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Impact of flash release treatment of lees on mannoprotein content during white wine ageing

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ABSTRACT

This study investigates the impact of adding treated wine lees processed by flash release (FD), with or without enzymatic treatment, on the polysaccharide composition, molecular weight distribution, and sensory properties of Chardonnay wines aged on lees for six months. Standard oenological parameters (pH, total and volatile acidity, SO_2 content, and alcohol) remained largely unaffected by lees addition, except for a slight pH and acidity increase in wines with 5 % untreated lees. Polysaccharide and oligosaccharide analyses revealed a significant increase in mannoproteins (MPs) content, especially in wines supplemented with FD-treated lees and FD-treated lees with enzymes, reaching concentrations up to 600 mg/L. FD treatment enhanced the extraction of high-molecular-weight polysaccharides, whereas enzymatic treatment by β-glucanase promoted their depolymerisation into mid-sized molecular fractions. High-performance size exclusion chromatography with a multi-angle laser light scattering (HPSEC-MALLS) analysis identified five major molar mass populations, with distribution patterns varying according to lees treatment. Sensory evaluation showed that wines aged with untreated lees exhibited more pronounced yeast and reductive aromas, whereas those aged with FD + enzyme-treated lees were perceived as more fruity, more acidic, and with greater aromatic persistence. Overall, the data demonstrate that flash release and enzymatic pre-treatment of lees significantly modify the colloidal composition of wine, with implications for both wine stability and sensory profile.

KEYWORDS: flash released treatment, mannoproteins, lees, white wines ageing, β-glucanase

INTRODUCTION

Wine lees are defined by European Regulation (EU Nº. 1308/2013) as the "residue that settles in containers holding wine after fermentation, during storage, or following an authorised treatment." Fresh lees obtained after racking consist of approximately 25 % of dry matter (Fornairon-Bonnefond *et al.*, 2001). The solid fraction of wine lees was mainly composed of yeast cells, tartaric crystals, bacteria, and organic residues from grape must deposits (Dimou *et al.*, 2015; Pérez-Bibbins *et al.*, 2015). The composition of lees can vary depending on grape composition, the winemaking process—particularly racking procedures—and the duration of ageing (Salgado *et al.*, 2010). Ageing on lees is a traditional winemaking method in which wine remains in contact with its lees for 2 to 14 months (Alexandre, 2022). During lees ageing, yeast autolysis occurs, releasing cellular content and cell wall components into the liquid phase. The release of these compounds into wine modifies its chemical composition, stabilising it and enhancing its organoleptic profile (Fornairon-Bonnefond *et al.*, 2001).

Yeast autolysis is a very slow process that can be defined as the hydrolysis of yeast macromolecules by intracellular enzymes (Fornairon-Bonnefond *et al.*, 2001; Babayan *et al.*, 1981). The hydrolysis of the cell wall by β-glucanases leads to the release of its constituents, including β-glucans and mannoproteins, which then diffuse into the extracellular medium. This process is also accompanied by a reduction in the molecular weight of the hydrolysis products. Lees ageing has a positive effect on the sensory and organoleptic qualities of wines (Fornairon-Bonnefond *et al.*, 2001). This technique enhances the structure, roundness, and body of wines (Alexandre, 2022) while also reducing their astringency and bitterness (Del Barrio-Galán *et al.*, 2011). Certain macromolecules released by yeast during autolysis contribute to these properties. Mannoproteins, in particular, are known for their interactions with tannins (Alexandre, 2022). The presence or addition of mannoproteins in wines helps reduce their astringency (Escot *et al.*, 2001; Wang *et al.*, 2021) as well as the perception of bitterness (Rinaldi *et al.*, 2019). Regarding astringency, mannoproteins inhibit interactions between the phenolic compounds responsible for this sensation and salivary proteins (Wang *et al.*, 2021). Mannoproteins influence the growth and size of aggregates formed by protein-tannin interactions, which are responsible for the astringent sensation (Assunção Bicca *et al.*, 2023). Furthermore, in sparkling wines, mannoproteins released through autolysis have been shown to improve foaming properties (Núñez *et al.*, 2006).

Moine-Ledoux *et al.* (1992) showed that ageing white wines on lees leads to an improvement in protein stability. A 32 kDa fragment derived from the *Saccharomyces cerevisiae* cell wall invertase, released during lees ageing, was identified for its stabilising role in relation to the heat-sensitive proteins of white wines (Moine-Ledoux & Dubourdieu, 1999). Similarly, Moine-Ledoux *et al.* (1992) demonstrated that ageing wines on lees improves tartaric stability. A highly glycosylated mannoprotein of approximately 40 kDa was identified for its role in inhibiting the crystallisation of potassium hydrogen tartrate (Moine-Ledoux *et al.*, 1997), acting as a protective colloid against tartrate precipitation. The yeasts present in the lees are also known to interact with and adsorb phenolic compounds found in wine. It has notably been demonstrated that anthocyanins are adsorbed onto the yeast cell wall (Morata *et al.*, 2003; Morata *et al.*, 2005). Tannins are also adsorbed by yeast lees, particularly the most polar condensed tannins. The antioxidant properties of wine lees have been demonstrated during ageing, particularly their ability to consume oxygen under simulated ageing conditions (Fornairon-Bonnefond *et al.*, 1999). The valorisation of by-products and waste from the viticulture and winemaking sector has become a major research challenge in recent years (Canalejo *et al.*, 2022). While research efforts to valorise grape pomace are favoured due to its polyphenol content, wine lees, especially those from white winemaking, have been much less studied.

The extraction of polysaccharides from yeast cell walls could represent a way of adding value to wine lees. Recently, De Iseppi *et al.* (2021a) showed that it was possible to extract macromolecules, notably mannoproteins, from wine lees using three different processes: enzymatic hydrolysis, ultrasonication, and autoclaving. Each of these methods yields proteoglycans with distinct properties and a variety of potential applications. The extract obtained by autoclave proved particularly suitable for tartaric stabilisation of wines, while ultra-sonication and enzymatic hydrolysis produced extracts more effective for stabilising heat-sensitive wine proteins. The same authors also demonstrated that mannoprotein-rich extracts obtained by autoclave extraction displayed emulsifying and foaming properties comparable to those of extracts derived from yeast biomasses grown in bioreactors, which is of interest to the food industry (De Iseppi *et al.*, 2021b).

Flash release *(flash détente)* was a technique with strong potential for the fruit processing (Ayala-Zavala *et al.*, 2024) and, of course, for the treatment of grapes and must during pre-fermentation stages (Ntuli *et al.*, 2022). This process involves rapidly heating the grape material to a high temperature (between 85 °C and 95 °C), followed by an instantaneous application of a deep vacuum (between 50 and 60 hPa). This sudden vacuum induces the instant vaporisation of intracellular water, particularly in the grape skin tissues, leading to cellular breakdown and disruption of the cell walls. This tissue disintegration significantly enhances the rapid extraction of valuable compounds present in the raw material. Although this technique has been extensively studied for the vinification of red grapes—known to contain high levels of bioactive compounds such as polyphenols (flavonols, anthocyanins, catechins, proanthocyanidins) (Morel-Salmi *et al.*, 2006; Samoticha *et al.*, 2016)—as well as polysaccharides

(Doco *et al.*, 2007), it also presents promising opportunities for the treatment and valorisation of white wine lees. By facilitating the extraction of these compounds, flash release transforms a commonly underutilised winemaking by-product into a valuable resource.

In addition to its high extraction efficiency, flash release was an environmentally friendly method: it requires no chemical additives, has low energy consumption, and aligns well with sustainable production practices. The technique also leads to must concentration through partial water evaporation, providing winemakers with flexibility for different oenological strategies. Furthermore, the valorisation of white wine lees through this process supports the circular economy in the wine industry by giving a second life to a rich and functional by-product. Lastly, this method meets current consumer demands for natural, healthy products with low environmental impact. Compounds extracted from lees treated with flash release may confer antioxidant, antimicrobial, texturising, or aromatic properties to finished products. Thus, flash release emerges as an effective technological tool for both enhancing product quality and supporting a more sustainable and innovative approach to winemaking.

In this paper, we describe the release of mannoproteins obtained from white wine lees treated or not by flash release, and added to wine at doses of 2 % or 5 %. In addition, to study the synergy between the two treatments, lees treated by flash release are subjected to enzymatic hydrolysis before addition to wine. Finally, a sensory analysis was carried out at the end of the six-month ageing period on the different wines obtained after addition of lees, treated or not by *flash détente*, enzymed or not, in comparison with the control wine.

MATERIALS AND METHODS

1. Wine samples and treatments of lees

A white Chardonnay wine 2022 (IGP Pays d'Oc) from the winery of south of France (NÉOTERA – Les Vignerons du Narbonnais, 11590 Ouveillan, France) was used after its final racking, along with its fine lees. The lees were then subjected to a *"flash détente"* (150 kg/hour FD prototype, Pera Pellenc) treatment, carried out under specific operating conditions: a vacuum pressure of 1 bar, a treatment flow rate between 150 and 200 kg/hL, and a temperature of 70 °C. Following this thermal treatment, the lees were treated (2 g/hL) with a β-glucanase enzyme (Rapidase® Batonnage, Oenobrands SAS, France). These treated lees (19.4 % dry matter) were subsequently added to the white wine at proportions of 2 % and 5 %, for a lees-ageing phase conducted in 30 L stainless steel beer barrels, in triplicate. The ageing lasted for six months in a temperature-controlled room at 15 °C. Lees resuspension was performed weekly: by rolling the barrels on the first three Thursdays of each month, and by *"bâtonnage"* on the last Thursday, with topping up performed if necessary. The ageing period was followed by bottling at the end of the experiment. During bottling, dissolved gas concentrations were controlled. Oxygen was adjusted to levels below 0.6 mg/L in wine and below 2 % O_2 in the headspace. Carbon dioxide concentrations in wines were maintained between 840 and 970 mg/L. The experimental design was described in Figure 1.

