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Recovery of phenolic compounds from wine lees using green processing: Identifying target molecules and assessing membrane ultrafiltration performance

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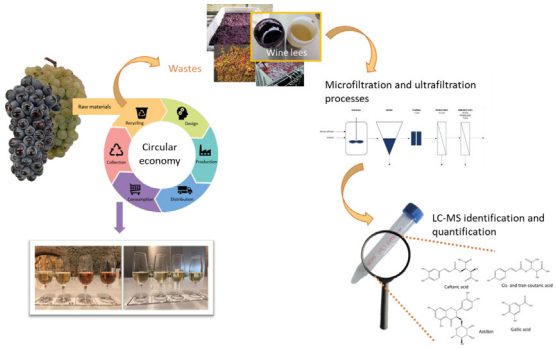
HIGHLIGHTS

- Recovery of polyphenols from winery wastes in the framework of the circular economy
- Extraction using green procedures and purification by micro- and ultrafiltration
- Polyphenol identification and quantification by LC-MS
- Caftaric, coutaric, caffeic and coumaric acids as main polyphenols in lees extracts
- Polyphenol filtrate free of impurities using the polyacrylonitrile membrane of 30 KDa

GRAPHICAL ABSTRACT



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ABSTRACT

Winery wastes are rich in polyphenols with high added value to be used in cosmetics, pharmaceuticals, and food products. This work aims at recovering and purifying the polyphenolic fraction occurring in the malolactic fermentation lees generated during the production of Albariño wines. Phenolic acids, flavonoids, and related compounds were recovered from this oenological waste by green liquid extraction using water as the solvent. The resulting extract solution was microfiltered to remove microparticles and further treated by ultrafiltration (UF) using membranes of 30 kDa and 5 kDa molecular weight cut-offs (MWCOs). The feed sample and the filtrate and retentate solutions from each membrane system were analyzed by reversed-phase liquid chromatography (HPLC) with UV and mass spectrometric (MS) detection. The most abundant polyphenols in the extracts were identified and quantified, namely: caftaric acid with a concentration of 200 μg g^{-1} and trans-coutaric acid, cis-coutaric acid, gallic acid, and astilbin with concentrations between 15 and 40 μg g^{-1}. Other minor phenolic acids and flavanols were also found. The UF process using the 30 kDa membrane did not modify the extract composition, but filtration through the 5 kDa polyacrylonitrile membrane elicited a decrease in polyphenolic content. Hence, the 30 kDa membrane was recommended to further pre-process the extracts. The combined extraction and purification process presented here is

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environmentally friendly and demonstrates that malolactic fermentation lees of Albariño wines are a valuable source of phenolic compounds, especially phenolic acids.

1. Introduction

In Southern Europe, and especially in France, Italy, and Spain, winemaking is an important agricultural activity that generates a large amount of waste, such as grape pomace, grape stalks, wine lees, or wastewater (Tapia-Quirós et al., 2022a). In many regions, there is a deeply rooted tradition in reusing winery waste, e.g. as fertilizers or for obtaining liquor. Currently, wine residues are also used in ethanol recovery by distillation, as animal feed or for biogas production. From the circular economy concept perspective, the valorization of wine processing waste is an excellent solution in terms of sustainability but also from an environmental point of view (Ahmad et al., 2020; Maicas and Mateo, 2020). In this scenario, any process that includes the recovery of higher-value compounds is more advantageous.

Winemaking waste can be considered a rich source of valuable bioactive compounds, such as polyphenols, which could be recovered before proceeding to other applications. Polyphenols are plant metabolites and comprise >8000 compounds (Crozier et al., 2006). They have antioxidant and anti-inflammatory properties, and many studies support that polyphenols could help to prevent or act as therapeutic agents for neurodegenerative, cardiovascular, or kidney diseases and certain types of cancer (Howell et al., 2018). As a consequence, there is great interest in using these compounds in nutraceuticals, pharmaceuticals, cosmetics, or as food additives, among others (Ferri et al., 2020; Galanakis, 2018).

The design of a process for the recovery of bioactive molecules from agri-food residues should consider both the extraction and purification stages. A wide range of techniques and solvents have been tested for the extraction stage from several agri-food wastes (Kumar et al., 2021; Pagano et al., 2021; Tapia-Quirós et al., 2022a). However, focusing on industrial-scale applications, only techniques such as conventional solid-liquid extraction, ultrasound-assisted extraction, or supercritical fluid extraction (SFE) are at present easily implementable (Belwal et al., 2020). On the other hand, if the final use of polyphenols is addressed to the food, pharmaceutical, or cosmetic industry sectors, there are scarce solvents compatible with the strict requirements of these sectors, with systems based on water and/or ethanol being the most applied. Thus, water-ethanol mixtures have already been shown to be efficient for the extraction of polyphenols (Montenegro-Landívar et al., 2021a, 2021b; Tapia-Quirós et al., 2020).

The purity of the extracts defines their added value and, therefore, the fractionation and purification of target bioactive molecules is the final goal of any recovery scheme. However, the purification of polyphenols remains a major challenge, due to the complexity of the extracts. As an alternative to conventional methods, membranes have been proposed to fractionate, purify or pre-concentrate compounds of interest (Arboleda Mejia et al., 2020; Conidi et al., 2018; Galanakis, 2015; Giacobbo et al., 2017; Kelly et al., 2019; Tapia-Quirós et al., 2022b; Yammine et al., 2018). Ultrafiltration (UF) is applied for clarification and fractionation before nanofiltration or reverse osmosis. Although separations by UF are ideally based on sieving processes, the molecular weight cut-off (MWCOs) of the membrane is not the only parameter to consider. Indeed, the performance of UF membranes relies on the size of both membrane pores and molecules, but the geometry (e.g., linear or globular), the cake layer formed along the filtration stages, the charge of the molecules, and the interaction of the molecules with the membrane polymer affect the retention processes (Cassano et al., 2011, 2017; Galanakis, 2015; Susanto et al., 2009; Yammine et al., 2018). Thus, after the UF stage, the rejection of polyphenols may be relevant, depending on the nature of the molecules, the membrane features, and the cake layer (Cassano et al., 2017; Tapia-Quirós et al., 2022b). For example, the retention of phenolic compounds (molecular weight < 1 kDa) from winery sludge extracts in UF with a polysulfone 100 kDa MWCO membrane was 69 % (Galanakis et al., 2013), while the rejection of total phenolics in kiwifruit juice with a cellulose acetate 30 kDa MWCO UF membrane was 13.5 % (Cassano et al., 2008). There is still a lack of information on the filtration performance in such complex extracts and the application of membrane processes for polyphenol valorization.

