gallic acid. Chemical reagents (Sigma–Aldrich) of analytical grade were used in all analyses, which were conducted in duplicate.

RESULTS AND DISCUSSION

The liquid stream obtained from the tartrate dissolution stage,[33] containing both dissolved tartaric acid and polyphenolic substances, was further treated either in bench-scale dead-end filtration test cells or in a laboratory-scale cross-flow filtration pilot unit, for the separation and recovery of tartaric acid and polyphenols.

Bench-scale dead-end filtration test cells

Dead-end filtration tests were performed in constant pressure mode using four types of membranes; UF runs were performed at TMP = 2 bar and NF runs at TMP = 10 bar. Table 3 summarizes

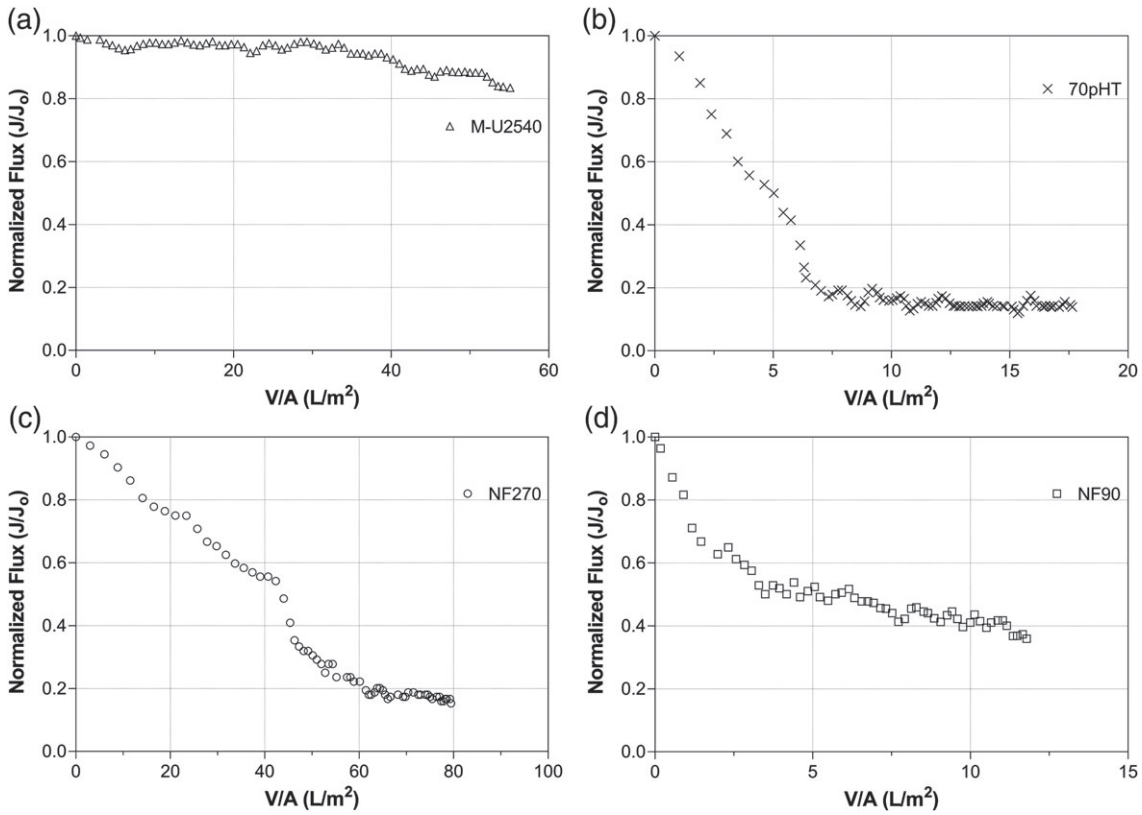

Figure 3. Normalized flux variation of different membranes during bench-scale dead-end filtration tests.

