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Characterization of polysaccharide extracts recovered from different grape and winemaking products

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ABSTRACT

One of the most important challenges of the oenological industry is the recovery and valorization of valuable compounds from grapes and grape by-products. Recent studies have focused on the obtention of phenolic compounds, but little attention has been paid to the extraction of grape polysaccharides, which could have a great potential as oenological products but also for their benefits to human health. This study aimed to recover polysaccharides from different grape and winemaking products and provide information about its composition. The results obtained with the white pomace and white lees revealed its potential to be exploited to obtain extracts rich in polysaccharides. White pomace revealed as a good source to obtain polysaccharides rich in arabinose and galactose (PRAG) and glucosyl polysaccharides. White lees showed a potential to be used to recover mannoproteins and glucans. Both extracts showed high polysaccharide purity (55.5% and 51.9%, respectively). Extracts rich in rhamnogalacturonan type II (RG-II) were obtained from a red wine (89.7% polysaccharide purity) and from the wash water used by the distillery after draining the distilled wine pomace (40.6% polysaccharide purity). Our results open new lines to obtain extracts with different polysaccharide composition, non-available in the market. Future studies are needed to evaluate their potential as stabilizing or finning agents and possible alternative solutions to traditional animal-origin protein fining agents.

1. Introduction

The recovery of bioactive compounds in the food industry is one of the greatest challenges of current research, and even more if they are recovered from the waste by-products. In the oenological industry, several by-products are generated and usually discharged as waste into the environment. However, some of these by-products such as grape pomace or lees contain some value-added molecules. Grape pomace is the main by-product, and include skins, pulps, seeds, and stems from white grapes (non-fermented waste) or red grapes (fermented waste). The reuse of these by-products will reduce the environmental impact and would be notable for the sustainable development of the wine industry (Bordiga, Montella, Travaglia, Arlorio, & Coïsson, 2019a; Petrović et al., 2016).

Polysaccharides are one of the main macromolecules found in grapes, musts, wines, and in grape pomace (Coelho, Pereira, Rodrigues, Texeira, & Pintado, 2020) and lees (De Iseppi et al., 2021a). Yeast mannoproteins (MP) and derivatives are produced from the cell walls of different yeast strains by the auxiliary oenological industry. These polysaccharides, permitted by the OIV as stabilizing agents for tartaric and protein haze (OIV Resolution Oeno 26/2004), are widely used in the winemaking to improve wine overall stability and sensory properties. On the contrary, grape polysaccharides are not commercially available, and its recovery and production are a challenge for the industry due to its potential as oenological products.

Grape polysaccharides arise from the pectocellulosic cell walls of grape berries and include polysaccharides rich in arabinose and galactose (PRAG), which comprise arabinans, arabinogalactans (AG), and arabinogalactan proteins (AGP), rhamnogalacturonans type I and II (RG-I and RG-II), and homogalacturonans (HG). PRAG and RG-II have shown

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to modulate tannin self-aggregation, and affect the body, structure, and mouthfeel of wines (Chong, Cleary, Dokoozlian, Ford, & Fincher, 2019; Brandão et al., 2017; Brandão et al., 2020; Quijada-Morín, Williams, Rivas-Gonzalo, Doco, & Escribano-Bailón, 2014; Vidal et al., 2004; Riou, Vernhet, Doco, & Moutounet, 2002). PRAG molecules have also shown to prevent against protein haze in white wines (Lankhorst et al., 2017) and improve the foaming properties in sparkling wines (Martínez-Lapuente, Guadalupe, Ayestarán, & Pérez-Magariño, 2015; Vincenzi, Crapisi, & Curioni, 2014). Other studies also report that AGP (Dufour & Bayonove, 1999), AG and MP (Mitropoulou, Hatzidimitriou, & Paraskevopoulou, 2011) can interact with wine aroma compounds and increase their volatility. RG-II affect tartrate crystallization (Gerbaud et al., 1997), and the dimer RG-II/boron can reduce the level of toxic cations in wines (Pérez, Rodríguez-Carvajal, & Doco, 2003). Oligosaccharides have also been reported to affect the astringency perception (Quijada-Morín et al., 2014) and interact with anthocyanins with stabilizing effects (Larsen, Buerschaper, Schieber, & Weber, 2019). In addition to the oenological functions, recent studies have shown the potential prebiotic activity of soluble carbohydrates and oligosaccharides extracted from grape seeds (Bordiga et al., 2019a; Bordiga et al., 2019b). Oligosaccharides from other fruits have been reported to have anticancer (Kapoor & Dharmesh, 2017) and cardioprotective effects (Zhang, Cai, & Ma, 2015); and different polysaccharide fractions isolated from wines have shown anti-inflammatory properties and a potential application to human health benefits (de Bezerra, 2018).

It is obvious that the effect of polysaccharides depends not only on their quantity but on the type of polysaccharide, chemical composition, molecular weight, and origin. Therefore, since the recovery of these compounds is a challenge, it is essential to have information on the composition of polysaccharides that could be recovered from different grape and winemaking products.

The aim of this study was to recover polysaccharides from different grape and winemaking products and provide information about its composition for possible oenological use. The matrixes used were white pomace (WP), red pomace (RP), white must (WM), red must (RM), red wine (RW), lees recovered after the red winemaking (RL), and lees recovered after the white winemaking (WL). Further fractionation techniques were used to obtain extracts with higher purification degrees (WPP and DWRP). Once obtained, all the fractions were analyzed in terms of monosaccharide composition, polysaccharide families, molecular weight distribution, polyphenolic composition and content, and protein content, compared with those formulates of mannoproteins commercially available (CM).

2. Material and methods

2.1. Materials

White grape pomace and white lees were obtained from the same winemaking process of Viura *Vitis vinifera* L. variety. Red grape pomace, red must, red wine and red lees were obtained from the same winemaking process of Tempranillo *Vitis vinifera* L. variety. Both winemakings were carried out during 2019 vintage in a winery of the Rioja Qualified Denomination of Origin. The process to obtain the fractions rich in RG-II was carried out in the INRAE Centre of Montpellier. Detailed information is provided below.

2.1.1. Grape pomace

White grape pomace was obtained from Viura *Vitis vinifera* L. variety after the pressing. Grapes were harvested at 22.8 °Brix, pH 3.32 and 6.54 g L^{-1} total acidity as g L^{-1} tartaric acid, and pressed in a pneumatic press (BucherVaslin XPro 8, France). After pressing, the grape pomace was obtained and preserved in a freezer at −5 °C. Red grape pomace was obtained from Tempranillo *Vitis vinifera* L. variety after the pressing. Grapes were harvested at 24.5 °Brix, pH 3.41 and 6.32 g L^{-1} total acidity as g L^{-1} tartaric acid. After the alcoholic fermentation, the solid parts were pressed, and the grape pomace was obtained and preserved in a freezer at −5 °C.

2.1.2. Musts

Red must was obtained from Tempranillo *Vitis vinifera* L. variety after the crushing and destemming of the grapes. White concentrated must (65° Brix) was supplied by Julian Soler S.A. (Cuenca, Spain).

2.1.3. Wine

Red wine was made by traditional winemaking from Tempranillo *Vitis vinifera* L. variety. Grapes were destemmed and pressed, and fermentation was conducted by adding 25 g hL^{-1} of *Saccharomyces cerevisiae* Uvaferm HPS™ (Lallemand Inc, Montreal, Canada).

2.1.4. Lees

White lees were recovered after the white winemaking of Viura *Vitis vinifera* L. variety. Red lees were recovered after the red winemaking of Tempranillo *Vitis vinifera* L. variety.

2.1.5. Fractions rich in RG-II

These fractions were obtained from a red wine from Carignan noir *Vitis vinifera* L. variety or from the washing residues used by distilleries (SFD, Vallon Pont d'arc, France) to drain wine marc after concentration by ultrafiltration and rotary evaporation. Total colloids were precipitated by five volumes of ethanol, the precipitate was redissolved in water, and freeze dried.

2.1.6. Commercial mannoproteins

Different commercial products (CM) derived from yeast cell walls and rich in mannoproteins, and usually employed for wine finning and wine aging, were analyzed. Mannolees and Noblesse were supplied by Lallemand Bio S.L (Madrid, Spain), Surlì Elevage was supplied by Enartis (San Martino, Italy) and Superbouquet MN was supplied by Agrovin (Ciudad Real, Spain).