This study focuses on the recovery of polyphenols from wine lees, the second-largest waste from oenological activity. Lees are sludges rich in yeasts, with a notable presence of polyphenols. They are generated throughout the vinification stages and are a valuable source of bioactive compounds. (Jara-Palacios, 2019). Hydroxycinnamic acids, flavonols and anthocyanidins have been identified as the main fractions of the polyphenolic content in wine lees (Barcia et al., 2014a, 2014b; Delgado De La Torre et al., 2015; López-Fernández-sobrino et al., 2021; Reis et al., 2016; Romero-Díez et al., 2018), with the polyphenolic profile being dependent on the grape variety and the oenological processing.

Unlike grape pomace, studies carried out in the context of the recovery of polyphenols from wine lees are scarce. Different pressure-driven membrane strategies have been evaluated for a range of membranes (Arboleda Meija et al., 2019; Giacobbo et al., 2013, 2015, 2017; López-Borrell et al., 2022; Romero-Díez et al., 2018; Tapia-Quirós et al., 2022b; Tapia-Quirós et al., 2020). The process performance is usually assessed in terms of total polyphenol content or families of polyphenols, but profiling each individual component is not performed. Since the value of the extracts strongly depends on the contents of phenolic compounds with remarkable antioxidant properties, identifying the target molecules is a key issue.

In this context, this work addresses (i) the identification of the main polyphenols present in aqueous extracts of lees from malolactic fermentation in wine production and (ii) the behavior of the target phenolic compounds in the UF stage of a pilot plant installed in a winery industry. Two UF membranes with different chemistries, i.e. polyether sulfone (PES) and poly-acrylonitrile (PAN), with two different MWCOs (5 and 30 kDa) were evaluated. The analysis of the membrane process streams (feed extract, permeate and retentate) was carried out by liquid chromatography with mass spectrometry (MS) and UV detection, encompassing the identification and quantification of polyphenols.

2. Materials and methods

2.1. Reagents

Standards of 32 polyphenols were used for identification and quantification (Table S1). A stock solution of each polyphenol was prepared in DMSO (Panreac, Darmstadt, Germany) at a concentration of 5000 mg L^{-1}. Working solutions were prepared by appropriate dilutions of stock solution with acetonitrile/water (50/50, *v*/v).

For chromatographic separation, the following solvents were used: water purified with an Elix 3 coupled to a Mili-Q system (Bedford, USA), formic acid (≥95 %, Sigma-Aldrich, St Louis, USA), and acetonitrile (99.9 %, UHPLC Supergradient, Panreac, Barcelona, Spain).

2.2. Instruments

A Dionex UHPLC system coupled to an LTQ Orbitrap Velos mass spectrometer with a HESI-II electrospray ionization source (Thermo Scientific, Ca, USA) was used for polyphenol identification.

The LC–MS system used mainly for quantitation purposes consisted of an Agilent 1100 Series liquid chromatograph (Agilent, Technologies, Palo Alto, CA, USA), equipped with a vacuum degasser (G1322A), binary pump (G1312A), autosampler (G1367A), and UV–visible diode array detector (G1315B), coupled to an Applied Biosystems 4000 QTrap hybrid triple

quadrupole/linear ion trap mass spectrometer (AB Sciex, Framingham, MA, USA).

2.3. Samples and sample treatment: pilot plant description

The agri-food industrial (AFI) waste sample under study consisted of malolactic fermentation lees of an Albariño cultivar (*Vitis vinifera* L.) from the Protected Designation of Origin (PDO) Rias Baixas, in Cambados, Galicia (North-Western Spain). After pressing the grapes, the vinification process comprised alcoholic fermentation (3 weeks) followed by malolactic fermentation (about 2 months). Once the malolactic fermentation finished, lees were filtered and processed according to a green extraction using water and a double filtration stage with MF and UF membranes in series for extract clarification; the filtration process was performed at a pilot plant scale (see the scheme in Fig. S1, supplementary material).

The lees extract was obtained by liquid extraction with water as the solvent, working at 40 °C for 30 min with stirring. The lees/water ratio was 1:10 (m/m). The resulting extract was then submitted to an MF step using a JX1812 membrane made of polyvinylidene difluoride (PVDF), from Biotech Test (Lenntech, Netherlands). The filtered solution was further subjected to UF using membranes of different chemical natures and molecular weight cut-off (MWCO), namely: polyacrylonitrile (PAN) MW1812 membrane with MWCO of 30 kDa and polyethersulfone (PES) PT1812 membrane with MWCO of 5 kDa, both from Biotech Test.

The membrane separation experiments were performed in a crossflow configuration where the permeate and concentrate streams are collected in different tanks to calculate mass balances and determine the total polyphenol content in each stream.

2.4. LC-UV-MS and LC-UV-MS/MS methods

The chromatographic separation was carried out with a Kinetex C18 column (150 mm length × 4.6 mm I·D, 2.6 μm particle size) from Phenomenex (Torrance, CA, USA) equipped with a SecurityGuard™ ULTRA cartridge C18 (Phenomenex). The mobile phase consisted of 0.1 % (*v*/v) formic acid (channel A) and acetonitrile (channel B). A constant flow rate of 0.7 mL min^{-1} was used. A linear gradient from 3 % to 15 % B was applied for the first 10 min and the percentage of B was increased linearly to 45 % from 10 min to 20 min. Then the percentage of B was increased up to 90 % in 2 min and kept constant for 2 additional min. Finally, the percentage of B was decreased to initial conditions (3 %) in 0.2 min and the column was conditioned under these conditions for 7.8 min before the next injection. The volume of injection was 5 μL. UV detection was performed in the spectral range from 190 to 400 nm.

High-resolution mass spectrometry (HRMS, with the LTQ Orbitrap) was initially used for the tentative identification (based on accurate mass errors below 5 ppm) of polyphenols present in wine lees extracts. After the tentative assignation of each given molecule, MS and MS/MS spectra were compared, when available, to those of pure standards for a definitive confirmation of the identities. Negative full scan mode (from *m/z* 100 to 1500) was recorded using a resolution of 60,000 full-width at half-maximum (FWHM) at *m/z* 200. In addition, a data-dependent product ion scan was activated when the full scan signal was higher than 1.0×10^5 (peak intensity threshold). Stepped normalized collision energies (NCE) of 17.5, 35.0, and 52.5 were applied and MS/MS spectra were recorded from an *m/z* of 50 Da. A mass resolution of 17,500 FWHM at *m/z* 200 was used for data-dependent analysis. Nitrogen (purity higher than 99.98 %) was used as HESI-II sheath gas, ion-sweep gas, and auxiliary gas, at flow rates of 60, 0 and 10 arbitrary units, respectively. Capillary and S-Lens RF voltages were set at −2.5 kV and 50 V, respectively. Source temperature was kept at 350 °C and capillary temperature at 320 °C. The HRMS analyzer was tuned and calibrated every 3 days by using the calibration solution supplied by Thermo Fisher Scientific. LC-HRMS data were acquired and processed with Xcalibur 2.2 (Thermo Scientific, Ca, USA).