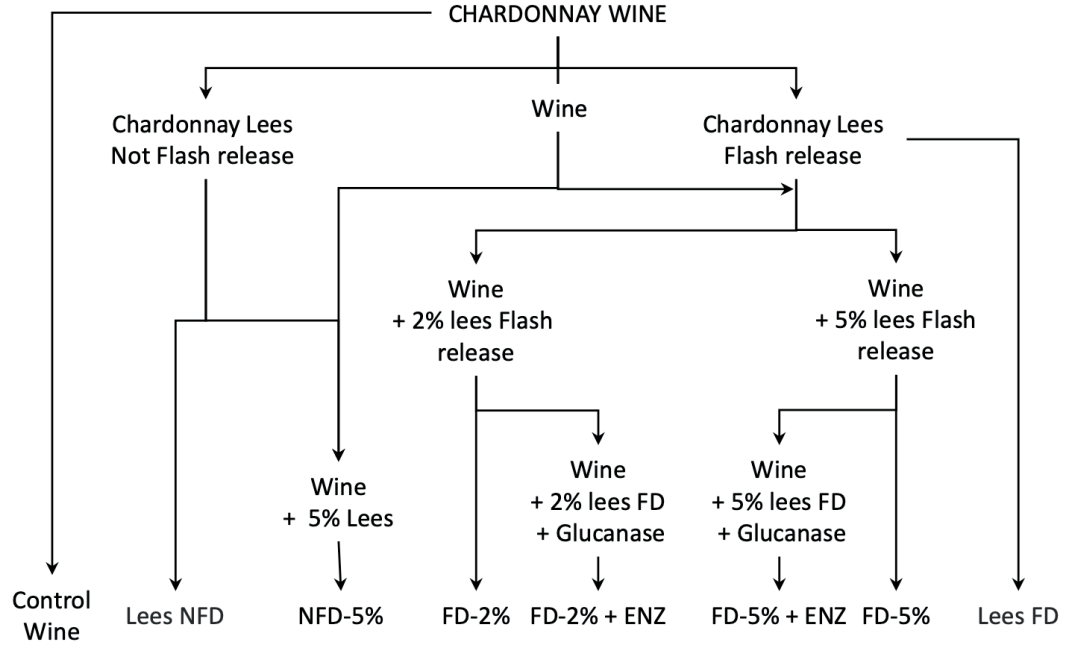


FIGURE 1. Experimental design of lees treated by FD and glucanase and added to Chardonnay wine. Control wine; NFD-5 % = wine with 5 % of lees not treated; FD-2 % = wine with 2 % of lees flash release; FD-2 % + ENZ = wine with 2 % of lees flash release and treated by enzymes (glucanase); FD-5 % = wine with 5 % of lees flash release; FD-5 % + ENZ = wine with 5 % of lees flash release and treated by enzymes (glucanase); Lees FD = lees treated by flash release; Lees NFD = lees without treatment by flash release. All procedures were carried out in triplicate.

Oenological parameters were obtained according to the official methods described by the International Organisation of Vine and Wine (OIV 2016; In *Compendium of International Methods of Wine and Must Analysis*; International Organisation of Vine and Wine: Paris, France, 2016), and measured in all wines in triplicate after six months of ageing.

2. Isolation of polysaccharide and oligosaccharide fractions

The total wine carbohydrate fractions were isolated as previously described (Ducasse *et al.*, 2010; Quijada-Morín *et al.*, 2014; Apolinar-Valiente *et al.*, 2013; Apolinar-Valiente *et al.*, 2014; Apolinar-Valiente *et al.*, 2018; Doco *et al.*, 2015; Martínez-Lapuente *et al.*, 2016). Wines (5 mL) were depigmented in polyamide CC6 columns, and wine polysaccharides and oligosaccharides not retained in the polyamide column were eluted by two bed volumes of 1 M NaCl. High-performance size-exclusion chromatography (HPSEC) was performed on a Superdex-30 HR column (60 × 1.6 cm, Pharmacia, Sweden) with a precolumn (0.6 × 4 cm) equilibrated at 1 mL/min with 30 mM ammonium formate, pH 5.6. The elution of polysaccharides and oligosaccharides was monitored with an Erma-ERC 7512 (Erma, Tokyo, Japan) refractive index. The isolated fractions were freeze-dried, dissolved in water, and freeze-dried again four times to remove the ammonium salt.

3. Polysaccharide and oligosaccharide analysis

The neutral glycosyl-residue composition of wine polysaccharides was determined by gas chromatography after hydrolysis with trifluoroacetic acid (Albersheim *et al.*, 1967) and neutral sugar conversion in their alditol acetate derivatives (Blakeney *et al.*, 1983). Inositol and allose were added to the samples, after hydrolysis and before reduction and acetylation, as internal standards for quantification (Doco *et al.*, 1999). The concentrations of wine polysaccharides took into account the composition of characteristic monosaccharides as well as the hydrolysis yield as previously described for wine PS compositions (Doco *et al.*, 1999). The concentrations of mannoproteins (MPs), polysaccharides rich in arabinose and galactose (PRAGs), and rhamnogalacturonan type II (RG-II) families were estimated from the concentration of individual glycosyl residues, as determined by GC after acidic hydrolysis, reduction, and acetylation. All mannoses were attributed to yeast MPs. The PRAGs were estimated from the sum of galactose and arabinose residues. RG-II was calculated from the concentrations of 2-*O*-methyl-fucose and 2-*O*-methyl-xylose. (Apolinar-Valiente *et al.*, 2013; Doco *et al.*, 1999; Ducasse *et al.*, 2010).

The neutral and acidic sugar compositions of oligosaccharide fractions were determined after solvolysis with anhydrous MeOH containing 0.5 M HCl (80 °C, 16 h), by GC-MS of their per-*O*-trimethylsilylated methyl glycoside derivatives (Doco *et al.*, 2001).

4. Determination of the molar mass of wine polysaccharides

Physicochemical parameters of wine polysaccharides were determined according to the protocol described in a previous laboratory publication (Martínez-Lapuente *et al.*, 2016; Apolinar-Valiente *et al.*, 2013; Apolinar-Valiente *et al.*, 2014; Apolinar-Valiente *et al.*, 2018; Nigen *et al.*, 2019; Doco *et al.*, 2025). Briefly, molar mass distributions, molar weight and number-average mass (M_w and M_n in g/mol), polydispersity index (M_w/M_n), and intrinsic viscosity ([η] in mL/g) were determined at 25 °C by coupling size exclusion chromatography with a multiangle light scattering device (MALLS), a differential viscometer, and a differential refractive index detector. SEC elution was performed on an OH-pack guard followed by two serial Shodex OH-pack KB-804 and KB-805 columns (0.8 × 30 cm; Shodex Showa Denkko, Japan) at a 1 mL/min flow rate in 0.1 M $LiNO_3$ after filtration through a 0.1 µm filter unit. The MALLS photometer, a DAWN-HELEOS from Wyatt Technology Inc. (Wyatt Technology Corporation, Santa Barbara, CA, USA), was equipped with a GA–AS laser (λ = 658 nm). The differential viscometer detector (Viscostar II, Wyatt Technology Inc., USA) was equipped with a 4-capillary bridge design. The concentration of each eluted polysaccharide was determined using the differential refractive index detector (Optilab TrEX, Wyatt Technology Inc., USA). All collected data were analysed using Astra V 6.0.6 software with the Zimm plot (order 1) technique for molar-mass estimation and a differential refractive index increment of the polymer in the solvent used. A d*n*/d*c* classical value was employed for polysaccharides (0.146 mL/g) (Redgwell *et al.*, 2005).

5. Sensorial analysis

Panel training and selection: The panel was composed of 17 panellists (4 men and 13 women, aged between 30 and 68), selected based on their sensory performances and interest, and trained in the descriptive sensory analysis of wines. The judges were further trained to perform wine descriptive analyses (Norm ISO 8586-2, 1994).

Quantitative Descriptive Analysis (QDA): The wines were subjected to QDA to assess their similarities and differences, following the guidelines of ISO 13299 (2003). To characterise wine samples, five olfactory descriptors and five gustatory descriptors were used. To ensure panel uniformity, judges underwent training to understand and consistently use these attributes and familiarise themselves with the product space. Olfactory and gustatory reference standards were introduced to aid judges in identifying and memorising sensory attributes. The evaluations were conducted in individual testing booths. Each sample was served at 18.0 ± 0.7 °C in black glasses to eliminate any potential visual bias. The glasses were covered with plastic caps and labelled with random three-digit codes. The wines were presented in a comparative series of four samples (Control; FD-5 %; FD-5 % + ENZ; NFD-5 %). The three sets of triplicates were analysed. For data reliability, wines were analysed twice, with assessors rating each attribute on unstructured linear scales that ranged

from "low" on the left to "high" on the right. Data acquisition was facilitated using FIZZ software (v.2.518; Biosystème, France).

Sensory statistics: Sensory descriptor data were converted by the FIZZ software into marks from 0 to 10. First, the jury's performance analyses (discrimination, repeatability, and consensus) were carried out to ensure robustness. A three-way ANOVA (three factors: wine, judge, repeat) and an analysis of their interactions were performed. Then descriptors analysis was performed using a two-way ANOVA (two factors: zone, judge). Mean values for wines were then compared through the Tukey multiple comparison test. A principal component analysis (PCA) type covariance was performed on sensorial profiles of wines grouping the triplicate series obtained with control wine (Control), wine with 5 % of lees (NFD-5 %), wine with 5 % of lees flash release (FD-5 %), and wine with 5 % of lees flash release and treated by enzymes (FD-5 % + ENZ) to evaluate the differences between wines.

6. Statistical treatment

Statistical data analyses were performed using XLSTAT software (version 2022, Lumivero, Paris). One-way analysis of variance (ANOVA) using the Tukey HSD post-hoc test was carried out to evaluate the significant differences between wines. Differences were considered statistically significant at p-value < 0.05.

RESULTS AND DISCUSSION

The main oenological parameters of wines obtained at the end of the ageing period are given in Table 1. The addition of lees treated by FD or FD + ENZ to Chardonnay wine does not lead to changes in pH or in the various oenological parameters tested (total acidity (g H_2SO_4/L), volatile acidity (mg H_2SO_4/L), alcohol (% *v/v*), free SO_2 (mg H_2SO_4/L), and total SO_2 (g H_2SO_4/L). Only the untreated lees (NFD-5 %) slightly modify the pH compared to the control wine, increasing it from 3.57 to 3.62, as well as the total acidity, which rose from 3.09 in the control to 3.21 in the wine with added lees (traditional method NFD-5 %).

Table 2 presents the quantitative composition (mg/L) and molar ratio of glycosyl residues in polysaccharides isolated from six wine samples. These include the control wine; wine with 5 % untreated lees (NFD-5 %); wine with 2 % flash release lees (FD-2 %); wine with 2 % flash release lees treated with enzymes (FD-2 % + ENZ); wine with 5 % flash release lees (FD-5 %); and wine with 5 % flash release lees treated with enzymes (FD-5 % + ENZ). The same table also provides the molar percentage composition of monosaccharides in flash release-treated lees (Lees FD) and untreated lees (Lees NFD). The presence of rhamnose, arabinose, galactose, fucose, apiose, 2-*O*-methyl-fucose, and 2-*O*-methyl-xylose indicates the cell wall origin of grape berry polysaccharides. The identification of these sugars confirms the presence of structures similar to polysaccharides rich in arabinose and galactose (PRAGs), rhamnogalacturonans, and mannoproteins (Ducasse *et al.*, 2010; Quijada-Morín *et al.*, 2014; Apolinar-Valiente *et al.*, 2013; Apolinar-Valiente *et al.*, 2014; Apolinar-Valiente *et al.*, 2018; Doco *et al.*, 2015; Doco *et al.*, 2025; Martínez-Lapuente *et al.*, 2016). The presence of xylose residues suggests that traces of hemicelluloses may have been solubilised from grape berry cell walls (Vidal *et al.*, 2003). The identification of several rare sugars such as apiose, 2-*O*-methyl-fucose, and 2-*O*-methyl-xylose indicates the presence of RG-II (Doco *et al.*, 1993; Vidal *et al.*, 2003).

Substantial differences were observed in the composition of neutral sugars. As expected, mannose was the major monosaccharide in all wines analysed, as well as in both treated and untreated lees (Lees FD and Lees NFD). The presence of mannose indicates the yeast origin of this fraction of polysaccharides in the studied wines.