2.2. Obtainment of polysaccharide fractions

2.2.1. Extraction of polysaccharides from grape pomaces

The extraction of polysaccharides from pomaces was performed following the procedure recently published by our research group (Canalejo, Guadalupe, Martínez-Lapuente, Ayestarán, & Pérez-Magariño, 2021). Firstly, pomaces were frozen at −5 °C. After defrosting, they were homogenized using an UltraTurrax at 18,000–20,000 rpm (15–20 min). The extraction was carried out for 18 h with an acidic solution of tartaric acid (pH 1, solid to liquid ratio 1:4) in a thermostatic ultrasonic bath at 22 °C and 35 kHz. Then, the samples were centrifuged (13,600*g* for 20 min, 4 °C), and the supernatants were concentrated five times with a rotary evaporator at maximum 32 °C. Polysaccharides were recovered in the supernatants by precipitation with four volumes of cold 96% ethanol containing 0.3 M HCl (pH 0.6) for 20 h at 4 °C. The samples were centrifuged (33,000*g* for 20 min), the supernatants discarded, and the pellets freeze-dried. These precipitates contained the polysaccharides extracted from the white grape pomace (WP) and the red grape pomace (RP). All the reagents used were food-grade and food-safe.

2.2.2. Extraction of polysaccharides from musts

Polysaccharides from must were recovered by precipitation after ethanolic dehydration (Ayestarán, Guadalupe, & León, 2004; Guadalupe, Martínez-Pinilla, Garrido, Carrillo, & Ayestarán, 2012). For the extraction from red must, the must was previously concentrated five times to ensure the quantitative precipitation of all polysaccharide families (Ayestarán et al., 2004; Guadalupe et al., 2012). For the extraction in white must, a commercial white concentrated must (65° Brix) was used as it is commercially available. Polysaccharides were precipitated with four volumes of cold 96% ethanol containing 0.3 M HCl (pH 0.6) for 20 h at 4 °C. The samples were then centrifuged, the

supernatants discarded, and the pellets washed with ethanol several times and freeze-dried. These precipitates contained the polysaccharides extracted from the white must (WM) and the red must (RM). All the reagents used were food-grade and food-safe.

2.2.3. Extraction of polysaccharides from wine

Wine polysaccharides were recovered by precipitation after ethanolic dehydration as previously described (Ayestarán et al., 2004; Guadalupe et al., 2012). The wine was concentrated five times and precipitated with four volumes of cold 96% ethanol containing 0.3 M HCl (pH 0.6) for 20 h at 4 °C. The pellets were washed with ethanol several times and freeze-dried. The precipitates contained the polysaccharides extracted from the red wine (RW). All the reagents used were food-grade and food-safe.

2.2.4. Obtainment of the fractions rich in RG-II

A first fraction was obtained from the freeze-dried polysaccharides extracted from a Carignan noir wine by two successive steps of anion-exchange performed following the procedure described by Buffetto et al., 2014 and Vidal, Williams, Doco, Moutounet, & Pellerin, 2003. Polysaccharides were solved in water and dialyzed against 50 mM sodium citrate buffer pH 4.6 before being loaded on a Fractogel EMD DEAE 650 (M) (Merck, Germany) column (18 × 24 cm^2) equilibrated with the same buffer. An unbound fraction was recovered, and the bound polysaccharides were eluted by stepwise gradient of NaCl (10, 50, 150 and 250 mM in the starting buffer). The fraction eluted by 50 mM of NaCl on the Fractogel EMD DEAE 650 was loaded on a concanavalin A-Sepharose (Pharmacia, Sweden) column equilibrated 50 mM sodium acetate buffer pH 5.6 containing 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 1 mM $MnCl_2$, the unbound fraction was collected, dialyzed against water, freeze dried and named WPP (Wine Purified Polysaccharides). A second fraction was obtained from the wash water used by the distillery after draining the distilled wine pomace. It was concentrated by ultrafiltration on a membrane with a 20 kg mol^{-1} cut off and rotary evaporation, total colloids were precipitated by four volumes of ethanol. The precipitate was redissolved in water and dialyzed against water. A 50 g L^{-1} solution was precipitated with final 40% ethanol overnight at 4 °C. After centrifugation, the pellet and supernatant were recovered. Finally, the supernatant was concentrated and then lyophilized to obtain the DWRP fraction (Distilled Washing Residues Polysaccharides).

2.2.5. Wine lees production

Wine lees were recovered from the winemaking of Viura and Tempranillo wines. Viura wines were made by traditional winemaking. Grapes were destemmed and pressed, and fermentation was conducted by adding 25 g hL^{-1} of *Saccharomyces cerevisiae* Uvaferm HPS™ (Lallemand Inc, Montreal, Canada). The fermentation was conducted at 18 °C. After 10 days from the end of the alcoholic fermentation, the wine was racked, and the lees collected from the bottom of the tank, centrifuged and freeze-dried (WL sample). Tempranillo wines were made by traditional winemaking. Grapes were destemmed and crushed, and fermentation was conducted by adding 25 g hL^{-1} of *Saccharomyces cerevisiae* Uvaferm HPS™ (Lallemand Inc, Montreal, Canada). The fermentation was conducted at 22 °C. Thereafter, malolactic fermentation was spontaneously made. Thereafter, the wine was racked, and the lees collected from the bottom of the tank. They were centrifuged and the pellet was freeze-dried (RL sample).

2.3. Quantification of monosaccharides by GC–MS and estimation of polysaccharide families

The monosaccharide composition of the fractions was determined by gas chromatography with mass detector (GC–MS) of their trimethylsilyl-ester *O*-methyl glycosyl-derivates (TMS) obtained after acidic methanolysis and derivatization as described by Guadalupe et al., 2012. The chromatographic column was an Agilent HP-5 ms fused silica GC column (30 m × 0.25 mm × 0.25 μm), and the chromatograph an Agilent Technologies 7890A (Agilent Technologies, Waldbronn, Germany) coupled to a 5975C VL quadrupole mass detector. The content of each polysaccharide family was estimated from the concentration of their individual glycosyl residues which are characteristic of structurally identified wine polysaccharides (Doco, Quellec, Moutounet, & Pellerin, 1999; Doco, Williams, Pauly, O'Neill, & Pellerin, 2003; Guadalupe et al., 2012; Guadalupe, Ayestarán, Williams, & Doco, 2015). Briefly, all the mannose content was attributed to yeast mannoproteins, and the glucose content was used to estimate de concentration of glucosyl polysaccharides. RG-II content was calculated from the sum of its diagnostic sugars, which represent approximately 25% of the RG-II molecule, for one residue of 2-*O*-methyl-fucose, RG-II contains 5 rhamnosyl, 3 arabinosyl, 2 galactosyl, 1 glucuronosyl and 10 galacturonosyl residues. PRAG, which is a large family that contains structurally well-defined polysaccharides such as arabinans, AG, and AGP, was estimated from the sum of galactosyl and arabinosyl residues remaining when we removed RG-II monosaccharides. The remaining galacturonosyl residues were attributed to homogalacturonans. The validity of the used methods, their repeatability as well as the hydrolysis yield were checked according to Vidal et al., 2003.

2.4. Analysis of polysaccharides by HPSEC-RID

The molecular weight (Mw) distributions of the extracts were estimated by high-resolution size-exclusion chromatography in a modular 1100 Agilent liquid chromatograph (Agilent Technologies, Waldbronn, Germany) with a refractive index detector by using two serial Shodex SB-803 and SB-805 columns (0.8 × 30 cm, Pharmacia, Sweeden) equilibrated at 1 mL min^{-1} with 0.1 M lithium nitrate, and the rest of conditions previously described (Ayestarán et al., 2004; Guadalupe et al., 2012). The Mw were determined by calibration of the Shodex columns with narrow pullulan standards (Shodex P-82, Waters, Barcelona, Spain) P-5, Mw = 5.9 kg mol^{-1}; P-10, Mw = 11.8 kg mol^{-1}; P-50, Mw = 47.3 kg mol^{-1}, P-100, Mw = 112 kg mol^{-1} and P-200, Mw = 212 kg mol^{-1}.

2.5. Quantification of monomeric phenolic compounds by HPLC-DAD

The extracts were solved in methanol:water (50:50, v/v) and filtered through a 0.45 μm filter before use in the HPLC-DAD. The analysis of the phenolic compounds was determined by using the Agilent 1100 liquid chromatograph equipped with a Lichrospher 100 RP-18 reversed phase column (0.4 × 25 cm, 5 μm) following the method of Portu, López, Baroja, Santamaría, and Garde-Cerdán (2016).