Low-resolution mass spectrometry (LRMS, with the 4000Qtrap spectrometer) working in Multiple Reaction Monitoring (MRM) mode was exploited aiming at confirming major components with standards and at identifying other minor polyphenols as well as for quantification purposes. Polyphenols were detected in negative mode. The ion spray voltage was set at −2500 V and the source temperature was 700 °C. Nitrogen was used as nebulizer and auxiliary gas and was set at 20, 50 and 50 arbitrary units for the curtain gas, the ion source gas 1 and the ion source gas 2, respectively. Declustering potential (DP), collision energy (CE), collision exit cell potential (CXP) and ion transitions pairs were optimized for 32 polyphenolic compounds and are depicted in the supplementary material (Table S2). The LC-MS/MS data were acquired and processed with Analyst 1.6.2 (AB Sciex, Framingham, MA, USA). For quantitative purposes, standard solutions of each analyte were prepared in the range of 0.02 to 10 mg L^{-1}.

3. Results and discussion

This section is divided into two parts focused on: (i) the analytical composition of the aqueous extract of the malolactic fermentation lees from the production of Albariño wines and (ii) the distribution of phenolic compounds in the filtrate and retentate fractions resulting from ultrafiltration through MW1812 (30 kDa MWCO) and PT1812 (5 kDa MWCO) membranes to assess the viability of this approach to pre-concentrate and purify the extracts.

3.1. Identification of polyphenols in microfiltered wine lees extracts

The lees extract obtained by mechanical stirring at 40 °C for 30 min using water as the solvent (see details in Section 2.3) was cleaned by MF (Fig. S1) to retain large macromolecules and particles above 0.3 μm of diameter using a JX1812 PVD membrane. The microfiltered lees extract was then analyzed by LC-UV-MS for the identification of polyphenols. As shown in Fig. 1, the chromatogram at 280 nm is complex, with a high number of peaks, most of them attributable to phenolic compounds. Hydroxybenzoic acids, flavanols, and flavanones show a maximum absorption at 280 nm, pointing out that most of the compounds detected at this wavelength might belong to one of these polyphenol families. A simpler profile was observed at 325 nm (Fig. 1b), where hydroxycinnamic acids and stilbenes show a maximum absorbance. The shortage of peaks with maximum absorbance at 370 nm (or higher) suggests a limited presence of flavonoids other than flavanols and flavanones, meaning that the contents of flavonols, flavones, and isoflavones were residual.

This section was essentially focused on the identification of the main peaks labeled in Fig. 1 by MS. Besides, efforts were also made to identify other minor polyphenols present in such types of samples. Two strategies were followed for this purpose, relying on liquid chromatography coupled with high- and low-resolution MS (i.e. HRMS and LRMS).

The microfiltered lees extract and the supposed (available) phenolic standards were analyzed by LC-HRMS using a data-dependent acquisition mode. In general, two fragmentation patterns were observed for the studied polyphenols. Carboxylic acids tend to suffer a decarboxylation, while esters undergo the rupture of the ester bond as the primary fragmentation. Table 1 shows the assignation and HRMS information of main peaks (those numbered in Fig. 1) and available MS/MS data is given in Table S3. The overall strategy for compound elucidation is here detailed with various representative cases. As can be seen in Table 1, the $[M\text{-}H]^-$ of compound 1 was observed at *m/z* 169.01360, which matched with that of the gallic acid with an error of −3.7 ppm. In addition, the MS/MS spectrum showed only a peak at *m/z* 125.02412, which corresponds to the decarboxylated moiety. Both retention time and MS and MS/MS spectra agree with those of the pure standard, confirming the identification of peak 1. In another illustrative example, MS spectra of the chromatographic peak at retention time 15.5 min (compound 13) showed a peak at *m/z* 179.03437. In addition, a single ion in the MS/MS spectrum at *m/z* 135.04465 was found, corresponding, again, to the decarboxylation process (Fig. S3). Retention time, $[M\text{-}H]^-$ and fragment ion were the same as for the caffeic acid reference standard. In this way, compounds such as 9, 16, 18, 20, and 22 were

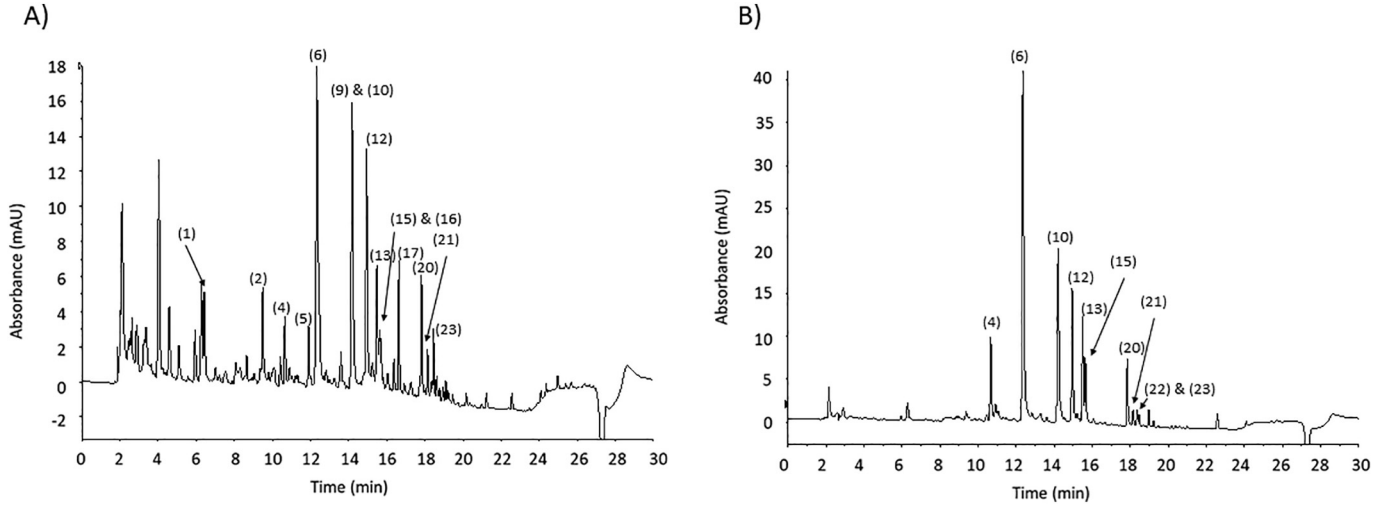

Fig. 1. Microfiltered wine lees extract chromatogram at 280 nm (A) and 325 nm (B). For peak identification see Table 1.

confirmed to correspond to catechin, epicatechin, ethyl gallate, *p*-coumaric acid, and ferulic acid, respectively.