TABLE 1. Basic oenological parameters according to the official methods described by the International Organisation of Vine and Wine (OIV, 2016) measured in control wine, wine with 5 % of lees (NFD-5 %), wine with 2 % of lees flash release (FD-2 %), wine with 2 % of lees flash release and treated by enzymes (FD-2 % + ENZ), wine with 5 % of lees flash release (FD-5 %), and wine with 5 % of lees flash release and treated by enzymes (FD-5 % + ENZ).

	Control wine		NFD-5 %		FD-2 %		FD-5 %		FD-2 % + ENZ		FD-5 % + ENZ	
pH	3.57 ± 0.00	*c*	3.62 ± 0.00	*a*	3.58 ± 0.00	*bc*	3.59 ± 0.00	*b*	3.57 ± 0.00	*c*	3.59 ± 0.00	*b*
Total acidity (g H_2SO_4 L^{-1})	3.09 ± 0.03	*b*	3.21 ± 0.02	*a*	3.08 ± 0.02	*b*	3.08 ± 0.00	*b*	3.07 ± 0.04	*b*	3.07 ± 0.00	*b*
Volatil acidity (g H_2SO_4 L^{-1})	0.29 ± 0.02	*a*	0.27 ± 0.00	*a*	0.27 ± 0.00	*a*	0.28 ± 0.00	*a*	0.29 ± 0.02	*a*	0.28 ± 0.00	*a*
Alcohol (% *v/v*)	13.64 ± 0.01	*a*	13.55 ± 0.01	*b*	13.51 ± 0.00	*b*	13.32 ± 0.05	*c*	13.49 ± 0.00	*b*	13.35 ± 0.01	*c*
Free SO_2 (g H_2SO_4 L^{-1})	6.00 ± 0.00	*a*	5.00 ± 1.00	*a*	5.33 ± 0.57	*a*	4.00 ± 0.00	*a*	5.00 ± 1.00	*a*	4.33 ± 1.15	*a*
Total SO_2 (g H_2SO_4 L^{-1})	53.33 ± 1.15	*a*	49.33 ± 3.05	*ab*	50.66 ± 2.88	*ab*	45.00 ± 0.00	*b*	50.33 ± 3.21	*ab*	46.33 ± 2.88	*b*

All data are expressed as average values ± standard deviation (n = 3). Different Latin letters in the same row indicate statistically significant differences (p < 0.05).

TABLE 2. Quantitative composition (mg/L) and molar ratio of glycosyl-residue of polysaccharides and oligosaccharides isolated from control wine, wine with 5 % of lees (NFD-5 %), wine with 2 % of lees flash release (FD-2 %), wine with 2 % of lees flash release and treated by enzymes (FD-2 % + ENZ), wine with 5 % of lees flash release (FD-5 %), and wine with 5 % of lees flash release and treated by enzymes (FD-5 % + ENZ). Quantitative composition and molar ratio of polysaccharides from lees treated by flash release (Lees FD), and lees without treatment by flash release (Lees NFD). (part 1/2)

	Control		NFD-5 %		FD-2 %		FD-5 %		FD-2 % + ENZ		FD-5 % + ENZ		Lees FD		Lees NFD	
2-*O*Me-Fuc (molar %)	1.1 ± 0.0	a	0.5 ± 0.0	c	0.7 ± 0.2	b	0.4 ± 0.0	c	0.4 ± 0.1	c	0.2 ± 0.0	d	0.1 ± 0.0	e	0.0 ± 0.0	e
Rha (molar %)	6.6 ± 0.1	a	3.0 ± 0.1	c	4.0 ± 0.4	b	2.7 ± 0.2	c	2.6 ± 0.5	c	1.2 ± 0.0	d	0.6 ± 0.0	d	0.6 ± 0.0	d
Fuc (molar %)	0.7 ±0.1	a	0.4 ± 0.0	bc	0.5 ± 0.1	b	0.5 ± 0.2	b	0.4 ± 0.1	bc	0.2 ± 0.0	c	0.2 ± 0.0	c	0.2 ± 0.0	c
2-*O*Me-Xyl (molar %)	0.9 ± 0.0	a	0.4 ± 0.0	b	0.5 ± 0.1	b	0.3 ± 0.0	b	0.4 ± 0.1	b	0.2 ± 0.0	c	0.0 ± 0.0	d	0.0 ± 0.0	d
Ara (molar %)	10.5 ± 0.7	a	5.8 ± 0.7	bc	6.6 ± 1.6	b	5.8 ± 0.6	bc	3.7 ± 0.2	de	2.6 ± 0.3	e	3.7 ± 0.1	de	4.5 ± 0.3	cd
Api (molar %)	0.3 ± 0.0	bc	0.1 ± 0.0	bc	0.1 ± 0.1	bc	0.1 ± 0.0	bc	0.1 ± 0.0	bc	0.0 ± 0.0	c	0.7 ± 0.6	ab	1.1 ± 0.2	a
Xyl (molar %)	2.1 ± 0.1	a	0.9 ± 0.0	c	1.5 ± 0.2	b	0.7 ± 0.1	c	0.7 ± 0.1	c	0.4 ± 0.1	cd	0.3 ± 0.6	cd	0.0 ± 0.0	d
Man (molar %)	54.9 ± 0.7	e	79.4 ± 0.5	c	72.3 ± 3.4	d	79.5 ± 1.1	c	85.8 ± 0.7	b	91.2 ± 0.2	a	48.9 ± 0.3	f	46.5 ± 2.2	f
Gal (molar %)	17.9 ± 1.2	a	8.0 ± 0.1	c	11.7 ± 0.4	b	8.2 ± 0.1	c	4.6 ± 0.2	d	2.7 ± 0.1	e	3.1 ± 0.1	e	3.6 ± 0.4	de
Glc (molar %)	5.0 ± 2.8	bc	1.4 ± 0.0	c	2.2 ± 1.5	bc	1.8 ± 0.6	bc	1.4 ± 0.3	c	1.3 ± 0.1	c	42.5 ± 0.2	a	43.5 ± 1.4	a
Ara/Gal ratio	0.58 ± 0.0	d	0.73 ± 0.09	cd	0.57 ± 0.16	d	0.71±0.06	cd	0.81 ± 0.09	cd	0.97 ± 0.15	bc	1.20 ± 0.04	ab	1.26±0.14	a
MPs mg/L [a]	73 ± 4.9	e	244 ± 21.0	c	154 ± 12.9	d	271 ± 24.4	c	290 ± 8.5	c	602 ± 32.3	a	354 ± 17.7	b	276 ± 12.2	c
RG-II mg/L [b]	66 ± 4.2	a	66 ± 6.0	a	61 ± 12.2	a	66 ± 4.5	a	68 ± 19.4	a	65 ± 3.7	a	10 ± 8.4	b	4 ± 6.1	b
PRAGs mg/L [c]	29 ± 0.6	de	34 ± 4.7	cd	31 ± 1.1	de	38 ± 1.6	bc	19 ± 2.3	f	25 ± 0.9	ef	46 ± 2.7	a	45 ± 4.8	ab
Total PolySacs mg/L	168 ± 9.0	e	344 ± 31.7	c	246 ± 1.7	d	375 ± 27.6	bc	377 ± 18.3	bc	692 ± 32.9	a	410 ± 20.1	b	325 ± 16.6	c
Oligos mg/L	312.4 ± 93.1	b	393.1 ± 48.7	ab	393.2 ± 63.8	ab	531.9 ± 59.2	a	381.1 ± 15.3	b	481.3 ± 60.8	a	nd [f]		nd	
% NS [d]	71.9 ± 1.7	d	83.9 ± 1.5	a	77.1 ± 3.3	bc	81.6 ± 0.5	ab	75.5 ± 1.7	cd	85.2 ± 0.5	a	nd		nd	
% AS [e]	28.1 ± 1.7	a	16.1 ± 1.5	d	22.9 ± 3.3	bc	18.4 ± 0.5	cd	24.5 ± 1.7	ab	14.8 ± 0.5	d	nd		nd	
NS/AS ratio	2.6 ± 0.2	c	5.2 ± 0.6	ab	3.4 ± 0.7	c	4.4 ± 0.2	b	3.1 ± 0.3	cd	5.8 ± 0.2	a	nd		nd	
Ara (molar %)	2.7 ± 1.4	a	3.4 ± 0.8	a	4.8 ± 4.2	a	5.1 ± 2.4	a	0.6 ± 0.9	a	3.9 ± 1.3	a	nd		nd	
Rha (molar %)	3.8 ± 1.4	a	2.2 ± 0.2	a	4.1 ± 2.4	a	3.9 ± 1.5	a	2.1 ± 0.0	a	2.5 ± 0.9	a	nd		nd	
Gal (molar %)	14.6 ± 2.4	a	12.0 ± 1.3	ab	11.6 ± 1.9	ab	9.6 ± 1.2	b	12.4 ± 0.7	ab	9.2 ± 0.4	b	nd		nd	

All data are expressed as average value ± standard deviation (n = 3). Different Latin letters in the same row indicate statistically significant differences ($p < 0.05$); 2-*O*Me-Fuc = 2-*O*-methyl-fucose; Rha = rhamnose; Fuc = fucose; 2-*O*Me-Xyl = 2-*O*-methyl-xylose; Ara = arabinose; Api = apiose; Xyl = xylose; Man = mannose; Gal = galactose; Glc = glucose; GalA = galacturonic acid; GlcA = glucuronic acid; 4-*O*-Me-GlcA = 4-*O*-methyl-glucuronic acid. [a] MPs: mannoproteins. [b] RG-II: rhamnogalacturonans type II. [c] PRAGs: polysaccharides rich in arabinose and galactose. Total PolySacs = sum of wine MPs, RG-II, and PRAGs. [d] NS = Neutral Sugars. [e] AS = Acidic Sugars. [f] nd = not determine.