2.6. Quantification of protein and phenolic content by colorimetric assays

The protein concentration of the extracts was determined by the Bradford Protein Assay (BPA) (Bradford, 1976) using bovine albumin as standard. The content of total phenolics was estimated by the Folin assay (Singleton & Rossi, 1965) using gallic acid as standard.

2.7. Statistical analyses

All the extractions and analytical determinations were made in triplicate. Statistical analyses were carried out using SPSS Statics 23 (IBM Corp., Armonk, NY, USA). A one-way analysis of variance (ANOVA) was applied at a confidence level of 95 % (p-value of 0.05) to determine significant differences among the extracts using the Duncan post-hoc testing.

3. Results and discussion

3.1. Monosaccharide composition of the extracts

Table 1 shows the carbohydrate composition obtained in the different extracts. Significant differences were observed in the amount of monosaccharides, which indicated differences in their polysaccharide composition. The most prevalent glucosyl residue detected in both white and grape pomaces (WP and RP) was glucose, followed by galactose and galacturonic acid, in agreement with the data described in bibliography for grape pomace (Canalejo et al., 2021) and grape skins (Apolinar-Valiente, Romero-Cascales, Gómez-Plaza, López-Roca, & Ros-García, 2015 and 2017). Glucose is the main component of major structural polysaccharides from the grape cell walls such as cellulose and hemicelluloses, and the most prevalent residue in both the skin and the pulp cell walls (Vidal, Williams, O'Neill, & Pellerin, 2001). The high content of glucose was thus attributed to grape structural glucosyl polysaccharides (GP). After glucose, the most prevalent sugars were galacturonic acid and galactose. Rhamnose, arabinose and glucuronic acid were also detected in the ranges described in bibliography for white pomace (Canalejo et al., 2021). The contents of galactose and arabinose were used to estimate the content of the pectic PRAG, also considering the molar ratios of the RG-II; and the content of galacturonic acid was attributed to HG. The content of mannose agreed with the data obtained in other studies for red and white grape skins (Apolinar-Valiente et al., 2015 and 2017), and was attributed to mannans and hemicellulose from the grape pericarp (Arnous & Meyer, 2009; Minjares-Fuentes, Femenia, Garau, Candelas-Cadillo, & Simal, 2016).

The presence of minor carbohydrates, 2-*O*-methyl xylose, 2-*O*-methyl fucose, aceric acid, apiose, 3-deoxy-D-lyxo-hepyulosaric acid (DHA) and 2-keto-3-deoxy-D-*manno*-octulosonic acid (Kdo), were also detected in WP and RP, and were used to estimate the content of RG-II. Results showed that the content of total monosaccharides was higher in WP than in RP, indicating a higher extraction of polysaccharides from the WP, which was attributed to differences in the grape variety but also to the obtainment of the pomace, as red pomace is obtained after the alcoholic fermentation and, probably, most grape polysaccharides has already been released into the must during the fermentation.

Glucose, galactose, mannose and galacturonic acid were the major carbohydrates detected in both extracts from musts, and their content was significantly higher in the WM extract, indicating that the extraction of polysaccharides was higher with the white must. This was attributed to the cultivar and to the fact that the white must is obtained once the pressing of the grapes is done, which facilitates the release of grape cell-wall polysaccharides into the must. However, red must is obtained after the grapes have been just crushed and destemmed, meaning that some of the polysaccharides still remain adhered to the pulp and skin of the grapes, so they are not released into the must.

Galactose, glucose, mannose, and arabinose were the major carbohydrates detected in the RW extract, followed by galacturonic and glucuronic acid. The amounts obtained were like those described in bibliography for red wines (Apolinar-Valiente et al., 2013; Guadalupe, Palacios, & Ayestarán, 2007; Martínez-Lapuente, Guadalupe, Ayestarán, & Pérez-Porras, 2021), and were used to estimate the content of grape PRAG and GP, and in the case of mannose, to calculate the amount MP released by yeast during fermentation.

Galacturonic acid and rhamnose were the most prevalent sugar in WPP and DWRP. These extracts showed the highest concentrations of galacturonic acid, rhamnose, glucuronic acid, arabinose, and the rare sugars of RG-II.

The commercial mannoprotein-based products (CM) and the extracts obtained from white and red lees (WL and RL) were mainly composed of mannose and showed the highest content of it among all the extracts. The percentage of mannose in CM extracts was between 67 and 70%, and the percentage of glucose between 30 and 33%, confirming that these products were mainly mannoproteins enriched products. The monosaccharides forming grape polysaccharides were not detected in CM. The content of mannose was also higher than glucose in the extracts from lees, and their contents were similar in both WL and RL.

Different characteristic ratios were calculated to elucidate the polysaccharide and oligosaccharide sugar structure of each extract: Arabinose to Galactose (Ara/Gal), Rhamnose to Galacturonic acid (Rha/GalA) and Arabinose + Galactose to Rhamnose (Ara + Gal)/Rha (Table 2). The Ara/Gal ratio is characteristic of PRAG-like structures, and higher values of this ratio indicate higher contents of arabinose or structures rich in arabinose arising from the pectic framework (Vidal et al., 2003). The Rha/GalA ratio could be an indicator of the relative richness of polysaccharides as homogalacturonans versus rhamnogalacturonan-like structures (Arnous & Meyer, 2009). Except for the fractions WPP and DWRP, the Ara/Gal and Rha/GalA were below 0.5 in all the extracts, indicating that these extracts contained a low content of polysaccharides rich in arabinose and a majority of homogalacturonan-like structures. The WPP extract showed the highest Ara/Gal and Rha/GalA ratio, with values of 0.94 and 1.1 respectively, indicating a major presence of structures rich in arabinose and a similar richness in homogalacturonan and rhamnogalacturonan-like structures. The Ara + Gal/Rha ratios were used to estimate the relative importance of the neutral side-chains to the rhamnogalacturonan backbone, since most of the arabinose and galactose content is associated with pectin hairy regions (Apolinar-Valiente et al., 2015). These ratios were significantly higher in extracts from WP, WM, RM and RW, which might indicate that the rhamnogalacturonan-like structures in these extracts carry more neutral lateral chains. Previous studies have shown that the composition of certain polysaccharides in grapes may vary according to the grape cultivar (Apolinar-Valiente et al., 2015, Apolinar-Valiente, Gómez-Plaza, Terrier, Doco, & Ros-García, 2017). The results of our study suggest that this structure was also dependent on the matrix used for the polysaccharide extraction.

3.2. Polysaccharide composition of the extracts

Table 2 shows the concentrations of the polysaccharide families in the extracts. It is important to highlight that the presence of mannose in the grape pomace and must extracts was associated to the mannans of the grape pericarp, which are basically composed of mannose units. Mannose is not a constituent of any other plant cell wall polysaccharide as it is assumed that mannose is not a side-chain substituent of rhamnogalacturonan I (RG-I) (Arnous & Meyer, 2009; Nunan, Sims, Bacic, Robinson, & Fincher, 1998). On the other hand, the presence of mannose in the extracts from wines and lees was attributed to mannoproteins from yeast. In the same way, GP were attributed to structural celluloses and hemicelluloses in the extracts from pomaces (Canalejo et al., 2021) and musts, glucans in the CM extracts (Pérez-Magariño et al., 2015), and both in the extracts obtained from wine and lees. Total polysaccharides (TP) were calculated as the sum of PRAG, RG-II, HG, MP, and GP.

The highest quantities of TP were obtained in the extract from WM and the WPP extract, with values above 870 mg g^{-1}, followed by the extracts obtained from WP and WL, with values above 500 mg g^{-1}. These extracts showed values in the range obtained for the commercial products (489 – 834 mg g^{-1}). The lowest values were obtained in extracts from RP, RM, RL and DWRP.