The molecular elucidation of unknown compounds whose structures could not be confirmed by the comparison of the MS and MS/MS spectra with those of standards was also attempted following a similar strategy. As a matter of example, the MS and MS/MS spectra of the chromatographic peak 6 at a retention time of 12.3 min are shown in Fig. 2. As can be seen, a pseudo-molecular ion at *m/z* 311.04083 was observed which is compatible (error lower than 5 ppm) with the chemical formula $C_{13}H_{11}O_9^-$. In addition, two fragments at *m/z* 149.00862 and 179.03437 were observed in the MS/MS spectrum which are related to the tartaric and caffeic acids, respectively. Thus, compound 6 probably corresponded to caftaric acid (ester formed from caffeic and tartaric acids). The identification of this compound was confirmed afterwards by MRM analysis. Similarly, compounds 10 and 12 are related to tartaric acid since peaks at *m/z* 149 ($[M-H]^-$ of tartaric acid) and 163 ($[M\text{-H-tartaric acid}]^-$) appeared in the MS/MS spectrum (Fig. 3). With this in mind and the $[M-H]^-$ ion at *m/z* 295.0447, coutaric acid can be proposed as the most plausible structure. In this case, trans-coutaric acid standard was the only one available for confirmation by MRM, having an identical retention time as compound 12. Regarding compound 10, the spectral data were similar to trans-coutaric, so we reasonably assumed that it should be cis-coutaric acid, although no standards were available for its experimental confirmation. The evaluation of HRMS data enabled the tentative identification of other minor components (compounds 3, 7, 8, 11, and 23) which were, afterwards, confirmed by the MRM-based LC-MS/MS method using standards.

Finally, although the exact structure of other components cannot be established, MS data provided sufficient information to assign compounds 17, 21, and 24 to a hydroxybenzoic acid glucoside, a coumaric acid isomer, and a hydroxybenzoic acid isomer, respectively.

Quantitative results showed that caftaric acid was the most important polyphenol in wine lees extract used as the feed of the UF processes, with

Table 1
Polyphenols identified in wine lees extracts.

Peak number	t_R (min)	Proposed chemical formula	$[M-H]^-$ *m/z* calculated value	$[M-H]^-$ *m/z* observed value	Mass error (ppm)	Identification (polyphenol family)
1	6.41	$C_7H_6O_5$	169.01422	169.01360	−3.7	Gallic acid[a] (hydroxybenzoic acid)
2	9.49	$C_{28}H_{14}O_2$	382.09883	382.09895	−2.5	Unknown
3	10.05	$C_7H_6O_4$	153.01931	153.01870	−4.0	3,4-Dihydroxybenzoic acid[a] (hydroxybenzoic acid)
4	10.64	NA	NA	NA	NA	Unknown (hydroxycinnamic acid)
5	11.90	$C_{21}H_{31}O_{10}$	443.19227	443.19072	−3.4	Unknown
6	12.33	$C_{13}H_{12}O_9$	311.04083	311.03942	−4.5	Caftaric acid[a] (hydroxycinnamic acid)
7	13.73	$C_7H_6O_3$	137.02439	137.02368	−5.2	4-hydroxybenzoic acid[a] (hydroxybenzoic acid)
8	14.05	$C_{16}H_{18}O_9$	353.08778	353.08631	−4.2	Chlorogenic acid[a] (hydroxycinnamic acid)
9	14.11	$C_{15}H_{14}O_6$	289.07174	289.07054	−4.1	Catechin[a] (flavanol)
10	14.18	$C_{13}H_{12}O_8$	295.04592	295.04476	−3.9	cis-Couratic acid[b] (hydroxycinnamic acid)
11	14.33	$C_7H_6O_4$	153.01931	153.01870	−4.0	2,5-Dihydroxybenzoic acid[a] (hydroxybenzoic acid)
12	14.93	$C_{13}H_{12}O_8$	295.04592	295.04474	−4.0	t-Couratic acid[a] (hydroxycinnamic acid)
13	15.48	$C_9H_8O_4$	179.03496	179.03437	−3.2	Caffeic acid[a] (hydroxycinnamic acid)
14	15.52	$C_9H_{10}O_5$	197.04552	ND	NA	Syringic acid[a,c] (hydroxybenzoic acid)
15	15.62	NA	NA	NA	NA	Unknown
16	15.80	$C_{15}H_{14}O_6$	289.07174	289.07054	−4.1	Epicatechin[a] (flavanol)
17	16.63	$C_{14}H_{23}O_{11}$	366.11674	366.11826	4.1	Unknown (hydroxybenzoic acid glucoside)
18	17.27	$C_9H_{10}O_5$	197.04552	197.04468	−4.3	Ethyl gallate[a] (hydroxybenzoic acid)
19	17.55	$C_{27}H_{30}O_{16}$	609.14609	ND	NA	Rutin[a,c] (flavonol)
20	17.81	$C_9H_8O_3$	163.04004	163,03944	−3.7	p-Coumaric acid[a] (hydroxycinnamic acid)
21	18.12	$C_9H_8O_3$	163.04004	163,03939	−4.0	p-Coumaric acid isomer[b] (hydroxycinnamic acid)
22	18.34	$C_{10}H_{10}O_4$	193.05061	193.04983	−4.0	Ferulic acid[a] (hydroxycinnamic acid)
23	18.44	$C_{21}H_{22}O_{11}$	449.10891	449.10769	−2.7	Astilbin[a] (flavanone)
24	20.98	$C_7H_6O_3$	137.02439	137.02378	−4.5	4-Hydroxybenzoic acid isomer[b] (hydroxybenzoic acid)

[a] Confirmed with standards.
[b] Tentative identification.
[c] Not detected by LC-HRMS (orbitrap) but by LC-MS/MS (MRM mode).