the compositions of all streams (feed, permeate and concentrate) from the bench-scale dead-end filtration runs. Concerning tartaric acid, the first two membranes tested (M-U2540 and 70pHT) exhibit negligible rejection of tartaric acid, whereas NF270 and especially NF90 significantly reduce its concentration in the permeate. These results can be explained by the molecular size of the tartaric acid molecule (150 Da); M-U2540 and 70pHT pore sizes are too large to retain it. The same trend holds for potassium ion; i.e. it freely passes through UF membrane (M-U2540) and it is moderately to highly rejected by the NF membranes with increasing rejection at smaller pore sizes. It can also be noted that potassium removal is enhanced due to its positive charge; indeed, potassium rejection from 70pHT membrane is higher, compared with tartaric acid, despite its much smaller ion size (39 Da). On the other hand, both polyphenolic substances and polysaccharides are rejected by both UF and NF membranes tested, given that they are mainly macromolecules. As expected, the rejection efficiency increases with decreasing membrane pore size (M-U2540 → 70pHT → NF270 → NF90).

Figure 2 presents the rejection coefficients for all the membranes tested in the dead-end filtration mode. As already mentioned, the main objective of this study is to achieve separation and recovery of both tartaric acid and polyphenolic substances. In these tests, the maximum separation efficiency is achieved with the membrane that exhibits the greatest possible recovery of tartaric acid in the permeate stream, while maximizing rejection of polyphenols (and of other compounds) in the concentrate stream. As shown in Fig. 2, ultrafiltration membrane M-U2540 shows the higher recovery of tartaric acid (96.4%); nonetheless, the main drawback of M-U2540 is the very low selectivity regarding the other components of the feed effluent, as it fails to reject/separate an acceptable amount of potassium cations (14.0% rejection), polyphenols (47.0% rejection) and polysaccharides (61.8% rejection). However, the concentrate from UF 20 kDa membrane exhibited the higher anti-oxidant activity.

From the nanofiltration membranes tested, the best separation/selectivity was achieved by the 70pHT membrane since it led to 90.4% recovery of the tartaric acid while rejecting an acceptable amount of potassium cations (48.9%). Furthermore, 70pHT rejected the majority of polyphenols (91.9%) and polysaccharides (86.3%) from the feed effluent. These data are comparable with results published by Galanakis *et al.*, where retention coefficients in the range of 56–85% (for total phenols) and 61–87% (for total sugars) were measured by various types of UF membranes (with MWCO 100, 20 and 1 kDa, respectively).[32] The performance of the other two NF membranes tested (NF270 and NF90) were not favorable for this separation, since the rejection rate of tartaric acid was quite high, i.e. 67.7% and 95.7%, respectively. The major mass portion of tartaric acid was in the concentrate stream together with the other substances, for which the rejection rate was also high. The aforementioned conclusions are corroborated by computation of S_F of the tartaric acid and the polyphenols,[43] which led to the following results: $S_{F\ TA/TPP}$ is 5.12 for M-U2540, 12.64 for 70pHT, 7.16 for NF270, and 2.31 for NF90 membrane.

Concerning membrane filtration performance, all three types of nanofiltration membranes exhibit a substantial reduction in normalized flux (J/J_0) linked to permeate volume per membrane surface (V/A) (Fig. 3). In general, it is observed that the initial flux reduction rate is more pronounced for NF270 and NF90 membranes. Furthermore, it is interesting to note that the initial sharp decline of permeate flux is followed by a plateau value for both membranes. This value is approx. 20% of the initial flux for the NF70 and 40% for the NF90. On the other hand, NF270 exhibits

Table 4. Physico-chemical characteristics of the feed, permeate and concentrate obtained in UF and NF cross-flow filtration experiments