As expected, the WPP extract showed the highest polysaccharide content and purity due to the purification steps used for its isolation. This extract was mainly composed of RG-II (74.7% of TP), followed by PRAG (14.7%), HG (6.9%) and GP (3.2%) (Table 5). The DWRP extract showed half the amount of total polysaccharides than WPP extract, but the majority polysaccharide was also RG-II, which constituted 53% of TP. It was concluded that the purification steps led to extracts with high concentrations of RG-II, mainly in the WPP extract. These results open new lines of interest since they allow testing the oenological application of extracts rich in RG-II, non-available in the market. RG-II is described as a strong accelerator of hydrogen tartrate crystallization at low

Table 1
Monosaccharide composition (mg carbohydrates g^{-1} of extract)[a] of the extracts determined by GC–MS of their trimethylsilyl-ester O-methyl glycosyl-derivates (TMS) obtained after acidic methanolysis and derivatization.

Extract[b]	2-*O*-Me Fuc[c]	2-*O*-Me Xyl[c]	Arabinose	Rhamnose	Fucose	Xylose	Mannose	Galactose	GalA[c]	Glucose	GlcA[c]	Ara/Gal	Rha/GalA	(Ara + Gal)/Rha
WP	0.53 (0.08) b	0.52 (0.02)b	3.9 (0.5)a	2.4 (0.2)a	0.14 (0.02) a	1.1 (0.0)a	2.9 (0.3)a	120.3 (21.4) cd	74.7 (0.7) e	224.3 (40.6)e	1.4 (0.2)a	0.03 (0.00)a	0.03 (0.00)a	51.3 (8.0)c
RP	0.64 (0.06) b	0.58 (0.14)b	16.1 (3.6) b	9.9 (3.3)ab	0.65 (0.12) a	6.3 (0.6)c	19.3 (5.2)ab	70.1 (3.6)b	64.3 (5.6) d	126.7 (28.1)c	9.3 (1.0) bc	0.23 (0.05)b	0.15 (0.05)b	9.4 (3.3)a
WM	0.09 (0.01) a	0.05(0.00) a	27.4 (5.5)c	13.4 (1.5)b	0.74 (0.13) a	2.6 (0.4)ab	171.4 (6.4)c	361.1 (60.4) f	26.3 (2.7) b	183.9 (16.2)e	17.4 (2.6) d	0.08 (0.00)a	0.51 (0.07)d	28.9 (2.0)b
RM	0.58 (0.14) b	0.57 (0.08)b	2.4 (0.4) a	1.3 (0.1)a	0.08 (0.01) a	0.3 (0.0)a	37.8 (4.2)ab	209.7 (17.2) e	33.7 (9.0) c	45.3 (5.6)b	0.8 (0.1)a	0.01 (0.00)a	0.04 (0.01)a	158.6 (10.6) d
RW	0.36 (0.09) ab	0.49 (0.09)b	45.8 (2.4) d	4.0 (0.3)a	0.13 (0.01) a	0.8 (0.0)a	45.1 (3.2)b	128.0 (24.6) d	24.7 (2.1) b	61.9 (30.7)b	8.9 (0.4) bc	0.36 (0.09)c	0.16 (0.02)b	43.5 (8.9)c
WPP	7.88 (0.62) d	7.99 (0.64)d	76.5 (5.4)e	147.5 (5.4) d	15.06 (1.33)c	17.0 (4.6)d	3.0 (0.8)a	81.6 (4.4)bc	132.8 (2.0)g	11.8 (2.6)a	71.1 (11.6)e	0.94 (0.03)d	1.11 (0.06)f	1.1 (0.1)a
DWRP	2.41 (0.15) c	1.93 (0.04)c	28.8 (6.0)c	59.2 (13.9) c	6.33 (1.85) b	4.1 (0.8)bc	3.6 (0.5)a	67.7 (1.0)b	85.8 (2.2)f	6.7 (0.5)a	15.6 (3.1) cd	0.43 (0.08)c	0.69 (0.15)e	1.7 (0.3)a
WL	0.03 (0.01) a	0.08 (0.02)a	2.4 (0.3)a	1.2 (0.4)a	0.10 (0.04) a	0.9 (0.3)a	202.5 (60.6)c	11.0 (3.2)a	4.1 (0.8)a	167.5 (31.6)d	0.8 (0.3)a	0.22 (0.07)b	0.29 (0.06)c	11.3 (1.1)a
RL	0.08 (0.02) a	0.03 (0.01)a	5.4 (1.9)a	2.7 (0.3)a	0.23 (0.07) a	1.4 (0.4)ab	191.0 (17.9)c	12.9 (3.4)a	9.4 (0.9)a	136.7 (10.4)cd	2.1 (0.4) ab	0.42 (0.07)c	0.29 (0.02)c	6.8 (1.4)a
CM[d]	nd	nd	nd	nd	nd	0.28 (0.06) –0.38 (0.07)a	318.3 (19.1) –484.1 (38.6)d	nd	nd	134.3 (15.3) –229.0 (14.0)e	nd			
F-value	388.315*	396.811*	143.525*	273.238*	132.855*	33.660*	120.364*	64.194*	358.388*	48.296*	87.365*	80.685*	91.563*	244.921*

[a] All parameters are given with their standard deviation (n = 3). Different letters in the same column indicate statistically significant differences ($p < 0.05$). A one-way analysis of variance (ANOVA) with the Duncan post-hoc test was used. Level of significance * indicates significance at $p < 0.001$.

[b] Extracts obtained from the different matrixes. WP: White Pomace; RP: Red Pomace; WM: White Must; RM: Red Must; RW: Red Wine; WPP: Wine Purified Polysaccharides; DWRP: Distilled Washing Residues Polysaccharides; WL: White Lees; TL: Red Lees; CM: Commercial Mannoproteins.

[c] 2-*O*-Me Fuc: 2-*O*-Me-Fucose; 2-*O*-Me Xyl: 2-*O*-Me-Xylose; GalA: Galacturonic acid; GlcA: Glucuronic acid, TC: Total carbohydrates as the sum of individual monosaccharides.

[d] Range of values obtained in the commercial products.

Table 2
Polysaccharide concentration (mg polysaccharide g^{-1} of extract)[a] of the extracts.

Extracts[b]	PRAG[c]	RG-II[c]	HG[c]	MP[c]	GP[c]	TP[c]
WP	198.8 (59.6)c	50.5 (4.9)b	75.7 (17.2)d	4.4 (1.0)a	225.5 (40.6)d	554.9 (74.3)ab
RP	107.5 (7.6)b	60.6 (4.8)b	60.7 (11.8)c	24.1 (5.2)a	133.4 (27.5)c	386.3 (31.7)a
WM	445.2 (53.5)e	7.4 (1.0)a	25.5 (5.5)c	214.2 (33.9)b	186.5 (16.2)d	878.7 (65.6)d
RM	277.4 (21.4)d	55.5 (0.3)b	28.4 (2.8)c	47.4 (5.2)a	46.0 (5.7)b	454.7 (22.9)ab
RW	197.7 (45.1)c	38.9 (4.0)b	21.4 (5.0)bc	55.7 (2.8)a	62.7 (27.7)b	376.5 (53.45)a
WPP	132.2 (6.9)b	670.3 (36.8)d	61.8 (4.4)cd	3.7 (0.8)a	28.8 (5.3)ab	896.8 (38.1)d
DWRP	97.6 (6.5)b	215.8 (7.5)c	77.69 (8.2)d	4.5 (0.5)a	10.8 (1.0)a	406.4 (12.9)a
WL	13.0 (4.5)a	3.9 (0.6)a	3.8 (1.3)a	329.5 (60.6)b	168.4 (31.6)cd	518.6 (68.4)ab
RL	15.4 (1.1)a	5.9 (1.3)a	8.8 (1.3)ab	264.5 (17.9)b	138.1 (10.4)c	432.7 (20.8)a
CM[d]	nd	nd	nd	397.9 (20.4)–605.7 (38.6)c	134.6 (11.6)–229.4 (14.0)d	532.4 (26)–834.4 (41.1)c
F-value	84.213*	609.718*	40.295*	74.423*	39.567*	40.426*

[a] All parameters are given with their standard deviation (n = 3). Different letters in the same column indicate statistically significant differences ($p < 0.05$). A one-way analysis of variance (ANOVA) with the Duncan post-hoc test was used. Level of significance: * indicates significance at $p < 0.001$.

[b] Extracts obtained from the different matrixes. WP: White Pomace; RP: Red Pomace; WM: White Must; RM: Red Must; RW: Red Wine; WPP: Wine Purified Polysaccharides; DWRP: Distilled Washing Residues Polysaccharides; WL: White Lees; TL: Red Lees; CM: Commercial Mannoproteins.