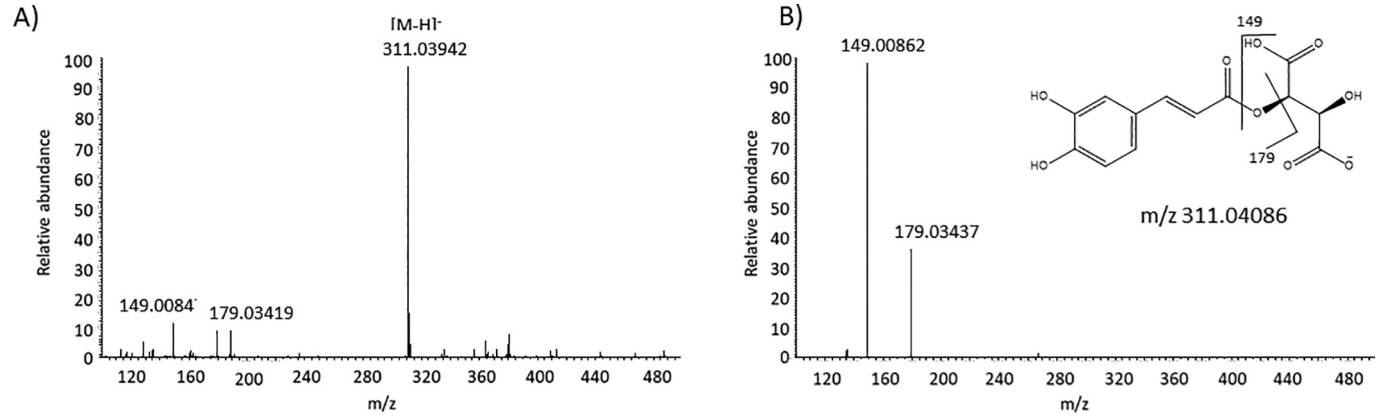

Fig. 2. HRMS (A) and HRMS/MS (B) spectra of compound 6 (caftaric acid) from wine lees extract.

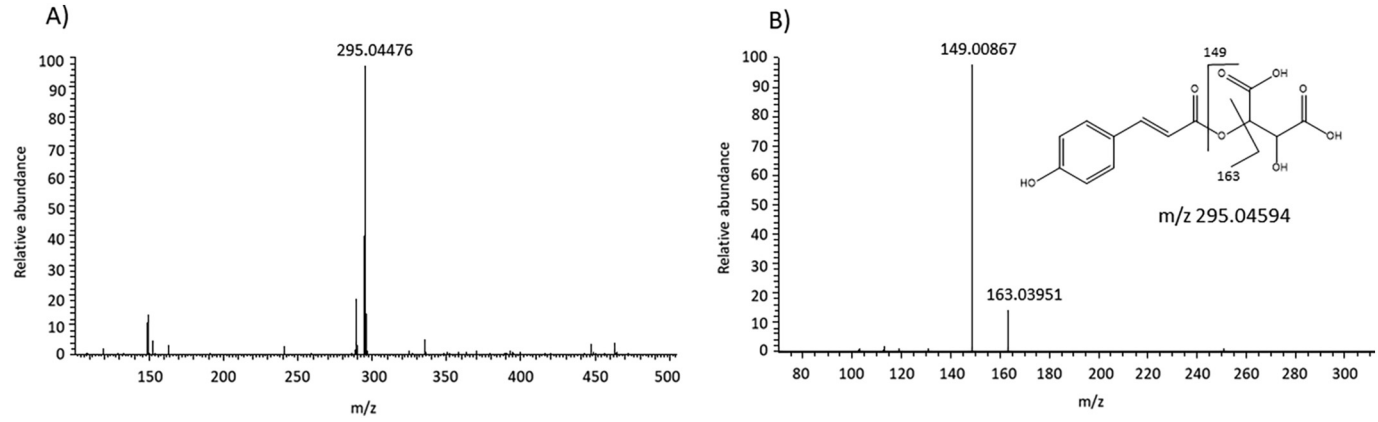

Fig. 3. HRMS (A) and HRMS/MS (B) spectra of compound 10 (cis-coutaric acid) from wine lees extract.

a concentration of almost 200 μg g^{-1} (Table 2). Other relevant components were (cis and trans) coutaric acids and gallic acid and astilbin, with concentrations ranging from 20 to 40 μg g^{-1}. The concentrations of the other polyphenols identified were below 10 μg g^{-1}, among which other hydroxycinnamic acids (caffeic and coumaric acid) and flavanols (catechin and epicatechin) stood out. In summary, phenolic acids are the

Table 2
Phenolic concentration of wine lees after microfiltration (MF) and ultrafitration (UF) of the aqueous extract. Concentrations are expressed as μg of compound per g of wine lees.

Peak no.	Compound	Concentration (μg g^{-1})				
		JX1812 MF	MW1812 UF30-R	MW1812 UF30-P	PT1812 UF5-R	PT1812 UF5-P
1	Gallic acid	20.7	20.1	20.3	18.9	12.1
3	3,4-Dihydroxybenzoic acid	2.1	2.2	2.0	2.0	1.7
6	Caftaric acid[a]	189.5	179.5	181.0	167.5	73.1
7	4-Hydroxybenzoic acid	0.9	1.0	0.8	0.9	0.3
8	Chlorogenic acid	0.2	0.2	0.2	0.2	0.1
9	Catechin	6.5	2.1	7.1	1.3	1.4
10	cis-Couratic acid[b]	39.8	39.8	39.7	37.6	22.9
11	2,5-Dihydroxybenzoic acid	6.6	6.0	6.1	5.0	3.7
12	trans-Couratic acid	37.7	35.7	36.2	34.2	19.4
13	Caffeic acid	7.7	6.4	6.9	6.3	3.2
14	Syringic acid	0.4	0.6	0.4	0.5	0.4
16	Epicatechin	2.2	0.5	2.6	0.4	0.5
18	Ethyl gallate	2.5	2.5	2.4	2.0	1.2
19	Rutin	BLQ	BLQ	BLQ	BLQ	BLQ
20	p-Coumaric acid	9.1	7.6	7.4	7.5	3.5
22	Ferulic acid	1.8	1.7	1.5	1.5	0.8
23	Astilbin	19.3	16.6	19.3	14.8	03.7

Mean values ($n = 2$).
BLQ: below limit of quantification (<0. 2 μg g^{-1}).
P: permeate stream; R: retentate stream.
[a] MS quantification from 10 to 100 μg g^{-1}; UV (325 nm) quantification for concentration higher than 100 μg g^{-1}.
[b] Quantified using standard curve of trans-coutaric acid.