TABLE 2. Quantitative composition (mg/L) and molar ratio of glycosyl-residue of polysaccharides and oligosaccharides isolated from control wine, wine with 5 % of lees (NFD-5 %), wine with 2 % of lees flash release (FD-2 %), wine with 2 % of lees flash release and treated by enzymes (FD-2 % + ENZ), wine with 5 % of lees flash release (FD-5 %), and wine with 5 % of lees flash release and treated by enzymes (FD-5 % + ENZ). Quantitative composition and molar ratio of polysaccharides from lees treated by flash release (Lees FD), and lees without treatment by flash release (Lees NFD). (part 2/2)

Glc (molar %)	27.0 ± 4.0	d	44.6 ± 1.5	ab	35.4 ± 3.9	c	41.6 ± 3.5	abc	37.6 ± 0.9	bc	46.7 ± 2.6	a	nd	nd
Man (molar %)	18.3 ± 0.1	bc	19.2 ± 1.7	ab	16.7 ± 1.1	c	17.6 ± 0.9	bc	21.0 ± 0.9	a	20.8 ± 0.8	a	nd	nd
Xyl (molar %)	4.7 ± 1.5	a	2.2 ± 0.0	ab	3.9 ± 1.3	ab	3.3 ± 0.8	ab	1.9 ± 0.0	b	1.8 ± 0.8	b	nd	nd
Xylitol (molar %)	0.7 ± 0.6	a	0.3 ± 0.4	a	0.6 ± 0.7	a	0.6 ± 0.1	a	0.0 ± 0.0	a	0.2 ± 0.2	a	nd	nd
GalA (molar %)	24.6 ± 1.5	a	14.2 ± 0.7	d	20.3 ± 2.8	bc	16.3 ± 0.9	cd	21.9 ± 1.9	ab	12.9 ± 0.5	d	nd	nd
GlcA (molar %)	1.8 ± 0.3	a	1.0 ± 0.4	b	1.3 ± 0.4	ab	1.3 ± 0.3	ab	1.5 ± 0.2	ab	1.0 ± 0.1	b	nd	nd
4-*O*-Me-GlcA (molar %)	1.7 ± 0.4	a	0.9 ± 0.4	ab	1.4 ± 0.2	ab	0.8 ± 0.6	ab	1.1 ± 0.0	ab	0.8 ± 0.1	b	nd	nd
Ara/Gal ratio	0.18 ± 0.10	a	0.29 ± 0.04	a	0.41 ± 0.42	a	0.53 ± 0.30	a	0.05 ± 0.07	a	0.43 ± 0.16	a	nd	nd
Rha/GalU ratio	0.16 ± 0.06	a	0.16 ± 0.02	a	0.20 ± 0.13	a	0.24 ± 0.08	a	0.09 ± 0.01	a	0.20 ± 0.07	a	nd	nd
(GalU-Rha)/2Rha ratio	39.75 ± 9.91	a	13.16 ± 0.07	b	32.9 ± 13.47	ab	23.95 ± 8.27	ab	20.35 ± 2.23	ab	13.24 ± 3.01	b	nd	nd
(Ara + Gal)/Rha ratio	4.50 ± 1.01	a	7.00 ± 0.46	a	4.04 ± 3.69	a	3.82 ± 0.55	a	6.35 ± 0.02	a	5.15 ± 1.25	a	nd	nd

All data are expressed as average value ± standard deviation (n = 3). Different Latin letters in the same row indicate statistically significant differences ($p < 0.05$); 2-*O*Me-Fuc = 2-*O*-methyl-fucose; Rha = rhamnose; Fuc = fucose; 2-*O*Me-Xyl = 2-*O*-methyl-xylose; Ara = arabinose; Api = apiose; Xyl = xylose; Man = mannose; Gal = galactose; Glc = glucose; GalA = galacturonic acid; GlcA = glucuronic acid; 4-*O*-Me-GlcA = 4-*O*-methyl-glucuronic acid. [a] MPs: mannoproteins. [b] RG-II: rhamnogalacturonans type II. [c] PRAGs: polysaccharides rich in arabinose and galactose. Total PolySacs = sum of wine MPs, RG-II, and PRAGs. [d] NS = Neutral Sugars. [e] AS = Acidic Sugars. [f] nd = not determine.

The concentrations (mg/L) of mannoproteins (MPs), polysaccharides rich in arabinose and galactose (PRAGs), and rhamnogalacturonan type II (RG-II) in wines were estimated based on the concentration of individual monosaccharides determined by GC after hydrolysis, reduction, and acetylation (Ducasse *et al.*, 2010). All mannose was attributed to yeast MPs. The sum of galactose and arabinose residues was used to estimate PRAG content in wines. RG-II concentration was calculated based on 2-*O*-methyl-fucose and 2-*O*-methyl-xylose levels. The sum of the individual polysaccharide families (MPs + PRAGs + RG-II) corresponds to the total polysaccharide content in wines, as shown in Table 2 and Figure 2.

The ageing on lees led to an increase in the amount of polysaccharides present in wine, in particular the contents of mannoproteins. Mannoproteins can be released from yeast cell walls into the medium at the beginning of alcoholic fermentation, and by the enzymatic action, producing high molecular mass mannoproteins during the six months of ageing on lees. The amount of mannoproteins released during the fermentation process (control wine) was even a little lower than that already described in the literature (Apolinar-Valiente *et al.*, 2013; Del Barrio-Galan *et al.*, 2011; Martínez-Lapuente *et al.*, 2016; Ntuli *et al.*, 2021; Ntuli *et al.*, 2022) or similar for a yeast that has fermented on a Chardonnay must (Pati *et al.*, 2010; Núñez *et al.*, 2006).

The concentrations of rhamnogalacturonan II (RGII) and protein-rich arabinogalactans (PRAGs) in wines supplemented with lees—regardless of treatment—remained relatively stable during ageing, averaging 60–65 mg/L for RGII and 25–30 mg/L for PRAGs. In contrast, the concentration of mannoproteins (MPs) increased significantly over time. In wines aged traditionally on lees at 5 % (NFD-5 %), MP content increased 3.3-fold, from 73 mg/L to 244 mg/L. Lees pre-treated by flash release (FD) prior to addition led to a 2-fold (FD-2 %) and 3.7-fold (FD-5 %) increase in MPs, corresponding to concentrations of 154 mg/L and 271 mg/L, respectively, as shown in Table 2 and Figure 2. The addition of β-glucanase after flash release treatment of lees further enhanced MP extraction. Wines with 2 % and 5 % lees treated with FD and β-glucanase (FD-2 % + ENZ and FD-5 % + ENZ) showed 4-fold and 8-fold increases in MPs, respectively, prior to ageing. After six months of lees ageing, the FD-5 % + ENZ condition yielded an MP concentration of 600 mg/L. The proportion of MPs in total polysaccharides also varied with treatment. In the control wine, MPs represented 43 % of total polysaccharides, increasing to 71 % under traditional lees ageing (NFD-5 %). Wines aged with flash-treated lees showed proportions ranging from 62 % (FD-2 %) to 87 % (FD-5 % + Enz), indicating a more efficient MPs release. Previous results reported that the FD technique increased the polysaccharide content of Grenache wines by 40–63 % depending on the time of FD treatment (Doco *et al.*, 2007), and similar results were also found in Merlot wine (Ntuli *et al.*, 2022). There is no doubt that the FD treatment can accelerate the extraction of MPs, and that, combined with the action of a β-glucanase, the quantity of MPs released was almost doubled, as shown in Figure 2. The presence of 15 % and 13 % of pectic polysaccharides (RG-II and PRAGs) in the fine lees of a Chardonnay wine

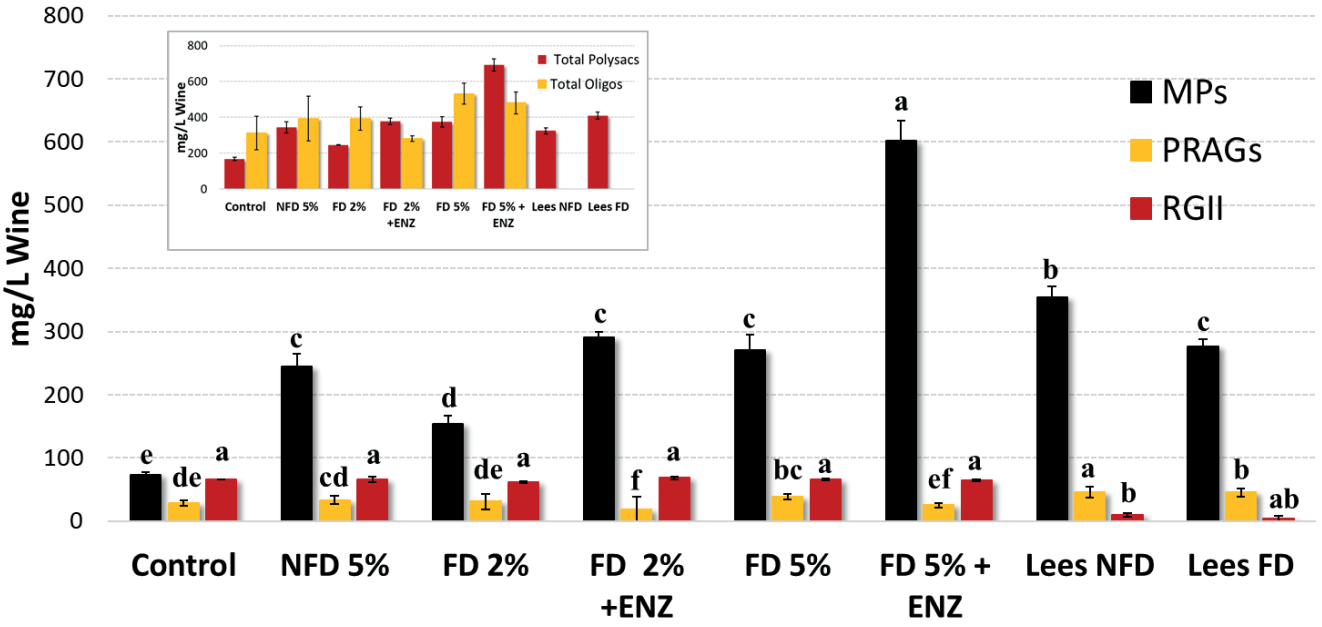


FIGURE 2. Concentration (mg/L) of mannoproteins (MPs), polysaccharides rich in arabinose and galactose (PRAGs) and rhamnogalacturonan II (RG-II), and in the window the concentration (mg/L) of total polysaccharides (sum of MPS, RG-II, and PRAGs) and oligosaccharides from control wine, wine with 5 % of lees (NFD-5 %), wine with 2 % of lees flash release (FD-2 %), wine with 2 % of lees flash release and treated by enzymes (FD-2 % + ENZ), wine with 5 % of lees flash release (FD-5 %), and wine with 5 % of lees flash release and treated by enzymes (FD-5 % + ENZ). Quantitative composition (mg/L) of Lees FD (lees treated by flash release) and Lees NFD (lees without treatment by flash release). Different Latin letters indicate statistically significant differences ($p < 0.05$).

untreated (Lees NFD) or treated by flash released (Lees FD) respectively, clearly indicates that the lees used come from the sedimentation of some of the pectic polysaccharides released from the cell walls of the grape berries during alcoholic fermentation and that they were recovered after its final racking.

The glycosyl residue composition of oligosaccharides isolated from control wine, wine NFD-5 %, wine FD-2 %, wine FD-2 % + ENZ, wine FD-5 %, and wine FD-5 % + ENZ was determined by GC after methanolysis and trimethylsilylation (Table 2). The two types of lees treated by flash release (Lees FD) and without treatment by flash release (Lees NFD) were not analysed for their oligosaccharide content, but only for their polysaccharide content. It contains most of the sugars identified in the composition of wine polysaccharides/oligosaccharides. The glycosyl composition includes sugars such as rhamnose, arabinose, galactose, xylose, galacturonic, and glucuronic acids from the pectocellulosic cell walls of grape berries, and also mannose and glucose released from yeast polysaccharides during ageing of wines on lees. Identification of xylose, glucuronic acid, and 4-*O*-methylglucuronic acid residues indicated that traces of hemicelluloses might be solubilised from grape berry cell walls and recovered as oligosaccharide structures in wines. These oligosaccharides of glucuronoxylan type were previously described in oligosaccharide fractions isolated from Carignan and Merlot wines (Ducasse *et al.*, 2010; Apolinar-Valiente *et al.*, 2021). The oligosaccharides isolated in wines show a high molar percentage of glucose, 27 % to 46.7 % and mannose, 18.3 % to 21 %. Current knowledge on the composition of oligosaccharides in wines remains limited (Apolinar-Valiente *et al.*, 2021), and even more so concerning oligomannans resulting from the degradation of yeast cell walls. Few studies have examined their influence on the sensory and physicochemical properties of wine, particularly regarding bitterness perception and interactions with polyphenols or aroma compounds. Boulet *et al.* (2024) proposed that oligosaccharides may promote the recovery and stabilisation of anthocyanins and tannins by forming protective colloidal layers, potentially modulating astringency. However, no clear relationship has yet been established between wine bitterness and the content, composition, or structure of oligosaccharides.