Membrane type		Volume (mL)	VFC	Conductivity (mS cm^{-1})	TA (ppm)	K^+ (ppm)	Total polyphenols (mg L^{-1} gallic acid)	Total polysaccharides (mg L^{-1} glucose)	Anti-oxidant activity (mg sample mg^{-1} DPPH$^{\cdot}$
UF (ESP04)	**Feed**	1500	3.6	3.27	10,929	300	157.6	252.2	-
	Permeate	1127		2.45	8,208	208	27.90	49.90	-
	Concentrate	420		2.63	10,280	243	98.90	442.6	11.6
NF (HFW1000)	**Feed**	3700	11.9	4.98	47 835	276	323.3	1718	-
	Permeate	3300		4.33	42 599	230	71.60	416.8	-
	Concentrate	310		6.04	54 235	381	2654	9592	10.3

a nearly linear flux reduction rate that is comparatively lower than the corresponding rate of the aforementioned nanofiltration membranes. Finally, the M-U2540 membrane, as expected, demonstrated the smallest fouling tendency, since only a 20% flux reduction was observed. This observation is in accord with the low rejection coefficient of UF membrane (presented in Figure 3).

Laboratory-scale cross-flow filtration pilot unit

The results from the bench scale filtration experiments suggest that favorable separation efficiencies concerning tartaric acids and polyphenols is achieved with nanofiltration membranes of pore size approximately 1000 Da. The 70pHT membrane, which exhibited the best performance among the tested membranes, has a pore size of approximately 720 Da; however, its geometrical configuration (spiral-wound) is considered inappropriate for such applications involving rather high concentration of suspended/colloidal solids in the feed. In contrast to the laboratory experiments when the suspended/colloid solids were removed during the centrifugation step for resin separation, in full scale applications, less energy intensive processes, like screening or sedimentation[31] are preferable. In the latter case, the suspended/colloid solids will be present in the membrane feed stream, thus tubular and hollow-fiber geometrical configurations seem to be more appropriate. Given the available tubular and hollow fiber membranes, two membrane types, i.e. ESP04 (4 kDa) and HFW1000 (1 kDa), were tested at pilot scale.

Cross-flow filtration runs were performed in concentration mode for both membrane types at TMP = 2.0 bar and cross-flow velocity of 0.34 m s^{-1}. Table 4 summarizes the characteristics of all streams (feed solution, permeate and concentrate) from the cross-flow filtration tests.

The permeate collected from both runs showed significantly lower concentration of polyphenols and polysaccharides compared with the feed, revealing that both TPP and TPS were rejected and concentrated in the concentrate streams. On the other hand, tartaric acid and potassium were slightly rejected by both membranes and were mainly found at the permeate side.

Figure 4 depicts the rejection of various components during the laboratory pilot-scale cross-flow filtration tests. Regarding tartaric acid, which is the main component of interest together with polyphenolic substances, HFW1000 showed a significantly lower rejection (10.9%) compared to ESP04 (24.9%), despite having smaller pore size. The same applied also for potassium, as HFW1000 exhibited 45.6% lower rejection compared to ESP04. On the other hand, ESP04 showed a slightly higher recovery of both polyphenols and polysaccharides compared to HFW1000 (approximately 5.5% higher recovery). Considering that polyphenols have low solubility in water, this high polyphenols recovery could be attributed to their intermolecular interactions with polysaccharides that are highly soluble in water, thus, favoring the polyphenols solubility in aqueous solutions.[44,45]

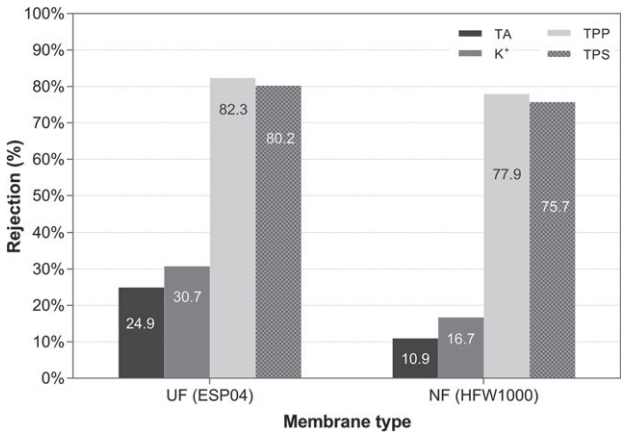

Figure 4. Membrane rejection of various components during laboratory pilot-scale cross-flow filtration tests.