[c] PRAG: Polysaccharide rich in arabinose and galactose; RG-II: Rhamnogalacturonans type II; HG: Homogalacturonans; MP: Mannans and Mannoproteins; GP: Glucosyl Polysaccharides; TP: Total Polysaccharides as the sum of PRAG, RG-II, HG, MP, and GP.

[d] Range of values obtained in the commercial products.

concentrations (Gerbaud et al., 1997) and could be used in winemaking to prevent it, or to reduce the level of toxic cations as it forms dimers RG-II/boron (Pérez et al., 2003). Even more interesting, the WPP extract could be a very effective finning agent for softening astringency in red wines as RG-II molecules have shown the greatest effect in reducing the astringency perception in wines (Brandão et al., 2017; Brandão et al., 2020; Quijada-Morín et al., 2014; Riou et al., 2002) by the formation of co-aggregates with tannins due to their branched side chains and unusual monosaccharides. Therefore, RG-II rich-extracts could be an efficient alternative to the finning agents of animal protein-origin, such as gelatins and albumins, because they are allergen-free and switch to sustainable and vegan-friendly wine production.

The results obtained with the WP revealed its potential to be exploited to obtain valuable grape by-products rich in polysaccharides. A good recovery of total polysaccharides was achieved from the WP, and all the types of polysaccharide families were isolated, being PRAG and GP the majority families (36 and 41% respectively) followed by HG (13.6%) and RG-II (9.1%). As RG-II, PRAG have also shown to modulate tannin self-aggregation, and affect the body, structure, and mouthfeel sensations of the wines (Chong et al., 2019; Brandão et al., 2017; Brandão et al., 2020; Quijada-Morín et al., 2014; Vidal et al., 2004; Riou et al., 2002). PRAG also prevent against protein haze in white wines (Lankhorst et al., 2017) and improve the foam stability in sparkling wines more than MP, HG and RG-II (Martínez-Lapuente et al., 2015). They have also been described to increase the volatility of certain aroma compounds (Dufour & Bayonove, 1999; Mitropoulou et al., 2011). Therefore, WP extract could have many applications. It could be used as a stabilizer to prevent protein haze in white wines, as an adjuvant in sparkling winemaking to improve the foaming properties, as a finning agent in red wines to decrease the perception of aggressive tannins and improve the mouthfeel sensations, as an oenological product to preserve wine aroma in white wines, etc. On the other hand, HG have shown higher binding affinities with some polyphenolic compounds than RG-I and AGP, improving the color stability of red wines (Fernandes et al., 2021).

The use of RP as a matrix to obtain grape polysaccharides led to extracts with lower contents of TP than the WP. As previously explained, this was attributed to differences in the obtaining of the pomace. Hence, WP is obtained after the pressing of the grapes before alcoholic fermentation. The pressing would facilitate the degradation of the grape pulp and skin cell walls and the release of polysaccharides. On the contrary, the RP is obtained after the alcoholic fermentation and, probably, most of the grape polysaccharides has already been released into the must during the fermentation process due to the action of grape and yeast endogenous enzymes. All polysaccharide families were recovered from RP, being again GP and PRAG the majority constituents, followed by RG-II, HG and mannans. The polysaccharide amounts obtained for WP was in the ranges previously described in our research (Canalejo et al., 2021).

White musts were revealed as the best matrix to recover grape polysaccharides. The highest quantities of polysaccharides were obtained from the extracts from WM. The PRAG represented more than 50% of the polysaccharides in this extract, followed by GP and mannans and small proportion of RG-II. Hence, these extracts could have the same uses as those described for WP. The amounts obtained from red must were half than those from WM because most of the grape polysaccharides remains in the solid cap and are released during the fermentation process.

The extracts obtained from red wine (RW) showed the lowest quantities of total polysaccharides, and were mainly composed of PRAG (54%), GP (16.7%), MP (14.8%) RG-II (10.3%) and lower quantities of HG (Table 5).

White and red lees showed a potential to be used to recover mannoproteins and glucans. Polysaccharide contents above 500 mg per g of extract were obtained from WL extracts, and around 430 from RL, obtaining extraction yields higher than those described in bibliography (De Iseppi et al., 2021a, De Iseppi, Marangon, Lomolino, Crapisi, & Curioni, 2021b). WL and RL extract were composed of MP (63.5 and 61.1% respectively) and glucans (32.5 and 31.9% respectively). These results revealed that lees could be a good matrix to recover yeast polysaccharides and a good alternative for the obtention of mannoprotein-rich products. Other studies also report the applications of these yeast polysaccharides in the food industry as emulsifiers and foaming agents (De Iseppi et al., 2021a; De Iseppi et al., 2021b); Varelas, Liouni, Calokerinos, & Nerantzis, 2016; Da Silva Araújo et al., 2014).

Finally, the commercial mannoprotein products analyzed were effectively composed of mannoproteins and around 25 % glucans. It is important to notice that the extracts from WM and WPP had more quantities of polysaccharides that the CM products. Moreover, extracts from WP and WL showed values in the range of the commercial products available in the market.

3.3. Molecular weight distribution of polysaccharides from the extracts

HPSEC-RID was performed to obtain the molecular weight (Mw) of the extracted polysaccharides and estimate their proportions (Guadalupe et al., 2012).

Different polysaccharide profiles were obtained for the different extracts (Fig. 1). Some extracts showed HPSEC profiles of 6 peaks while others showed fewer peaks. Peak 1 corresponded to high Mw

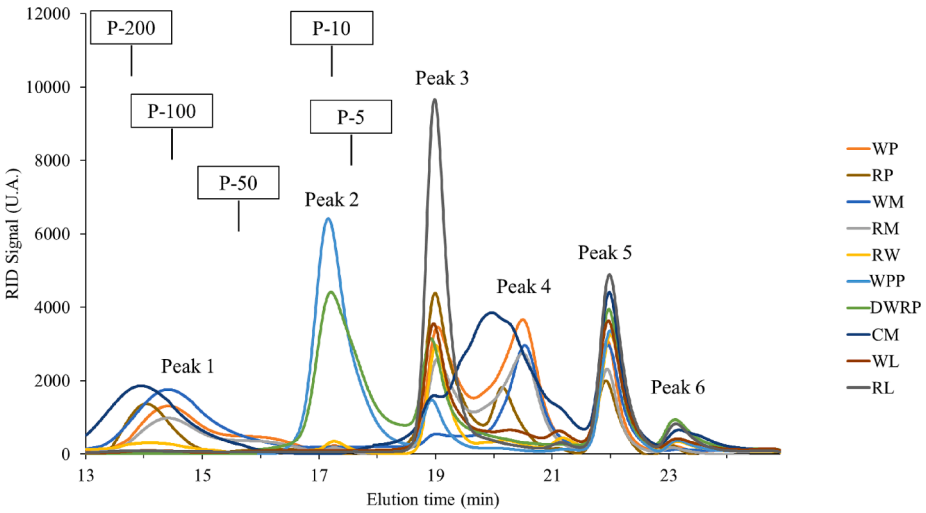

Fig. 1. HPSEC-RID chromatograms of the polysaccharides extracted from the different sources. Chromatograms obtained using two serial Shodex SB-803 and SB-805 columns. Elution times for the molecular weight markers (P-5 → P-200) are shown. Extracts obtained from the different matrixes: WP: White Pomace; RP: Red Pomace; WM: White Must; RM: Red Must; RW: Red Wine; WPP: Wine Purified Polysaccharides; DWRP: Distilled Washing Residues Polysaccharides; CM: Commercial Mannoproteins; WL: White Lees; RL: Red Lees. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

polysaccharides with an average molecular mass of 167 kg mol^{-1}. According to previous work (Canalejo et al., 2021; Guadalupe & Ayestarán, 2007), it corresponded to a complex mixture of high Mw PRAG, MP and GP. Peak 2 corresponded medium Mw polysaccharides, with an average Mw of 10.3 kg mol^{-1}. It was attributed to a mixture of RG- II dimers and PRAG, GP and MP and mannans of medium Mw (Ducasse et al., 2010; Guadalupe & Ayestarán, 2007). Signals eluting after 18 min (peak 3 to peak 6) corresponded to a Mw less than 5 kg mol $^{-1}$, and it was attributed to oligosaccharides and small fragments of PRAG, MP, HG and RG-II monomer (Guadalupe & Ayestarán, 2007; Guadalupe et al., 2012).