To try to gain a better understanding of the degradation of the yeast cell wall induced by flash release or by flash release with enzymes, the Glc/Man ratio was calculated. In all treated wines, the ratio was higher than in the control wine, indicating a release of molecules richer in glucose, increasing from 1.47 in the control wine to 2.33, 2.12, 2.37, 1.73, and 2.33 for NFD-5 %, FD-2 %, FD-5 %, FD-2 % + ENZ, and FD-5 % + ENZ, respectively. The yeast cell wall was composed of a complex structure consisting of three main fractions: β-glucans, mannoproteins (MPs), and chitin (Nakajima & Ballou, 1974).

These polysaccharides can be introduced into the wine matrix either through direct secretion from yeast cells during fermentation or through autolysis during wine ageing on lees (Ayestarán *et al.*, 2004; Guadalupe & Ayestarán, 2007; Marangon *et al.*, 2018). It has been reported that ageing on lees enriches wine with yeast-derived polysaccharides and that these compounds undergo a gradual evolution toward lower molecular weight fractions (Babayan *et al.*, 1981; Doco *et al.*, 1999). Hydrolysis by enzymes during ageing on lees leads to the degradation of the cell wall and the release of polysaccharides and oligosaccharides (Babayan *et al.*, 1981). *Flash détente*, by weakening and disrupting the cell wall, allows better access for enzymes, particularly β-glucanases that hydrolyse β-glucans. This was confirmed in the FD-2 % + ENZ and FD-5 % + ENZ treatments, which show a greater release of glucose-rich oligosaccharides compared to the control wine (Table 2). The molar percentage of glucose is increased by at least a factor of 1.6 in the oligosaccharides of wines aged on lees compared to those of the control wine (Table 2).

The HPSEC-MALLS chromatograms of the polysaccharide and oligosaccharide fractions, as well as the polysaccharide weight-average molar mass distributions for control wine, NFD-5 %, FD-2 %, FD-5 %, FD-2 % + ENZ, and FD-5 % + ENZ, are shown in Figure 3. The elution profiles (RI) of the polysaccharide and oligosaccharide fractions are displayed along with the molar mass (M_w) profile calculated from static light scattering measurements. All polysaccharide and oligosaccharide fractions presented populations of different sizes and proportions. The molar mass of the eluting polysaccharides and oligosaccharides decreased with increasing elution volume, which was consistent with the standard size-exclusion separation mechanism and corresponds to previously described profiles (Martínez-Lapuente *et al.*, 2016). The polysaccharide HPSEC profiles show in Figure 3 five principal populations, with concentration signal peaks in the range of approximately 14.0–19.5 minutes: Peak HMW (High Molecular Weight): between 6.2×10^5 and 2×10^5 g/mol; Peak MMW-1 (Middle Molecular Weight-1): between 2×10^5 and 8×10^4 g/mol; Peak MMW-2 (Middle Molecular Weight-2): between 8×10^4 and 4×10^4 g/mol; Peak LMW1 (Low Molecular Weight-1): between 4×10^4 and 1.5×10^3 g/mol, and Peak LMW2 (Low Molecular Weight-2): between 1.4×10^4 and 1.1×10^3 g/mol. The oligosaccharide profile shows several poorly resolved peaks between 20 and 22 minutes (Figure 2—fine line). The HPSEC profiles indicate that the three modalities with untreated lees (NFD-5 %) or lees treated by FD and added to wine at 2 % (FD-2 %) or 5 % (FD-5 %) have a higher MMW-1 polysaccharide population (2×10^5 to 8×10^4 g/mol) than the polysaccharides isolated from the control wine. The polysaccharide profiles of wines aged with lees treated by FD and then enzymed with a β-glucanase (FD-2 % + ENZ; FD-5 % + ENZ) show a significant chromatographic peak around 18 minutes, corresponding to the MMW-2 fraction (8×10^4 to 4×10^4 g/mol). Table 3 presents the molar mass distribution parameters (M_w: weight-average molar mass, M_n: number-average molar mass), polydispersity index (M_w/M_n), and hydrodynamic radius (R_w) values for the LMW1, LMW2, MMW-1, MMW-2, HMW-1, and HMW-2 peaks obtained from the

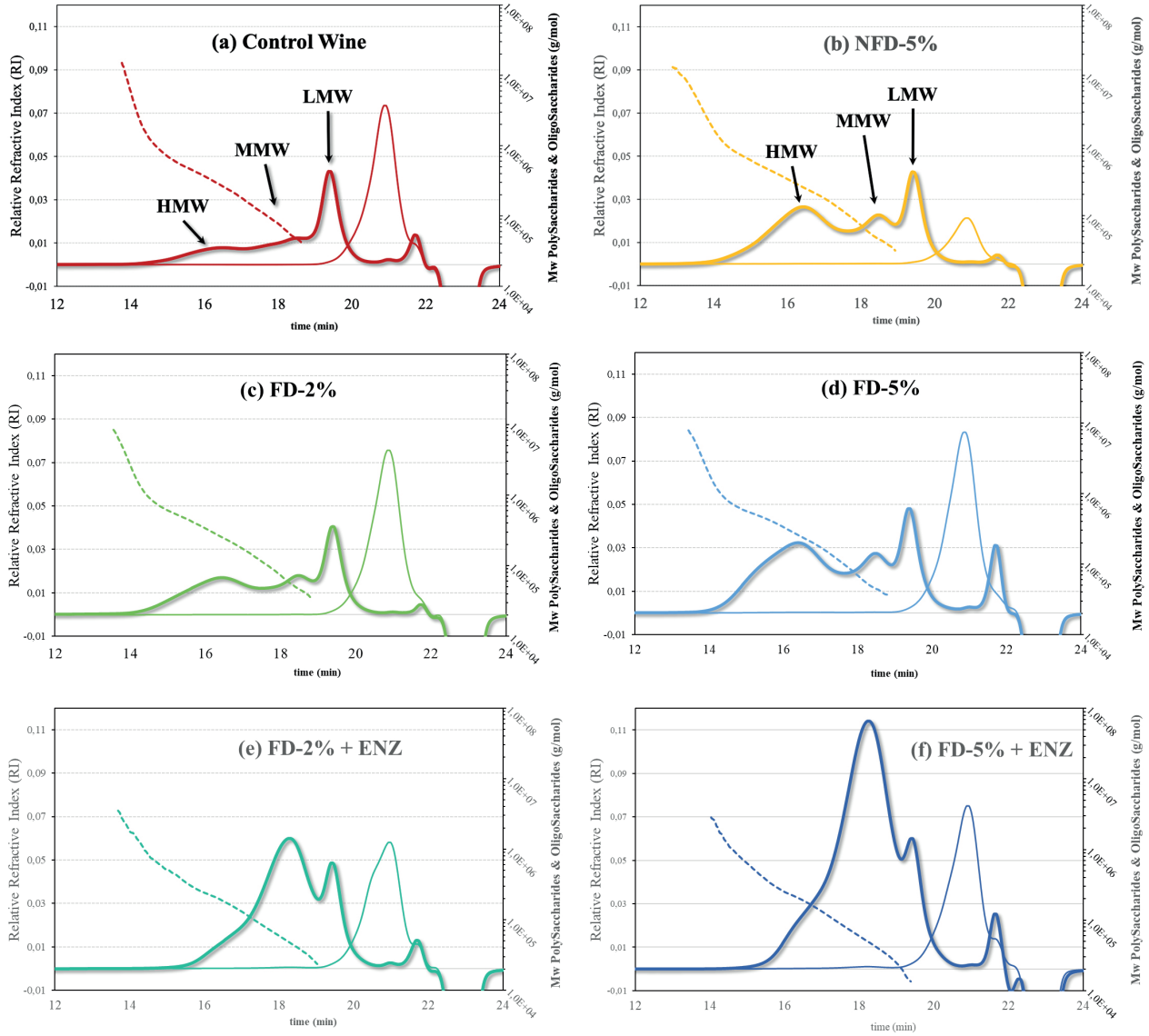


FIGURE 3. HPSEC-MALS chromatograms of the polysaccharide and oligosaccharide fractions and polysaccharide weight-average molar mass distributions from a) control wine, b) wine with 5 % of lees (NFD-5 %), c) wine with 2 % of lees flash release (FD-2 %), d) wine with 5 % of lees flash release (FD-5 %), e) wine with 2 % of lees flash release and treated by enzymes (FD-2 % + ENZ), and f) wine with 5 % of lees flash release and treated by enzymes (FD-5 % + ENZ). Polysaccharide molar weight distributions (M_w, g/mol, colour dashed line) and relative refractive index (DRI, relative scale), for polysaccharide fractions (full colour thick line) and oligosaccharide fractions (full colour fine line). Identified peaks corresponding to peaks of High Molecular Weight (HMW), Medium Molecular Weight (MMW-1 and -2), and Low Molecular Weight (LMW-1 and -2).

HPSEC-MALS profiles of: control wine; wine with 5 % lees (NFD-5 %); wine with 2 % flash release lees (FD-2 %); wine with 5 % flash release lees (FD-5 %); Wine with 2 % flash release lees treated with enzymes (FD-2 % + ENZ); and wine with 5 % flash release lees treated with enzymes (FD-5 % + ENZ).

The molar-mass distribution analysis determined by size exclusion chromatography coupled online to multi-angle laser light scattering of wine polysaccharides elaborated with different grape varieties allows us to delimit five mass ranges, which are illustrated in Figures 3 and 4. The five delimited ranges among different wines can be observed at Range 1 = 1×10^3–25×10^3 g/mol; Range 2 = 25×10^3–10×10^4 g/mol; Range 3 = 10×10^4–25×10^4 g/mol; Range 4 = 25×10^4–50×10^4 g/mol; Range 5 = up to 50×10^4 g/mol. The ranges have been selected due to their correspondence with values obtained from different purified polysaccharide families by SEC analysis: RG-II monomer, $M_w = 5 \times 10^3$ g/mol; RG-II dimer, $M_w = 10 \times 10^3$ g/mol; MP0c, $M_w = 58 \times 10^3$ g/mol; AGP0, $M_w = 14.5 \times 10^4$ g/mol; AGP2, $M_w = 16.5 \times 10^4$ g/mol; MP0a, $M_w = 35 \times 10^4$ g/mol; and MP3, $M_w = 1 \times 10^6$ g/mol. (Doco *et al.*, 1993; Vidal *et al.*, 2003; Martínez-Lapuente, *et al.*, 2016). As shown in Figure 4, the distribution of wine polysaccharide molar masses across the five defined ranges (Range 1 = 1×10^3–25×10^3 g/mol;

TABLE 3. Structural parameters of LMW1, LMW2, MMW-1, MMW-2, HMW-1 and HMW-2 peaks obtained in the HPSEC-MALS profiles of polysaccharides fractions from control wine, wine with 5 % of lees (NFD-5 %), wine with 2 % of lees flash release (FD-2 %), wine with 2 % of lees flash release and treated by enzymes (FD-2 % + ENZ), wine with 5 % of lees flash release (FD-5 %), and wine with 5 % of lees flash release and treated by enzymes (FD-5 % + ENZ).