main polyphenols detected in aqueous extracts from malolactic fermentation lees of Albariño. Similar studies, dealing with the phenolic profiling of red wine lees found flavonols and anthocyanidins as major components (Barcia et al., 2014a, 2014b; Delgado De La Torre et al., 2015; López-Fernández-sobrino et al., 2021; Reis et al., 2016; Romero-Díez et al., 2018). Indeed, quercetin and quercetin derivatives usually stand out over other polyphenols in this winemaking by-product (Delgado De La Torre et al., 2015; Reis et al., 2016). For instance, Reis et al. characterize *Vitis vinifera* L. cv Pinot Noir lees and reported levels of about 40 μg g^{-1} for quercetin, while the major phenolic acid was caffeic acid with a concentration of around 6 μg g^{-1} (Reis et al., 2016). In contrast, wine lees from Cabernet Sauvignon and Cabernet Franc grape varieties contained similar concentrations of flavonols (ca 375 μg g^{-1}) and hydroxycinnamic acids (ca 420 μg g^{-1}). Again, quercetin is the predominant flavonol accounting for 85 %. Regarding hydroxycinnamic acids, caftaric, t-coutaric, and caffeic acids account for around 20 % each (Barcia et al., 2014a, 2014b). Finally, stilbenes, found extensively in other wine by-products, are less common in wine lees.

The differences encountered between studies may be due to the type of wine lees, which depends on the grape variety and origin and the vinification process, as well as the extraction methodology, which relay on the final goal of the study. Indeed, most of the reported studies deal with the characterization and identification of phenolic compounds present in wine lees rather than the optimization of a methodology for the extraction and purification of polyphenols to be subsequently reused as bioactive compounds in the framework of a circular economy. Hence, organic solvents, such as ethanol, methanol, or acetone, were extensively used for their extraction. In our case, water was selected due to its full compatibility with food, pharmaceutical, or nutraceutical industries, boosting the extraction of more polar compounds such as phenolic acids and limiting the extraction of less polar compounds such as quercetin.

The environmentally friendly extraction and purification process presented here demonstrate that malolactic fermentation lees of grapevine cultivar Albariño can be a valuable source of phenolic compounds, mainly phenolic acids.

3.2. Ultrafiltration of lees extract

UF is an excellent option for processing large volumes of extracts such as those from agri-food industry wastes. After UF, the extracts are much simpler than the original ones, and they can be subjected to further steps, such as NF, RO, or adsorption processes to fractionate/purify the target compounds.

In this study, the microfiltered extract was treated by UF through membranes of different characteristics and different MWCO values: polyacrylonitrile (PAN) membrane with MWCO 30 kDa (MW1812) and polyethersulfone (PES) membrane with MWCO 5 kDa (PT1812). These two options were selected as representative examples to deal with different situations. In particular, the 30 KDa membrane was used to assure high extract fluxes with efficient removal of large macromolecules (proteins, polysaccharides, and others) that, despite not being monitored here, are certainly present in the extract. The 5 KDa counterpart was selected to increase the removal of medium-size biopolymers, also considering that the pass of some bigger polyphenols could be hindered in a certain extension, thus varying the composition of the resulting fractions.

Filtrate and retentate solutions were analyzed chromatographically according to the proposed method. As shown in Fig. S2, the samples obtained from the different UF membranes were qualitatively similar, with analogous compositional profiles to those found after the MF process. Thus, conclusions on the identity of the lees extract compounds (see Section 3.1) could be generalized to the UF samples. However, quantitative differences were encountered, as shown in Table 2. The content of the target polyphenols remained practically unchanged after UF with the 30 kDa PAN membrane, without appreciable losses in the permeate, except for the case of p-coumaric acid, in which a decrease of 9 % was observed. Conversely, for the 5 kDa PES membrane, relevant losses were observed in the permeate, in the range of 19–62 %, depending on the compound. The concentration of the retentate solutions of both 30 kDa and 5 kDa systems remained almost unaltered compared with the feeding. Since the molecular masses of the target molecules are in the range of 170–312 Da, well below the 5 kDa MWCO, the decrease in concentration for the permeate of the 5 kDa membrane cannot be attributed to a sieving effect but the adsorption of polyphenols onto the membrane or the cake layer formed on the membrane surface throughout the filtration cycle. This behavior has also been observed in other UF systems using PES membranes, with hydrogen bonding, aromatic π-π, and van der Walls interactions being the main mechanisms involved in adsorption processes due to the PES-polyphenols interaction (Cassano et al., 2017). In addition, the adsorption on the cake layer can also play a relevant role. Sousa dos S. et al. (2016) evaluated the performance of PES membranes (5-30 kDa) with catechins in green tea extracts. They reported a total polyphenol reduction in the permeate stream of 40 % for the 30 kDa membrane and about 95 % for the 5 kDa membrane. Recently, Tapia-Quirós et al. reported losses in the permeate of about 40 % when applying a PES membrane (Biomax 30kD) to winery lees extracts (Tapia-Quirós et al., 2022b).

Thus, considering these results, the MW1812 PAN membrane of 30 kDa was selected for the clarification of the extract before the extraction/concentration/fractionation stage using polymeric resins.

4. Conclusions

The solid-liquid extraction of malolactic fermentation lees by mechanical stirring using water as a green solvent followed by MF through a polyvinylidene difluoride membrane provided an extract solution especially rich in phenolic compounds, in particular the hydroxycinnamic acids. The comprehensive research by liquid chromatography with mass spectrometry under both high- and low-resolution platforms identified some major and minor compounds occurring in the sample. Around 20 compounds were assigned by comparing data such as exact mass, MS and MS/MS spectra with those of the corresponding standards. As a result, caftaric and (cis and trans) coutaric acids as well as their precursors (caffeic and *p*-coumaric acids, respectively) were the most abundant compounds in this type of sample. Various hydroxybenzoic acids, especially gallic and 2,5-dihydroxybenzoic acids, were also remarkable. Regarding flavonoids, astilbin (flavanone glycoside) and catechin (flavanol) were the most important molecules. The aqueous extract was further processed by ultrafiltration using PAN and PES membranes of 30 and 5 kDa molecular weight cut-offs, respectively. The PAN 30 kDa membrane allowed for removing microparticle impurities and macromolecules without altering the composition of the filtrate solution. In contrast, UF through the PES 5 kDa membrane decreased the concentration of polyphenols in the filtrate substantially, possibly because of the interaction of molecules with the membrane and/or the superficial cake layer. The permeated stream obtained with the PAN membranes has suitable properties for the subsequent processing stages of concentration and separation by membrane pressure-driven processes (e.g. NF and RO) and/or purification by chromatographic resins.