Table 3 shows the Mw distributions of the polysaccharides extracted from the different matrixes. A summary is also shown in Table 5. High Mw polysaccharides were only detected in important amounts in extracts from pomace, musts and wines, and commercial products. Extracts from musts showed the highest proportion of high Mw polysaccharides, indicating an important proportion of high Mw PRAG and GP. In these extracts, around 40% of the polysaccharides detected corresponded to high Mw PRAG or GP, 5% to medium Mw compounds and 55% to low Mw or oligosaccharides. RW extracts also showed an important amount of large and medium polysaccharides. In contrast, the extracts obtained from grape pomace showed a low proportion of large and medium polysaccharides (~22%) and were mainly composed of low Mw polysaccharides and oligosaccharides (~78%). Oligosaccharides have been related to the astringency perception. Some studies describe that oligosaccharides formed by mannose and galactose increase the astringency perception (Boulet et al., 2016; Quijada-Morín et al., 2014) by competition with polysaccharides to interact with tannins. On the contrary, RG-like and HG-like oligosaccharides are described to interact with anthocyanins and reduce the astringency perception (Quijada-Morín et al., 2014; Larsen et al., 2019).

The highest content of medium Mw polysaccharides was found in the WPP and DWRP fractions, being these compounds the predominant in both fractions. This agreed with the concentrations obtained for the polysaccharide families as both extracts were mainly composed of RG-II, which elute in the peak of medium Mw. The extracts from lees were exclusively composed of oligosaccharides and polysaccharides of low Mw, which agreed with the observations of De Issepi el al. (2021). This result indicated that the MP and glucans extracted were small fragments,

Table 3
Polysaccharide molecular weight distribution (%)[a] of the extracts determined by HPSEC-RID on two serial Shodex SB-803 HQ and SB-805 HQ columns.

		Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6
	Mw (kg mol^{-1})	546.30–31.81	24.5–3.6	< 5 kg mol^{-1}	< 5 kg mol^{-1}	< 5 kg mol^{-1}	< 5 kg mol^{-1}
	AMw (kg mol^{-1})[b]	167.6	10.3				
		High Mw[c]	Medium Mw[c]	Low Mw (Oligosaccharides)[c]			
Extracts[d]	**WP**	19.0 (4.7)b	3.6 (0.9)a	28.1 (3.7)de	45.5 (8.2)c	2.0 (1.1)a	1.8 (0.7)a
	RP	13.2 (1.8)b	6.9 (1.2)a	43.1 (3.1)e	12.7 (1.9)a	21.8 (3.5)de	2.3 (0.1)a
	WM	41.0 (4.6)c	4.6 (1.8)a	2.9 (0.3)a	36.9 (8.3)b	14.6 (3.8)bc	0.0
	RM	24.7 (6.0)b	20.4 (5.1)b	23.4 (2.4)cd	8.2 (3.4)a	20.6 (7.6)bcd	2.7 (0.9)a
	RW	24.7 (6.0)b	20.4 (5.1)b	23.4 (2.4)bc	8.2 (3.4)a	20.6 (7.6)bcd	2.7 (0.9)a
	WPP	0.0	71.2 (3.4)d	9.7 (1.7)ab	0.0	16.9 (5.0)bcd	2.2 (0.1)a
	DWRP	0.0	53.8 (5.6)c	22.8 (3.2)cd	0.0	19.1 (1.74)cde	4.3 (0.8)a
	WL	2.2 (0.7)a	0.0	49.6 (9.3)e	10.7 (1.6)a	32.4 (11.7)e	5.1 (1.4)a
	RL	0.0	0.0	82.8 (2.5)f	0.0	8.8 (4.2)ab	8.4 (3.2)b
	CM[e]	39.3 (1.3)–45.6 (2.1) c	1.0 (0.2)–1.6 (0.3) a	6.3 (1.1)– 6.7 (1.7) ab	18.1 (2.1)–22.9 (2.4) 2	17.3 (3.4)–19.3 (3.8) cde	1.7 (0.4)–3.9 (1.0) a
	F-value	21.471*	53.554*	43.139*	26.727*	5.803*	6.210*

[a] All parameters are given with their standard deviation (n = 3). Different letters in the same column indicate statistically significant differences ($p < 0.05$). A one-way analysis of variance (ANOVA) with a Duncan post-hoc test was used. Level of significance * indicates significance at $p \leq 0.001$.

[b] Average molecular weight (kg mol^{-1}).

[c] High Mw: polysaccharides of high molecular weight; Medium Mw: polysaccharides of medium molecular weight; Low Mw: polysaccharides of low molecular weight or oligosaccharydes.

[d] Extracts obtained from the different matrixes. WP: White Pomace; RP: Red Pomace; WM: White Must; RM: Red Must; RW: Red Wine; WPP: Wine Purified Polysaccharides; DWRP: Distilled Washing Residues Polysaccharides; WL: White Lees; TL: Red Lees; CM: Commercial Mannoproteins.

[e] Range of values obtained in the commercial products.

which was attributed to the fragmentation of these polysaccharides by endogenous enzymes during the winemaking process. In contrast, the MP and glucans from the commercial products showed around 40 to 45% high Mw polysaccharides.

3.4. Phenolic composition of the extracts

Carbohydrates are the main constituents of the grape cell walls and lees, but significant amounts of bound proteins and phenolic compounds are also present (Hernández-Hierro et al., 2014). In addition, the concentration of these compounds is also high in must and wines. Therefore, analyzing the proteins and phenolics was essential to know their content in the extracts. Table 4 shows the concentration of monomeric phenolic compounds.

Results indicated that the amount of phenolics extracted depended on the type of matrix and the extraction made. RW extract showed the highest content of monomeric phenolics, followed by RL, but the values were very low in both (25.8 and 17.3 mg g^{-1} respectively). No significant differences were found in the content of monomeric phenolics for RP, WP and RM extracts, with values between 9.2 and 12.4 mg g^{-1}; the rest of the extracts showed values below 3.6 mg g^{-1}. Anthocyanins were only detected in the extracts obtained from RP, RM, RW and RL, but in very low quantities. RW extract had the highest values of total flavonols followed by RL. Concretely, myricetin and syringetin compounds showed the highest quantities in both extracts. The rest of the extracts showed values of total flavonols below 3.8 mg g^{-1}. Regarding total flavanols, the highest content was detected in the pomace extracts, due to higher contents of epigallocatechin, catechin and procyanidin B1, although the values were below 9 mg g^{-1}. The content of hydroxybenzoic and hydroxycinnamic acids, and stilbenes was very low in all the extracts.

The content of total phenolic compounds, measured by the Folin assay, is shown in Table 5. These values were significantly higher than the contents of total monomeric phenolics as most of the quantified phenolic compounds by the Folin assay were tannins. As expected, the highest contents of total phenolics were obtained in RW followed by RM extracts. Except for these samples, the total phenolic content of the extracts was much lower than the total phenolic content described in the bibliography for extracts from grape skins (128.7–196.2 mg g^{-1}) (Apolinar-Valiente, Romero-Cascales, López-Roca, Gómez-Plaza, & Ros-García, 2010; Apolinar-Valiente et al., 2015 and 2017; Ferri et al., 2016; Hernández-Hierro et al., 2014). The extracts from lees (WL and RL), RP and WM showed similar contents of total phenolics, with values around 72 mg g^{-1}. The lowest values were found in WP and WPP.

In conclusion, the content of total phenolics in the extracts was significantly lower than the polysaccharide quantities and the contents described in bibliography, indicating that the procedures used were suitable to achieve a preferential extraction of polysaccharides, with very low presence of polyphenolic compounds.

3.5. Protein content of the extracts

Proteins are structural constituents forming a fibrillar net in grape cell walls. They are also the most important nitrogenous substances in the wines, being many of them glycoproteins derived from grape berries and mannoproteins from yeast. Table 5 shows the protein content expressed as mg of BSA per g of extract.

CM showed the highest protein content while the rest of the extracts did not show significant differences in their protein content. The values obtained (27.5–35.6 mg of BSA per g of extract) were significantly lower than those reported in other studies for extracts isolated from grape skins of different grape varieties and with different extraction procedures (66.8–175 mg of BSA per g of extract) (Apolinar-Valiente et al., 2010; 2015 and 2017). As reported in bibliography, the amount of proteins depends on the cultivar but also on the isolation procedure used (Apolinar-Valiente et al., 2010 and 2015). Our results indicated that the procedures used to isolate the polysaccharides from the pomace matrix led to lower co-precipitation of phenols and proteins than those described in the bibliography. The protein content obtained in the extracts from lees were like those reported in other studies for different extracts obtained from lees (De Iseppi et al., 2021a).