	Control Wine					NFD-5 %				
Peak name	**Peak HMW**	**Peak MMW-1**	**Peak MMW-2**	**Peak LMW-1**	**Peak LMW-2**	**Peak HMW**	**Peak MMW-1**	**Peak MMW-2**	**Peak LMW-1**	**Peak LMW-2**
Peak limits (min)	14.113 – 15.288	15.409 – 16.812	17.003 – 18.102	18.214 – 18.815	18.955 – 19.379	13.778 – 15.218	15.289 – 17.527	17.657 – 18.868	18.907 – 19.379	-
M_n [a]	7.473×10^5 (±0.226 %)	3.113×10^5 (±0.103 %)	1.018×10^5 (±0.208 %)	4.431×10^4 (±0.421 %)	1.496×10^4 (±0.616 %)	6.589×10^5 (±0.944 %)	2.195×10^5 (±1.373 %)	4.732×10^4 (±4.099 %)	1.682×10^4 (±7.386 %)	-
M_w [b]	8.406×10^5 (±0.264 %)	3.310×10^5 (±0.102 %)	1.100×10^5 (±0.192 %)	4.555×10^4 (±0.384 %)	1.552×10^4 (±0.649 %)	7.175×10^5 (±1.065 %)	2.572×10^5 (±1.031 %)	5.201×10^4 (±3.605 %)	1.771×10^4 (±7.248 %)	-
M_w/M_n [c]	1.125 (±0.347 %)	1.063 (±0.145 %)	1.081 (±0.283 %)	1.028 (±0.570 %)	1.037 (±0.895 %)	1.089 (±1.424 %)	1.172 (±1.717 %)	1.099 (±5.459 %)	1.053 (±10.349 %)	-
R_w [d]	24.6 (±0.3 %)	15.9 (±0.4 %)	14.3 (±0.7 %)	15.8 (±1.5 %)	16.2 (±2.3 %)	20.4 (±1.4 %)	10.4 (±4.4 %)	5.4 (±1,329.4 %)	24.3 (±3.2 %)	-
	FD-2 %					**FD-2 % + ENZ**				
Peak name	**Peak HMW**	**Peak MMW-1**	**Peak MMW-2**	**Peak LMW-1**	**Peak LMW-2**	**Peak HMW**	**Peak MMW-1**	**Peak MMW-2**	**Peak LMW-1**	**Peak LMW-2**
Peak limits (min)	13.810 – 15.652	15.710 – 17.460	17.526 – 18.077	18.857 – 19.416	18.118 – 18.792	13.679 – 16.327	16.371 – 17.096	17.133 – 17.858	19.094 – 19.427	17.903 – 19.020
M_n	5.427×10^5 (±0.079 %)	2.164×10^5 (±0.101 %)	8.350×10^4 (±0.200 %)	1.579×10^4 (±0.546 %)	4.472×10^4 (±0.341 %)	2.566×10^5 (±0.178 %)	1.514×10^5 (±0.071 %)	8.329×10^4 (±0.075 %)	1.428×10^4 (±0.481 %)	4.092×10^4 (±0.215 %)
M_w	6.114×10^5 (±0.102 %)	2.417×10^5 (±0.080 %)	8.548×10^4 (±0.195 %)	1.697×10^4 (±0.607 %)	4.582×10^4 (±0.314 %)	2.925×10^5 (±0.388 %)	1.545×10^5 (±0.072 %)	8.552×10^4 (±0.077 %)	1.466×10^4 (±0.518 %)	4.366×10^4 (±0.147 %)
M_w/M_n	1.126 (±0.129 %)	1.117 (±0.129 %)	1.024 (±0.279 %)	1.074 (±0.816 %)	1.025 (±0.463 %)	1.140 (±0.427 %)	1.021 (±0.101 %)	1.027 (±0.107 %)	1.026 (±0.707 %)	1.067 (±0.261 %)
R_w	19.6 (±0.1 %)	12.2 (±0.3 %)	9.9 (±2.0 %)	12.0 (±4.2 %)	8.5 (±4.1 %)	12.4 (±0.8 %)	9.3 (±0.7 %)	6.0 (±1.8 %)	3.8 (±184.9 %)	2.9 (±8.2 %)
	FD-5 %					**FD-5 % + ENZ**				
Peak name	**Peak HMW**	**Peak MMW-1**	**Peak MMW-2**	**Peak LMW-1**	**Peak LMW-2**	**Peak HMW**	**Peak MMW-1**	**Peak MMW-2**	**Peak LMW-1**	**Peak LMW-2**
Peak limits (min)	13.433 – 15.717	15.760 – 17.554	17.611 – 18.244	18.285 – 18.860	18.904 – 19.312	14.019 – 15.742	15.786 – 16.882	16.918 – 17.956	17.999 – 19.131	19.196 – 19.427
M_n	5.238×10^5 (±0.035 %)	2.121×10^5 (±0.072 %)	7.183×10^4 (±0.146 %)	4.225×10^4 (±0.390 %)	1.986×10^4 (±1.375 %)	3.058×10^5 (±1.376 %)	1.734×10^5 (±0.375 %)	8.041×10^4 (±0.251 %)	3.734×10^4 (±0.494 %)	1.306×10^4 (±1.510 %)
M_w	6.030×10^5 (±0.047 %)	2.380×10^5 (±0.050 %)	7.444×10^4 (±0.145 %)	4.283×10^4 (±0.339 %)	2.101×10^4 (±1.385 %)	3.308×10^5 (±1.466 %)	1.773×10^5 (±0.409 %)	8.441×10^4 (±0.258 %)	4.037×10^4 (±0.372 %)	1.321×10^4 (±1.532 %)
M_w/M_n	1.151 (±0.059 %)	1.122 (±0.088 %)	1.036 (±0.206 %)	1.014 (±0.517 %)	1.058 (±1.952 %)	1.082 (±2.011 %)	1.023 (±0.555 %)	1.050 (±0.360 %)	1.081 (±0.618 %)	1.012 (±2.151 %)
R_w	19.5 (±0.2 %)	12.8 (±0.5 %)	12.8 (±0.9 %)	14.7 (±1.6 %)	23.0 (±2.9 %)	14.0 (±3.6 %)	9.2 (±3.2 %)	2.3 (±51.1 %)	2.2 (±287.4 %)	14.2 (±7.2 %)

All data are expressed as average value ± standard deviation. [a] M_n = number-average mass. [b] M_w = molar weight. [c] M_w/M_n = polydispersity index, and [d] R_w = hydrodynamic radius for LMW (Low Molecular Weight), MMW-1, MMW-2 (Middle Molecular Weight-1 and -2) and HMW (High Molecular Weight) peaks obtained in the HPSEC-MALS profiles of polysaccharides fractions from control wine, wine NFD-5 %, wine FD-2 %, wine FD-2 % + ENZ, wine FD-5 %, and wine FD-5 % + ENZ.

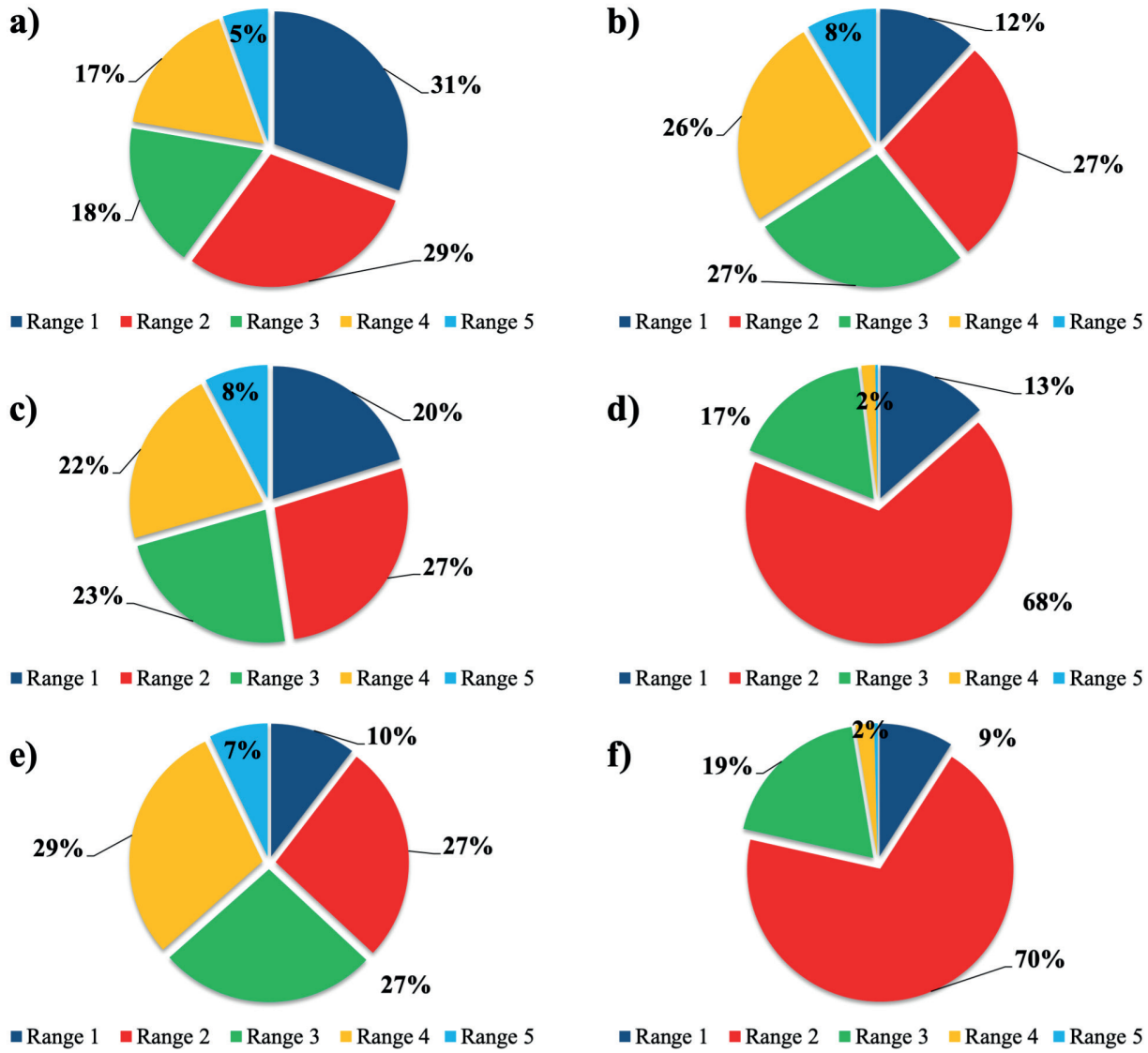


FIGURE 4. Distribution analysis of molar mass ranges determined by light scattering (d*n*/d*c* = 0.146 mL/g) of polysaccharides fractions isolated from wines produced from a) control wine, b) wine with 5 % of lees (NFD-5 %), c) wine with 2 % of lees flash release (FD-2 %), d) wine with 2 % of lees flash release and treated by enzymes (FD-2 % + ENZ), e) wine with 5 % of lees flash release (FD-5 %), and f) wine with 5 % of lees flash release and treated by enzymes (FD-5 % + ENZ). Molar mass range: Range 1 = 1 × 10^3–25 × 10^3 g/mol; Range 2 = 25 × 10^3–10 × 10^4 g/mol; Range 3 = 10 × 10^4–25 × 10^4 g/mol; Range 4 = 25 × 10^4–50 × 10^4 g/mol; Range 5 = up to 50 × 10^4 g/mol.