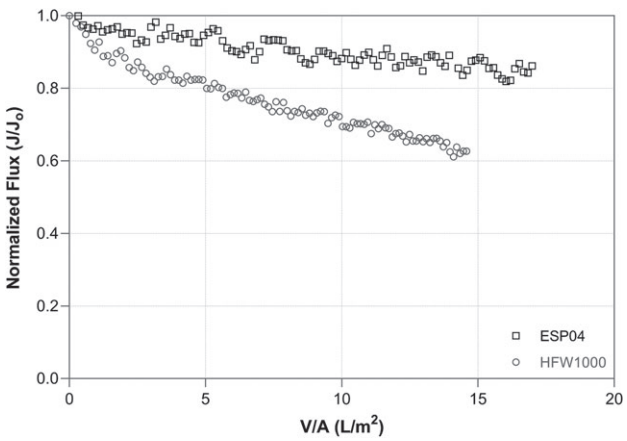

Figure 5. Normalized flux of different membranes during laboratory-scale cross-flow filtration tests.

The separation factor of the tartaric acid and the polyphenols for ESP04 and HFW1000 membrane is 2.83 and 29.1, respectively. The main objective of this process is to separate tartaric acid from the other ingredients of the solution (mainly polyphenols and polysaccharides); therefore, it is clear from Fig. 4 that HFW1000 is more suitable for this kind of separation since it exhibits significantly smaller tartaric acid rejection, while achieving adequately high recovery rates of TPP and TPS.

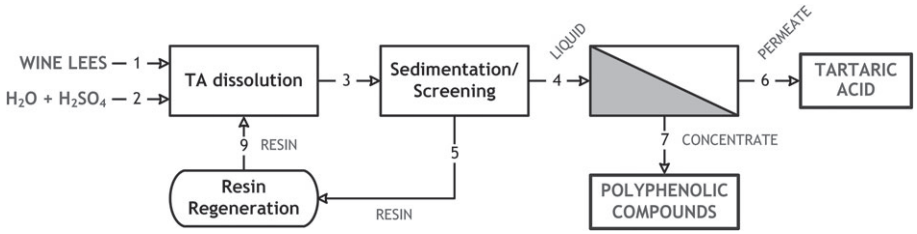

Figure 6. Proposed process flowchart.

As depicted in Fig. 5, there was a constant reduction in permeability over the operating period of both membranes. The reduction was more pronounced for the HFW1000 membrane as it decreased by approximately 37.3%, compared with the initial flux after 1.8 hours of operation. ESP04 showed a significantly smaller decrease in permeability (11.8%), over the same time period. Both membranes were chemically cleaned, and the clean water permeability was subsequently measured. The initially measured clean water permeabilities for ESP04 and HFW1000 membranes were 16.4 and 10.5 LMH/bar, respectively. After chemical cleaning, the clean water permeability was fully restored, thus indicating the effective removal of the membrane fouling layer.

Proposed process for valorization of wine lees

Based on the aforementioned results of this study, as well as those already reported,[33] a proposed process scheme is presented for an environment-friendly process for the separation and recovery of tartaric acid and polyphenolic substances from wine lees. The proposed process is schematically depicted in Fig. 6. As a first step, wine lees as is (without drying) are mixed with acidified H_2O; further, in the presence of cationic resins, the potassium bitartrate is dissolved yielding tartaric acid, whereas polyphenolic substances that are embedded/entrapped in the wine lees matrix are also dissolved in the acidified water. The resin is separated from the resulting liquor through sedimentation or screening and recycled to the regeneration stage employing H_2SO_4. The main salt resulting from resin regeneration is K_2SO_4 which can be used as a raw material for potassium fertilizers.