3.6. Polysaccharide purity

Previous studies of our workgroup (Pérez-Magariño et al., 2015) analyzed the polysaccharide purity of commercial dry yeast products rich in polysaccharides and how they affect the chemical composition, foam, and sensory quality of sparkling wines. It was concluded the commercial product with the highest purity and mannoprotein content produced the most significant changes in the volatile composition of the wines and enhanced the fruity aromas.

Table 5 shows the polysaccharide purity of the extracts, expressed as the total amount of polysaccharides in relation to the weight of the product analyzed. The extracts obtained from WM and the fraction rich in the RG-II (WPP) showed the highest purity, with values higher than 85%. The purity of the extract from the WP was also very good, with values around 55%, which was in the range of the values detected in the CM. The purity of the extracts from WL was also above 50%, being higher than those described in bibliography De Iseppi et al., 2021a, De Iseppi et al., 2021b)). The rest of the extracts showed similar polysaccharide purities, with values around 40%.

Table 5 also shows a summary of the polysaccharide composition of each fraction, its polysaccharide Mw distribution, protein, and phenolic content. All these data have been discussed in the previous sections.

4. Conclusions and perspectives

The present paper provides information about the composition of polysaccharide extracts recovered from different grape and winemaking products. The highest polysaccharide purity was obtained in the fraction WPP (89.7%) followed by the extract recovered from the white must (WM), white grape pomace (WP), and white lees (WL) (87.9, 55.5 and 51.9%, respectively). The procedures used were suitable to achieve a preferential extraction of polysaccharides, with very low presence of polyphenolic compounds and proteins. WPP extract showed the highest content of RG-II (74.7%), which makes it a very good alternative to be used as a finning agent for softening red wine astringency. WM extract contained high contents of PRAG (50.7%) and polysaccharides of high and low Mw. All polysaccharide families were isolated in WP extract, being PRAG and GP the major families. The polysaccharide composition of WM and WP extracts revealed a great potential to be used as stabilizing or finning agents during the winemaking, constituting a possible alternative to traditional animal-origin protein fining agents. WP and WL revealed as a valuable source to obtain polysaccharides thus opening the way to a new type of exploitation of these by-products. Future studies must evaluate their potential as stabilizing or finning agents during wine production as well as the use of by-products from other grape varieties.

Uncited references

CRediT authorship contribution statement

Diego Canalejo: Investigation, Formal analysis, Writing – original draft. **Zenaida Guadalupe:** Investigation, Formal analysis. **Leticia Martínez-Lapuente:** Investigation, Formal analysis. **Belén Ayestarán:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Silvia Pérez-Magariño:** Conceptualization, Funding acquisition. **Thierry Doco:** Conceptualization, Methodology, Visualization, Supervision, Funding acquisition, Writing – review & editing.

Table 4
Concentration of monomeric phenolic compounds (mg phenols g^{-1} of extract) [a] of the extracts determined by HPLC on a LiChrospher 100 RP18 column.

Flavonoids[c]	Extracts[b] WP	RP	WM	RM	RW	WPP	DWRP	WL	RL	CM[d]	F-value
Anthocyanins											
Dephinidin-3-glc	nd	0.40 (0.01)a	nd	0.56 (0.01)c	0.50 (0.02)b	nd	nd	nd	0.74 (0.04)d	nd	127.420*
Cyanidin-3-glc	nd	nd	nd	nd	nd	nd	nd	nd	0.29 (0.01)	nd	ns
Petunidin-3-glc	nd	0.40 (0.02)a	nd	0.55 (0.01)c	0.47 (0.02)b	nd	nd	nd	0.63 (0.03)d	nd	70.252*
Peonidin-3-glc	nd	0.29 (0.00)b	nd	0.28 (0.01)a	0.29 (0.01)ab	nd	nd	nd	0.33 (0.01)c	nd	47.066*
Malvidin-3-glc	nd	0.67 (0.05)a	nd	1.33 (0.01)c	0.85 (0.03)b	nd	nd	nd	1.36 (0.06)c	nd	191.197*
Total non-acylated	nd	*1.76 (0.06)a*	nd	*2.72 (0.11)c*	*2.10 (0.04)b*	nd	nd	nd	3.35 (0.08)d	nd	*634.030**
Malvidin-3-acglc	nd	nd	nd	0.28 (0.01)a	0.28 (0.01)a	nd	nd	nd	0.31 (0.01)b	nd	1039.956*
Total acylated	nd	nd	nd	*0.28 (0.01)a*	*0.28 (0.01)a*	nd	nd	nd	*0.31 (0.01)b*	nd	*4531.280**
Petunidina-3-cmglc	nd	nd	nd	0.28 (0.01)a	0.41 (0.01)c	nd	nd	nd	0.30 (0.01)b	nd	1505.536*
Peonidina-3-cmglc	nd	nd	nd	0.30 (0.01)a	0.36 (0.01)c	nd	nd	nd		nd	244.183*
Malvidina-3-*trans*-cmglc	nd	nd	nd	0.41 (0.02)b	0.38 (0.01)a	nd	nd	nd	0.44 (0.01)c	nd	545.976*
Total-coumaroylated	nd	nd	nd	*0.98 (0.02)b*	*1.15 (0.01)c*	nd	nd	nd	*0.74 (0.01)a*	nd	*2200.063**
Total anthocyanins	nd	*1.76 (0.06)a*	nd	*3.98 (0.03)c*	*3.53 (0.04)b*	nd	nd	nd	*4.40 (0.03)d*	nd	*692.086**
Flavonols											
Myricetin-3-gal	nd	0.72 (0.01)a	nd	0.80 (0.03)b	0.80 (0.01)b	nd	nd	nd	0.89 (0.01)c	nd	1532.590*
Free myricetin	nd	0.71 (0.01)a	nd	1.09 (0.05)b	2.49 (0.08)d	nd	nd	nd	1.36 (0.08)c	nd	736.687*
Quercetin-3-glcU	nd	nd	nd	0.12 (0.01)a	0.27 (0.01)c	nd	nd	nd	0.17 (0.01)b	nd	1504.115*
Quercetin-3-glc	nd	0.15 (0.01)a	nd	0.19 (0.01)b	0.57 (0.01)c	nd	nd	nd	0.20 (0.01)b	nd	1696.700*
Free quercetin	nd	nd	nd	0.12 (0.01)b	0.15 (0.01)c	nd	nd	nd	0.11 (0.01)a	nd	830.040*
Kaempferol-3-gal	nd	0.11 (0.01)a	nd	0.22 (0.02)b	0.23 (0.02)b	nd	nd	nd	0.22 (0.01)b	nd	96.245*
Kaempferol-3-glcU	0.07 (0.00)b	nd	nd	0.06 (0.01)a	0.09 (0.01)c	nd	nd	nd	0.06 (0.01)a	nd	121.000*
Syringetin-3-glc	nd	nd	nd	0.86 (0.01)a	11.3 (0.28)c	nd	nd	nd	4.91 (1.49)b	nd	154.787*
Free syringetin	nd	nd	nd	nd	4.36 (0.90)b	nd	nd	nd	1.35 (0.36)a	nd	739.500*
Isorhamnetin	2.42 (0.45)c	nd	nd	0.05 (0.01)a	0.20 (0.01)b	nd	nd	nd	0.06 (0.01)a	nd	80.920*
Laricetina-3-glc	nd	nd	nd	0.26 (0.01)b	0.19 (0.01)a	nd	nd	nd	0.28 (0.01)c	nd	57.887*
Total myricetin	nd	*1.43 (0.01)a*	nd	*1.89 (0.06)b*	*3.30 (0.08)d*	nd	nd	nd	*2.25 (0.08)c*	nd	*1043.623**
Total quercetin	nd	*0.15 (0.01)a*	nd	*0.43 (0.01)b*	*0.98 (0.02)d*	nd	nd	nd	*0.48 (0.01)c*	nd	*2619.521**
Total kaempferol	*0.07 (0.00)a*	*0.11 (0.01)b*	nd	*0.28 (0.02)c*	*0.32 (0.04)d*	nd	nd	nd	*0.28 (0.02)c*	nd	*85.694**
Total syringetin	nd	nd	nd	*0.86 (0.01)a*	*15.67 (0.94)c*	nd	nd	nd	*6.26 (1.53)b*	nd	*151.938**
Total Flavonols	*2.49 (0.45)a*	*1.69 (0.01)a*	nd	*3.78 (0.07)b*	*20.65 (0.94)d*	nd	nd	nd	*9.61 (1.53)c*	nd	*57.032**
Flavanols											
Epigallocatechin	2.63 (0.03)e	2.64 (0.03)e	0.42 (0.02)a	0.87 (0.06)c	0.78 (0.03)b	nd	nd	1.11 (0.03)d	2.79 (0.02)f	nd	5337.899*
Catechin	0.28 (0.03)d	1.92 (0.02)h	nd	0.19 (0.04)b	0.38 (0.02)e	0.23 (0.029)c	0.44 (0.03)f	0.52 (0.01)g	0.12 (0.01)a	nd	1852.291*
Procyanidin B1	6.01 (0.92)f	1.65 (0.01)e	0.21 (0.04)c	0.04 (0.01)b	0.04 (0.01)b	0.01 (0.00)a	0.01 (0.00)a	0.29 (0.00)d	nd	nd	1000.361*
Total Flavanols	*8.96 (0.92)f*	*6.21 (0.03)f*	*0.63 (0.04)b*	*1.10 (0.07)c*	*1.19 (0.04)c*	*0.24 (0.03)a*	*0.45 (0.03)a*	*1.91 (0.03)d*	*2.92 (0.02)e*	nd	*1268.588**
Hydroxybenzoicacids											
Gallic acid	0.68 (0.01)e	0.31 (0.07)c	0.43 (0.04)cd	0.22 (0.01)b	0.37 (0.04)c	0.48 (0.06)d	0.84 (0.01)f	1.43 (0.08)g	0.13 (0.01)a	0.43 (0.02)–8.07 (0.34)h	751.103*
Hydroxycinnamicacids											