Range 2 = 25 × 10^3–10 × 10^4 g/mol; Range 3 = 10 × 10^4–25 × 10^4 g/mol; Range 4 = 25 × 10^4–50 × 10^4 g/mol; Range 5 = up to 50 × 10^4 g/mol) reveals clear differences depending on the treatment applied to lees. In the control wine, the molar mass distribution was relatively balanced, with a predominance of lower molar mass polysaccharides (Range 1, 30.7 %) and mid-range fractions (Range 2, 29.4 %), and a smaller proportion of high molecular weight fractions (Ranges 4 and 5, 16.8 % and 5.5 %, respectively). In contrast, wines supplemented with lees treated by flash release (FD-2 % and FD-5 %) show a significant shift towards higher molar mass fractions. Notably, FD-5 % wines exhibit a marked increase in Range 3 (10 × 10^4–25 × 10^4 g/mol) and Range 4 (25 × 10^4–50 × 10^4 g/mol) populations (26.5 % and 29.5 %, respectively), indicating enhanced extraction of high molecular weight polysaccharides, suggesting that FD lees contribute to the enrichment of these fractions. This trend is also observed in wines with untreated lees (NFD-5 %), similar to the traditional ageing, which present comparable levels in Range 3 (26.6 %) and Range 4 (25.7 %).

However, the application of enzymatic treatment after flash release of lees (FD + ENZ 2 % and FD + ENZ 5 %) drastically alters the profile. Both wines show a sharp increase in the Range 2 (25 × 10^3–10 × 10^4 g/mol) fraction (67.5 % and 69.5 %, respectively), with a decrease in all other ranges, particularly those corresponding to higher molar masses. Range 4 and Range 5 fractions are nearly absent in these samples (≤ 2.2 % and ≤ 0.9 %, respectively), indicating an extensive degradation of high molecular weight polysaccharides into smaller fragments. This suggests that

the enzymatic hydrolysis, likely by a β-glucanase activity, promotes depolymerisation of large polysaccharides into mid-sized polysaccharides. Overall, the data suggest that the flash release of lees enhances the extraction of high-molecular-weight polysaccharides. While enzymatic treatment reduces their size distribution by breaking them down into smaller polysaccharides. These structural modifications could have important implications for the mouthfeel, stability, and ageing potential of wines, but not only that. Chalier *et al.* (2007) showed that mannoproteins of different sizes interact and do not have the same retention capacity with the wine's aroma compounds. These interactions vary depending on the MPs (mannoproteins) from the yeast strains used, which could influence the aromatic quality of the wine. The pre-treatment of lees by FD and enzymes leads to mannoproteins of different sizes, with different properties, and an impact on the final perception of the product.

For the sensory analysis conducted by the panel on the different wines aged with treated or untreated lees, only the 5 % addition modalities were evaluated. Performance analyses show that the jury was repeatable, consensual, and generally discriminant, specifically those that revealed perceptible differences in the aromatic profile. Principal Component Analysis (PCA) (Figures 5a and 5b) shows the sensory profiles of the control wine, wine with 5 % of untreated lees (NFD-5 %), wine with 5 % of flash release lees (FD-5 %), and wine with 5 % of flash release lees treated with enzymes (FD-5 % + ENZ). The first principal component (F1) axis accounts for 41.02 % of the variability, with variables such as fruity, yeast, and reduced/sulphur aromas strongly correlated with this axis. This axis likely reflects the effect of lees treatment prior to ageing. The second component (F2), which accounts for 24.83 % of the variance, is correlated with odour intensity, bitterness, and aqueous perception. Wines aged with 5 % untreated lees ("traditional method"—NFD-5 %) show more pronounced yeast and reductive/sulphur aromas compared to the control wine, which was instead characterised as more fruity and more acidic. Modalities involving flash-release-treated lees (FD-5 %) tend to result in a higher perception of bitterness and a more aqueous character than the other two lees modalities (NFD-5 % and FD-5 % + ENZ). It has previously been demonstrated that the perception of astringency in wine decreases with increasing mannoprotein content, as well as with decreasing concentrations of mannose- and galactose-rich oligosaccharides (Quijada-Morin *et al.*, 2014; Boulet *et al.*, 2016). Chong *et al.* (2019) reported that the presence of poly- and oligosaccharides in Cabernet-Sauvignon wines contributes to a reduction in perceived acidity and bitterness. Bitterness perception has also been associated with the presence of gentiobiose in *koji amazake*, a traditional Japanese fermented rice beverage (Oguro *et al.*, 2019). The release of mannoproteins of varying molecular sizes from polysaccharides to oligosaccharides into wine, depending on the pre-treatment applied to lees, may influence the colloidal stability of wine and its organoleptic properties, particularly the perception of bitterness. The modality using flash release lees treated with β-glucanase (FD-5 % + ENZ) shows little difference from the control wine and was perceived as more fruity, more acidic, and with a longer finish. Among all modalities with lees addition, the one perceived as having the most pronounced odour was the untreated lees modality (NFD-5 %), which most closely resembles traditional ageing practices.

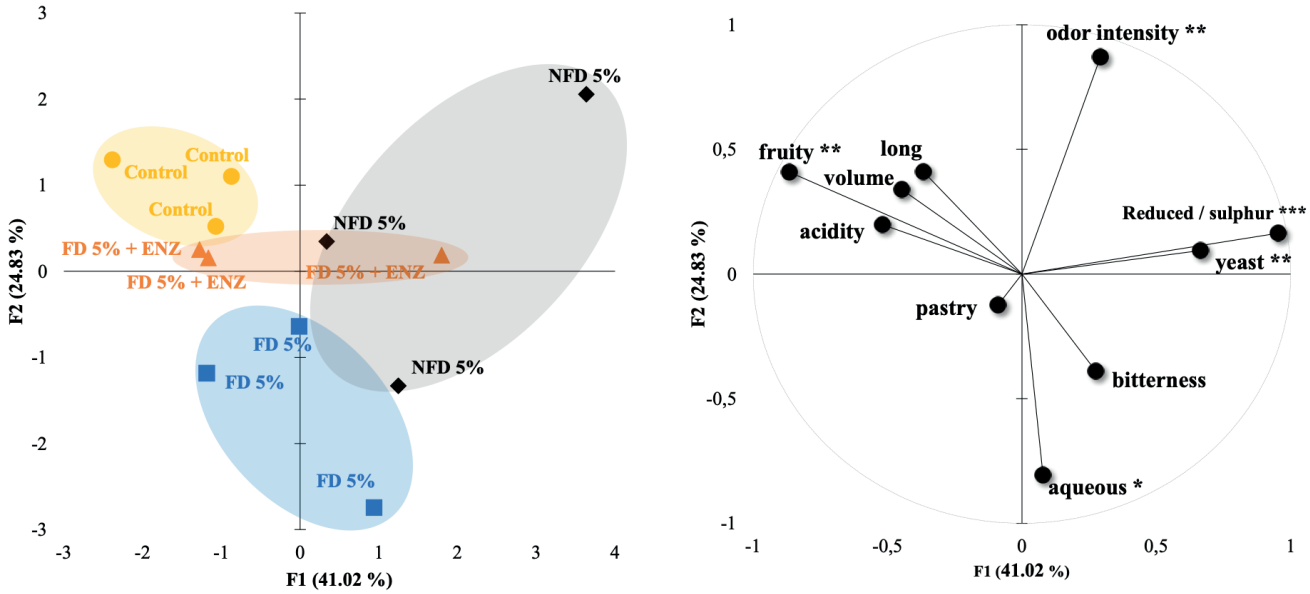


FIGURE 5. Individuals map (a) and correlation circle (b) of the principal component analysis (PCA) of sensorial profiles of wines (triplicates) obtained with control wine (Control), wine with 5 % of lees (NFD-5 %), wine with 5 % of lees flash release (FD-5 %), and wine with 5 % of lees flash release and treated by enzymes (FD-5 % + ENZ). Variables used: fruity, volume, long (long in the mouth), acidity, pastry, aqueous, bitterness, yeast, reduced/sulphur, and odour intensity.

CONCLUSION

This study examines the impact of different lees treatments with the FD prototype of Pera Pellenc (untreated, treated by *"flash détente"* (FD) with or without the addition of enzymes) on the polysaccharide composition and sensory characteristics of Chardonnay wines after six months of ageing. The results show that the addition of lees does not significantly affect classical oenological parameters (pH, acidity, SO_2, alcohol), except in the case of untreated lees (NFD-5 %), which slightly increases pH and total acidity. In terms of polysaccharides, FD treatment on lees before adding to wines, especially when combined with an enzyme (β-glucanase), significantly enhances the release of mannoproteins (up to 600 mg/L), while levels of pectic polysaccharides PRAGs and RG-II remain stable. Molar mass analysis after SEC-MALs reveals that enzymatic treatment following FD promotes the "breakdown" of high molecular weight polysaccharides into smaller fragments, and leads to an increase in low molecular weight MPs. Sensory-wise, untreated lees enhance yeast aromas and sulphur-like notes, while FD lees treated with enzymes provide a more fruity and acidic profile with greater length on the palate, slightly weaker than that of the control wine. Thus, FD treatment of lees, particularly when combined with enzymes, improves targeted mannoproteins extraction while positively influencing the wine's sensory profile.

The FD process helps preserve the physicochemical and biochemical properties essential to the final product. However, controlling sensory characteristics remains one of the most challenging aspects of the process, as adjusting parameters like temperature or pressure can compromise the quality of the treated product in this case, white wine lees, by causing loss of aromatic compounds or the formation of undesirable notes (*e.g.*, sulphur or reductive odours). Furthermore, traditional ageing of wines on lees was time-consuming, energy-intensive, and costly. Using *flash détente* as a pre-treatment for lees appears to be an effective solution to shorten ageing time. This method also offers the added advantage of valorising a naturally occurring by-product in wineries by enabling its rapid reintegration into wine through a short supply chain. Further research should explore the effects of flash release and enzymatic treatments on lees from different grape varieties, optimising treatment conditions and ageing parameters to better understand their impact on the aromatic profile of white wines.