All in all, the presence of relevant levels of polyphenols, especially hydroxycinnamic and hydroxybenzoic acids, in the aqueous extracts of the malolactic fermentation lees of the Albariño grape variety, confirms that lees are valuable by-products of wine production. Therefore, the recovery of bioproducts with known beneficial properties, using green chemistry and circular economy approaches, would support sustainable and profitable management of the waste generated.

CRediT authorship contribution statement

Aina Mir-Cerdà: Investigation. **Iris Carretero:** Investigation. **José Rubén Coves:** Investigation, Writing - Review & Editing. **Alba Pedrouso:** Investigation, Writing - Review & Editing. **Celia María Castro-Barros:** Investigation. **Teresa Alvarino:** Supervision. **José Luis Cortina:** Conceptualization, Writing - Review & Editing, Funding acquisition. **Javier Saurina:**

Conceptualization, Supervision, Writing - Original Draft, Writing - Review & Editing, Funding acquisition. **Mercè Granados:** Conceptualization, Supervision, Writing - Original Draft, Writing - Review & Editing, Funding acquisition **Sonia Sentellas:** Conceptualization, Supervision, Writing - Original Draft, Writing - Review & Editing.

Data availability

No data was used for the research described in the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Ahmad, B., Yadav, V., Yadav, A., Rahman, M.U., Yuan, W.Z., Li, Z., Wang, X., 2020. Integrated biorefinery approach to valorize winery waste: a review from waste to energy perspectives. Sci. Total Environ. 719, 137315. https://doi.org/10.1016/j.scitotenv.2020.137315.

Arboleda Meija, J.A., Parpinello, G.P., Versari, A., Conidi, C., Cassano, A., 2019. Microwave-assisted extraction and membrane-based separation of biophenols from red wine lees. Food Bioprod. Process. 117, 74–83. https://doi.org/10.1016/j.fbp.2019.06.020.

Arboleda Mejia, J.A., Ricci, A., Figueiredo, A.S., Versari, A., Cassano, A., Parpinello, G.P., de Pinho, M.N., 2020. Recovery of phenolic compounds from red grape pomace extract through nanofiltration membranes. Foods 9. https://doi.org/10.3390/foods9111649.

Barcia, M.T., Pertuzatti, P.B., Gomez-Alonso, S., Godoy, H.T., Hermosin-Gutierrez, I., 2014a. Phenolic composition of grape and winemaking by-products of Brazilian hybrid cultivars BRS Violeta and BRS Lorena. Food Chem. 159, 95–105. https://doi.org/10.1016/j.foodchem.2014.02.163 CO - FOCHDJ.

Barcia, M.T., Pertuzatti, P.B., Rodrigues, D., Gómez-Alonso, S., Hermosín-Gutiérrez, I., Godoy, H.T., 2014b. Occurrence of low molecular weight phenolics in Vitis vinifera red grape cultivars and their winemaking by-products from São Paulo (Brazil). Food Res. Int. 62, 500–513. https://doi.org/10.1016/j.foodres.2014.03.051.

Belwal, T., Chemat, F., Venskutonis, P.R., Cravotto, G., Jaiswal, D.K., Bhatt, I.D., Devkota, H.P., Luo, Z., 2020. Recent advances in scaling-up of non-conventional extraction techniques: learning from successes and failures. TrACTrends Anal. Chem. 127. https://doi.org/10.1016/j.trac.2020.115895.

Cassano, A., Donato, L., Conidi, C., Drioli, E., 2008. Recovery of bioactive compounds in kiwi-fruit juice by ultrafiltration. Innov. Food Sci. Emerg. Technol. 9, 556–562. https://doi.org/10.1016/j.ifset.2008.03.004 CO - IFSEBO.

Cassano, A., Conidi, C., Drioli, E., 2011. Comparison of the performance of UF membranes in olive mill wastewaters treatment. Water Res. 45, 3197–3204. https://doi.org/10.1016/J.WATRES.2011.03.041.

Cassano, A., De Luca, G., Conidi, C., Drioli, E., 2017. Effect of polyphenols-membrane interactions on the performance of membrane-based processes. A review. Coord. Chem. Rev. 351, 45–75. https://doi.org/10.1016/J.CCR.2017.06.013.

Conidi, C., Drioli, E., Cassano, A., 2018. Membrane-based agro-food production processes for polyphenol separation, purification and concentration. Curr. Opin. Food Sci. 23, 149–164. https://doi.org/10.1016/J.COFS.2017.10.009.

Crozier, A., Jaganath, I.B., Clifford, M.N., 2006. Phenols, Polyphenols And Tannins: An Overview.

Delgado De La Torre, M.P., Priego-Capote, F., Luque De Castro, M.D., 2015. Characterization and comparison of wine lees by liquid chromatography-mass spectrometry in high-resolution mode. J. Agric. Food Chem. 63, 1116–1125. https://doi.org/10.1021/jf505331f.

Ferri, M., Vannini, M., Ehrnell, M., Eliasson, L., Xanthakis, E., Monari, S., Sisti, L., Marchese, P., Celli, A., Tassoni, A., 2020. From winery waste to bioactive compounds and new polymeric biocomposites: a contribution to the circular economy concept. J. Adv. Res. 24, 1–11. https://doi.org/10.1016/J.JARE.2020.02.015.

Galanakis, C.M., 2015. Separation of functional macromolecules and micromolecules: from ultrafiltration to the border of nanofiltration. Trends Food Sci. Technol. 42, 44–63. https://doi.org/10.1016/j.tifs.2014.11.005 CO - TFTEEH.

Galanakis, C.M., 2018. Polyphenols: properties, recovery, and applications. Polyphenols: Properties, Recovery, And Applications. https://doi.org/10.1016/C2016-0-05057-X.

Galanakis, C.M., Markouli, E., Gekas, V., 2013. Recovery and fractionation of different phenolic classes from winery sludge using ultrafiltration. Sep. Purif. Technol. 107, 245–251. https://doi.org/10.1016/j.seppur.2013.01.034 CO - SPUTFP.

Giacobbo, A., Oliveira, M., Duarte, E.C.N.F., Mira, H.M.C., Bernardes, A.M., de Pinho, M.N., 2013. Ultrafiltration based process for the recovery of polysaccharides and polyphenols from winery effluents. (Philadelphia, PA, United States) Sep. Sci. Technol. 48, 438–444. https://doi.org/10.1080/01496395.2012.725793 CO - SSTEDS.