(*continued on next page*)

Table 4 (*continued*)

Flavonoids[c]	Extracts[b] WP	RP	WM	RM	RW	WPP	DWRP	WL	RL	CM[d]	F-value
trans-Caftaric acid	0.24 (0.01)e	0.13 (0.01)c	0.13 (0.01)d	0.11 (0.01)a	nd	nd	nd	nd	0.11 (0.01)b	nd	2512.892*
trans-Coutaric acid	nd	nd	nd	nd	nd	nd	nd	0.12 (0.01)b	0.11 (0.01)a	nd	12483.643*
Total HCAS	*0.24 (0.01)f*	*0.13 (0.01)c*	*0.16 (0.01)d*	*0.11 (0.01)a*	nd	nd	nd	*0.12 (0.01)b*	*0.22 (0.01)e*	nd	*847.200**
Stilbenes											
Trans-Resveratrol	nd	0.003 (0.001)a	nd	0.03 (0.01)d	0.02 (0.01)b	nd	nd	nd	0.04 (0.01)c	nd	183.981*
Total monomericphenolics	12.4 (0.49)c	10.1 (0.03)c	1.2 (0.03)a	9.2 (0.16c)	25.8 (1.04)f	0.7 (0.09) a	1.3 (0.05)a	3.5 (0.07)b	17.3 (1.8)d	0.43 (0.02)–8.07 (0.34)b	585.073*

[a] All parameters are given with their standard deviation (n = 3). Different letters in the same row indicate statistically significant differences ($p < 0.05$) among the extracts content. A one-way analysis of variance (ANOVA) with the Duncan post-hoc test was used. Level of significance: * indicates significance at $p < 0.001$.

[b] Extracts obtained from the different matrixes. WP: White Pomace; RP: Red Pomace; WM: White Must; RM: Red Must; RW: Red Wine; WPP: Wine Purified Polysaccharides; DWRP: Distilled Washing Residues Polysaccharides; WL: White Lees; TL: Red Lees; CM: Commercial Mannoproteins.

[c] Nomenclature abbreviation: glc: glucoside; acglc: acetyl-glucoside; cmglc: coumaroyl-glucoside; gal: galactoside; glcU: glucuronide.

[d] Range of values obtained in the commercial products.

Table 5
Polysaccharide purity[a], polysaccharide composition, polysaccharide molecular weight distribution, protein content[a] and phenolic content[a] of the extracts.

Extracts[b]	Polysaccharide Purity (%)[c]	Polysaccharide Composition (%)[d]	Polysaccharide molecular weight Distribution (%)[e]	Total Proteins[f]	Total Phenolics[g]
WP	55.5 (7.4)c	35.8% PRAG, 9.1% RG-II, 13.6% HG, 40.6% GP	19.0% high Mw, 3.6% medium Mw, 77.4% low Mw	29.4 (2.4)ab	61.7 (0.5)a
RP	38.6 (3.2)a	27.8% PRAG, 15.7% RG-II, 15.7% HG, 6.2% MP, 34.5% GP	13.2% high Mw, 6.9% medium Mw, 79.9% low Mw	32.2 (2.9)bc	73.2 (0.2)c
WM	87.9 (6.6)e	50.7% PRAG, 2.9% HG, 24.4% MP, 21.2% GP	41.0% high Mw, 4.6% medium Mw, 54.4% low Mw	27.5 (0.6)ab	72.7 (0.2)c
RM	45.5 (4.2)ab	61.0% PRAG, 12.2% RG-II, 6.2 %HG, 10.4% MP, 10.1% GP	37.8% high Mw, 6.7% medium Mw, 55.5% low Mw	27.5 (1.5)ab	111.4 (0.6)f
RW	37.7 (5.3)a	52.5% PRAG, 10.3% RG-II, 5.7 %HG, 14.8% MP, 16.7% GP	24.7% high Mw, 20.4% medium Mw, 54.9% low Mw	35.6 (1.2)bc	161.6 (0.2)g
WPP	89.7 (3.7)e	14.7% PRAG, 74.7% RG-II, 6.9 %HG, 3.2% GP	71.2% medium Mw, 28.8% low Mw	29.8 (3.2)ab	66.4 (0.3)b
DWRP	40.6 (1.3)a	24.0% PRAG, 53.1% RG-II, 19.1 %HG, 2.7% GP	53.8% medium Mw, 46.2% low Mw	29.0 (2.9)ab	84.5 (0.3)d
WL	51.9 (6.8)bc	2.5% PRAG, 63.5% MP, 32.5% GP	2.2% high Mw, 97.8% low Mw	31.1 (1.9)ab	71.9 (0.2)c
RL	43.31 (2.1)ab	3.6% PRAG, 61.1% MP, 31.9% GP	100% low Mw	27.1 (5.5)a	72.0 (0.3)c
CM[h]	53.2 (2.3)–83.4 (4.1)cd	72.6–74.7% MP, 25.3–27.5% GP	39.3–45.6% high Mw, 43.4–52.8% low Mw	43.0 (2.0)–54.9 (0.8)c	85.6 (0.3)–87.4 (0.5)e
F-value	40.426**			2.890*	585.073**

[a] Parameters are given with their standard deviation (n = 3). Different letters in the same row and phenolic group indicate statistically significant differences ($p < 0.05$) among the extracts content. A one-way analysis of variance (ANOVA) using the Duncan post-hoc test was used. Level of significance: * and ** indicates significance at $p < 0.05$ $p < 0.001$ respectively.

[b] Extracts obtained from the different matrixes. WP: White Pomace; RP: Red Pomace; WM: White Must; RM: Red Must; RW: Red Wine; WPP: Wine Purified Polysaccharides; DWRP: Distilled Washing Residues Polysaccharides; WL: White Lees; TL: Red Lees; CM: Commercial Mannoproteins.

[c] Polysaccharide purity calculated as mg of total polysaccharides (calculated by GC–MS) per 100 mg of extract.

[d] Polysaccharide composition expressed as percentage of each polysaccharide family. Values below 2% are not shown.

[e] Polysaccharide molecular weight distribution (Mw) expressed as percentage of high Mw polysaccharides, medium Mw polysaccharides, and low Mw polysaccharides or oligosaccharides. Values below 2% are not shown.

[f] Total proteins expressed as mg BSA per g of extract.

[g] Total phenolics expressed as mg of gallic per g of extract and measured by the Folin assay.

[h] Range of values obtained in the commercial products.

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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