The liquid stream is subsequently directed to a membrane separation step involving a nanofiltration membrane with typical pore size approximately 1000 Da. The two outlet streams from the membrane process are the permeate that contains the bulk of the tartaric acid mass and some remaining potassium, and the concentrate stream comprising polyphenolic substances and other organic macromolecules (e.g. polysaccharides). The former can be used for the recovery of tartaric acid, after water removal (preferably through RO membrane filtration) that can be recycled back to the first step of the proposed process, whereas the polyphenolic-rich stream can be used for recovery of polyphenolic substances. The polyphenolic-rich stream obtained from the present tests, exhibits antioxidant activity characterized by an EC_{50} of approx. 10.0 mg sample mg^{-1} $DPPH^{\cdot}$; the antioxidant activity is quite small compared with pure antioxidants, such as gallic acid or trolox, with EC_{50} equal to 0.05 and 0.15 mg sample mg^{-1} $DPPH^{\cdot}$, respectively. The latter can be attributed to the observed antagonistic effect between hydroxycinnamic acid derivatives, flavonol and anthocyanin compounds in wine and winery wastes.[46] Nonetheless, it can be further purified to obtain polyphenolic-rich formulations with higher antioxidant activity.

CONCLUSIONS

An integrated method for valorization of wine lees, capable of recovering both tartaric acid and polyphenolic compounds, has been developed and demonstrated in this study, using membrane technology. The liquid stream of wine lees dissolution, obtained by a novel, patent pending method, has been further fractionated by membrane processes; with the latter, both harsh thermal processes (e.g. drying) and production of environmentally offensive chemical-waste sludge are avoided.

Nanofiltration membranes of typical pore size approximately 1000 Da, exhibit the best selective separation performance for tartaric acid and polyphenolic compounds, in both dead-end bench-scale filtration tests and cross-flow pilot plant experiments. Specifically, 70 pHT membrane (720 Da) in dead-end mode and HFW1000 (1000 Da) membrane exhibit separation factors 7.16 and 29.1 for tartaric acid and polyphenols, respectively. Tartaric acid is mainly recovered in the membrane permeate, whereas polyphenolic compounds and other macromolecules are retained in the concentrate stream. Membrane fouling is more pronounced in dead-end filtration experiments, with more than 80% reduction of the initial flux (70pHT); whereas in cross-flow mode the HFW1000 membrane exhibited less than 40% reduction of the initial flux after filtration of approx. 15 L m^{-2} permeate volume per membrane surface area. The applied cross-flow velocity (0.34 m s^{-1}), seems to partially remove the developing fouling layer, which appears to be totally removed by chemical cleaning with a standard protocol.

The results obtained in this, as well as a previous work[33] show that the proposed methodology, combining wine lees dissolution with acidified (pH = 3.0) water and cation resin (at optimized concentration 3.5 g resin g^{-1} dry lees), followed by a nanofiltration (1000 Da) separation step, yields two streams, i.e. a tartaric acid rich stream of approx. 42.6 g L^{-1} tartaric acid concentration, and another rich in polyphenolic substances of 2.65 g GAE L^{-1} TPP concentration. The former can be readily used for separation of pure tartaric acid (or possibly marketable TA concentrate), whereas the latter, which exhibits antioxidant activity with EC_{50} of approx. 10.0 mg sample mg^{-1} $DPPH^{\cdot}$, could be further purified to obtain polyphenolic-rich formulations. The valorization of wine lees, based on the proposed methodology using mild conditions and environment-friendly membrane processes, represents an interesting opportunity for the wine making industry, rendering a useless by-product, currently treated as a waste stream, a source of value-added marketable products.

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