Giacobbo, A., do Prado, J.M., Meneguzzi, A., Bernardes, A.M., de Pinho, M.N., 2015. Microfiltration for the recovery of polyphenols from winery effluents. Sep. Purif. Technol. 143, 12–18. https://doi.org/10.1016/j.seppur.2015.01.019.

Giacobbo, A., Bernardes, A.M., de Pinho, M.N., 2017. Sequential pressure-driven membrane operations to recover and fractionate polyphenols and polysaccharides from second racking wine lees. Sep. Purif. Technol. 173, 49–54. https://doi.org/10.1016/j.seppur.2016.09.007.

Howell, K., Bennett, L., Xue, L., Cory, H., Passarelli, S., Szeto, J., Tamez, M., Mattei, J., 2018. The role of polyphenols in human health and food systems: a mini-review. Front. Nutr. 1, 87. https://doi.org/10.3389/fnut.2018.00087 www.frontiersin.org.

Jara-Palacios, M.J., 2019. Wine lees as a source of antioxidant compounds. Antioxidants 8, 1–10. https://doi.org/10.3390/antiox8020045.

Kelly, N.P., Kelly, A.L., O'Mahony, J.A., 2019. Strategies for enrichment and purification of polyphenols from fruit-based materials. Trends Food Sci. Technol. 83, 248–258. https://doi.org/10.1016/J.TIFS.2018.11.010.

Kumar, M., Dahuja, A., Tiwari, S., Punia, S., Tak, Y., Amarowicz, R., Bhoite, A.G., Singh, S., Joshi, S., Panesar, P.S., Sharifi-Rad, J., Kaur, C., 2021. Recent trends in extraction of plant bioactives using green technologies: a review. Food Chem. 353. https://doi.org/10.1016/j.foodchem.2021.129431.

López-Borrell, A., López-Pérez, M.-F., Cardona, S.C., Lora-García, J., 2022. Membranes experimental Study and Mathematical Modeling of a Nanofiltration Membrane System for the Recovery of Polyphenols from Wine Lees. https://doi.org/10.3390/membranes12020240.

López-Fernández-sobrino, R., Soliz-Rueda, J.R., Margalef, M., Arola-Arnal, A., Suárez, M., Bravo, F.I., Muguerza, B., 2021. Ace inhibitory and antihypertensive activities of wine lees and relationship among bioactivity and phenolic profile. Nutrients 13, 1–17. https://doi.org/10.3390/nu13020679.

Maicas, S., Mateo, J.J., 2020. Sustainability of wine production. Sustainability 12. https://doi.org/10.3390/su12020559.

Montenegro-Landívar, M.F., Tapia-Quirós, P., Vecino, X., Reig, M., Valderrama, C., Granados, M., Cortina, J.L., Saurina, J., 2021a. Fruit and vegetable processing wastes as natural sources of antioxidant-rich extracts: evaluation of advanced extraction technologies by surface response methodology. J. Environ. Chem. Eng. 9. https://doi.org/10.1016/j.jece.2021.105330.

Montenegro-Landívar, M.F., Tapia-Quirós, P., Vecino, X., Reig, M., Valderrama, C., Granados, M., Cortina, J.L., Saurina, J., 2021b. Recovery of added-value compounds from orange and spinach processing residues: green extraction of phenolic compounds and evaluation of antioxidant activity. Antioxidants 10. https://doi.org/10.3390/antiox10111800.

Pagano, I., Campone, L., Celano, R., Piccinelli, A.L., Rastrelli, L., 2021. Green non-conventional techniques for the extraction of polyphenols from agricultural food by-products: a review. J. Chromatogr. A 1651. https://doi.org/10.1016/j.chroma.2021.462295.

Reis, G.M., Faccin, H., Viana, C., da Rosa, M.B., de Carvalho, L.M., 2016. Vitis vinifera L. cv pinot noir pomace and lees as potential sources of bioactive compounds. Int. J. Food Sci. Nutr. 67, 789–796. https://doi.org/10.1080/09637486.2016.1204595.

Romero-Díez, R., Rodríguez-Rojo, S., Cocero, M.J., Duarte, C.M.M., Matias, A.A., Bronze, M.R., 2018. Phenolic characterization of aging wine lees: correlation with antioxidant activities. Food Chem. 259, 188–195. https://doi.org/10.1016/j.foodchem.2018.03.119.

Sousa dos S., L., Cabral, B.V., Madrona, G.S., Cardoso, V.L., Reis, M.H.M., 2016. Purification of polyphenols from green tea leaves by ultrasound assisted ultrafiltration process. Sep. Purif. Technol. 168, 188–198. https://doi.org/10.1016/J.SEPPUR.2016.05.029.

Susanto, H., Feng, Y., Ulbricht, M., 2009. Fouling behavior of aqueous solutions of polyphenolic compounds during ultrafiltration. J. Food Eng. 91, 333–340. https://doi.org/10.1016/J.JFOODENG.2008.09.011.

Tapia-Quirós, P., Montenegro-Landívar, M.F., Reig, M., Vecino, X., Alvarino, T., Cortina, J.L., Saurina, J., Granados, M., 2020. Olive mill and winery wastes as viable sources of bioactive compounds: a study on polyphenols recovery. Antioxidants 9, 1074. https://doi.org/10.3390/antiox9111074.

Tapia-Quirós, P., Montenegro-Landívar, M.F., Reig, M., Vecino, X., Saurina, J., Granados, M., Cortina, J.L., 2022. Integration of membrane processes for the recovery and separation of polyphenols from winery and olive mill wastes using green solvent-based processing. J. Environ. Manag. 307, 114555. https://doi.org/10.1016/J.JENVMAN.2022.114555.

Tapia-Quirós, Paulina, Montenegro-Landívar, M.F., Reig, M., Vecino, X., Cortina, J.L., Saurina, J., Granados, M., 2022. Recovery of polyphenols from agri-food by-products: the olive oil and winery industries cases. Foods 11, 362. https://doi.org/10.3390/foods11030362.

Yammine, S., Brianceau, S., Manteau, S., Turk, M., Ghidossi, R., Vorobiev, E., Mietton-Peuchot, M., 2018. Extraction and purification of high added value compounds from by-products of the winemaking chain using alternative/nonconventional processes/technologies. Crit. Rev. Food Sci. Nutr. 58, 1375–1390. https://doi.org/10.1080/10408398.2016.1259982.