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REFERENCES

Albersheim, P., Nevins, D. J., English, P. D., & Karr, A. (1967). A method for the analysis of sugars in plant cell-wall polysaccharides by gas–liquid chromatography. *Carbohydrate Research*, *5*(3), 340–345. https://doi.org/10.1016/S0008-6215(00)80510-8

Alexandre, H. (2022). Aging on lees and their alternatives: Impact on wine. In Managing Wine Quality, Volume II: *Oenology and Wine Quality* (2nd ed., Vol. 2, p. 213–244), Elsevier. https://doi.org/10.1016/B978-0-08-102065-4.00010-9

Apolinar-Valiente, R., Romero Cascales, I., Williams, P., Gómez-Plaza, E., López Roca, J. M., Ros Garcia, J. M., & Doco, T. (2014). Effect of winemaking techniques on polysaccharide composition of Cabernet Sauvignon, Syrah, and Monastrell red wines. *Australian Journal of Grape and Wine Research*, *20*, 62–71. https://doi.org/10.1111/ajgw.12048

Apolinar-Valiente, R., Ruiz-García, Y., Williams, P., Gil-Munoz, R., Gómez-Plaza, E., & Doco, T. (2018). Preharvest application of elicitors to Monastrell grapes: Impact on wine polysaccharide and oligosaccharide composition. *Journal of Agricultural and Food Chemistry*, *66*(42), 11151–11157. https://doi.org/10.1021/acs.jafc.8b05231

Apolinar-Valiente, R., Williams, P., & Doco, T. (2021). Recent advances in our knowledge of wine oligosaccharides. *Food Chemistry*, *342*. https://doi.org/10.1016/j.foodchem.2020.128330

Apolinar-Valiente, R., Williams, P., Romero-Cascales, I., Gómez-Plaza, E., Lopez-Roca, J. M., Ros-Garcia, J. M., & Doco, T. (2013). Polysaccharide composition of Monastrell red wines from four different Spanish Terroirs: Effect of wine-making techniques. *Journal of Agricultural and Food Chemistry*, *61*(10), 2538–2547. https://doi.org/10.1021/jf304987m

Assunção Bicca, S., Poncet-Legrand, C., Roi, S., Mekoue, J., Doco, T., & Vernhet, A. (2023). Exploring the influence of *S. cerevisiae* mannoproteins on wine astringency and color: Impact of their polysaccharide part. *Food Chemistry*, 136160. https://doi.org/10.1016/j.foodchem.2023.136160

Ayala-Zavala, J., Castillo-Romero, T-de-J., Salgado-Cervantes, M., Marín-Castro, U., Vargas-Ortiz, M., Pallet, D., & Servent, A. (2024). Flash Vacuum Expansion: Effect on Physicochemical, Biochemical and Sensory Parameters in Fruit Processing. *Food Reviews International*, *40*:3, 833-866. https://doi.org/10.1080/87559129.2023.2197997

Ayestarán, B., Guadalupe, Z., & Leon, D. (2004). Quantification of major grape polysaccharides (Tempranillo v.) released by maceration enzymes during the fermentation process. *Analytica Chimica Acta*, *513*(1), 29-39. https://doi.org/10.1016/j.aca.2003.12.012

Babayan, T. L., Bezrukov, M. G., Latov, V. K., Belikov, V. M., Belavtseva, E. M., & Titova, E. F. (1981). Induced Autolysis of *Saccharomyces cerevisiae*: Morphological Effects, Rheological Effects, and Dynamics of Accumulation of Extracellular Hydrolysis Products. *Current Microbiology*, *5*, 163–168. https://doi.org/10.1007/BF01578522

Blakeney, A. B., Harris, P. J., Henry, R. J., & Stone, B. A. (1983). A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydrate Research*, *113*(2) 291-299. https://doi.org/10.1016/0008-6215(83)88244-5

Boulet, J.-C., Trarieux, C., Souquet, J.-M., Ducasse, M.-A., Caillé, S., Samson, A., Williams, P., Doco, T., & Cheynier, V. (2016). Models based on ultraviolet spectroscopy, polyphenols, oligosaccharides and polysaccharides for prediction of wine astringency. *Food Chemistry*, *190*, 357-363. https://doi.org/10.1016/j.foodchem.2015.05.062

Boulet, J.-C., Vernhet, A., Poncet-Legrand, C., Cheynier, V., & Doco, T. (2024). Exploring the role of grape cell wall and yeast polysaccharides in the extraction and stabilisation of anthocyanins and tannins in red wines. *OENO One*, *58*(1). https://doi.org/10.20870/oeno-one.2024.58.1.7793

Canalejo, D., Guadalupe, Z., Martínez-Lapuente, L., Ayestarán, B., Pérez-Magariño, S., & Doco, T. (2022). Characterization of grape polysaccharides extracted from different grape and winemaking products. *Food Research International*, *157*, 111480. https://doi.org/10.1016/j.foodres.2022.111480

Chalier, P., Angot, B., Delteil, D., Doco, T., & Gunata, Z. (2007). Interactions between aroma compounds and whole mannoprotein isolated from *Saccharomyces cerevisiae* strains. *Food Chemistry*, *100*(1), 22–30. https://doi.org/10.1016/j.foodchem.2005.09.004

Chong, H. H., Cleary, M. T., Dokoozlian, N., Ford, C. M., & Fincher, G. B. (2019). Soluble cell wall carbohydrates and their relationship with sensory attributes in Cabernet Sauvignon wine. *Food Chemistry*, *298*, 124745. https://doi.org/10.1016/j.foodchem.2019.05.020

De Iseppi, A., Marangon, M., Lomolino, G., Crapisi, A., & Curioni, A. (2021b). Red and white wine lees as a novel source of emulsifiers and foaming agents. *LWT-Food Science and Technology*, *152*, 112273. https://doi.org/10.1016/j.lwt.2021.112273

De Iseppi, A., Marangon, M., Vincenzi, S., Lomolino, G., Curioni, A., & Divol, B. (2021a). A novel approach for the valorization of wine lees as a source of compounds able to modify wine properties. *LWT-Food Science and Technology*, *136*(1). https://doi.org/10.1016/j.lwt.2020.110274

Del Barrio-Galán, R., Pérez-Magariño, S., Ortega-Heras, M., Williams, P., & Doco, T. (2011). Effect of Aging on Lees and of Three Different Dry Yeast Derivative Products on Verdejo White Wine Composition and Sensorial Characteristics. *Journal of Agricultural and Food Chemistry*, *59*(23), 12433–12442. https://doi.org/10.1021/jf204055u

Dimou, C., Kopsahelis, N., Papadaki, A., Papanikolaou, S., Kookos, I. K., Mandala, I., & Koutinas., A. A. (2015). Wine lees valorization: Biorefinery development including production of a generic fermentation feedstock employed for poly(3-hydroxybutyrate) synthesis. *Food Research International*, *73*, 81–87. https://doi.org/10.1016/j.foodres.2015.02.020

Doco, T., & Brillouet, J. M. (1993). Isolation and Characterisation of a Rhamnogalacturonan II from Red Wine. *Carbohydr. Res.*, *243*(2), 333–343. https://doi.org/10.1016/0008-6215(93)87037-S

Doco, T., O'Neill, M. A., & Pellerin, P. (2001). Determination of the neutral and acidic glycosyl-residue compositions of plant polysaccharides by GC–EI–MS analysis of the trimethylsilyl methyl glycoside derivatives. *Carbohydrate Polymers*, *46*(3), 249–259. https://doi.org/10.1016/S0144-8617(00)00328-3

Doco, T., Quellec, N., Moutounet, M., & Pellerin, P. (1999). Polysaccharide patterns during the aging of Carignan noir red wines. *American Journal of Enology and Viticulture*, *50*(1) 28–32. https://doi.org/10.5344/ajev.1999.50.1.25

Doco, T., Roi, S., Roy, A., Mouret, J-R., & Ducasse, M. A. (2025). Polysaccharide and oligosaccharide compositions of wines produced from traditional (Syrah and Cabernet-Sauvignon), disease-resistant (Artaban, G14 Bouquet and Resdur 2728k) and hybrid direct producer (Clinton, Isabelle and Jacquez) grape cultivars. *OENO One*, *59*(1):59-60. https://doi.org/10.20870/oeno-one.2025.59.1.8452

Doco, T., Williams, P., & Cheynier, V. (2007). Effect of Flash Release and Pectinolytic Enzyme Treatments on Wine Polysaccharide Composition. *Journal of Agricultural and Food Chemistry*, *55*, 6643-6649. https://doi.org/10.1021/jf071427t

Doco, T., Williams, P., Meudec, E., Cheynier, V., & Sommerer, N. (2015). Complex carbohydrates of red wine: Characterization of the extreme diversity of neutral oligosaccharides by ESI-MS. *Journal of Agricultural and Food Chemistry*, *63*, 671–682. https://doi.org/10.1021/jf504795g

Ducasse, M. A., Canal-Llauberes, R., De Lumley, M., Williams, P., Souquet, J., Fulcrand, H., Doco, T., & Cheynier, V. (2010). Effect of macerating enzyme treatment on the polyphenol and polysaccharide composition of red wines. *Food Chemistry*, *118*, 369–376. https://doi.org/10.1016/j.foodchem.2009.04.130

Escot, S., Feuillat, M., Dulau, L., & Charpentier, C. (2001). Release of polysaccharides by yeasts and the influence of released polysaccharides on colour stability and wine astringency. *Australian Journal of Grape and Wine Research*, *7*(3), 153–159. https://doi.org/10.1111/j.1755-0238.2001.tb00204.x

Fornairon-Bonnefond, C., Camarasa, C., Moutounet, M., & Salmon, J.-M. (2001). New trends on yeast autolysis and wine ageing on lees: A bibliographic review. *Journal International des Sciences de la Vigne et du Vin*, *57–78*(2), 22. https://doi.org/10.20870/oeno-one.2001.35.2.990

Fornairon-Bonnefond, C., Mazauric, J.-P., Salmon, J.-M., & Moutounet, M. (1999). Observations on the oxygen consumption during maturation of wines on lees. *OENO One*, *33*(2), 79. https://doi.org/10.20870/oeno-one.1999.33.2.1030

Guadalupe, Z., & Ayestarán, B. (2007). Polysaccharide profile and content during the vinification and aging of Tempranillo red wines. *Journal of Agricultural and Food Chemistry*, *55*, 10720–10728. https://doi.org/10.1021/jf0716782

Marangon, M., Vegro, M., Vincenzi, S., Lomolino, G., De Iseppi, A., & Curioni, A. (2018). A Novel Method for the Quantification of White Wine Mannoproteins by a Competitive Indirect Enzyme-Linked Lectin Sorbent Assay (CI-ELLSA). *Molecules*, *23*. https://doi.org/10.3390/molecules23123070

Martínez-Lapuente, L., Apolinar-Valiente, R., Guadalupe, Z., Ayestarán, B., Pérez-Magariño, S., Williams, P., & Doco, T. (2016). Influence of grape maturity on complex carbohydrate composition of red sparkling wines. *Journal of Agricultural and Food Chemistry*, *64*, 5020–5030. https://doi.org/10.1021/acs.jafc.6b00207

Moine-Ledoux, V., & Dubourdieu, D. (1999). An invertase fragment responsible for improving the protein stability of dry white wines. *Journal of the Science of Food and Agriculture*, *79*(4), 537–543. https://doi.org/10.1002/(SICI)1097-0010(19990315)79:4%3C537::AID-JSFA214%3E3.0.CO;2-B

Moine-Ledoux, V., Dulau, L., & Dubourdieu, D. (1992). Interprétation de l'amélioration de la stabilité protéique des vins au cours de l'élevage sur lies. *Journal International des Sciences de la Vigne et du Vin*, *26*(4), 239–251. https://doi.org/10.20870/oeno-one.1992.26.4.1188

Moine-Ledoux, V., Perrin, A., Paladin, I., & Dubourdieu, D. (1997). Premiers Résultats De Stabilisation Tartrique Des Vins Par Addition De Mannoprotéines Purifiées (Mannostab). *Journal International des Sciences de la Vigne et du Vin*, *31*(1), 23–31. https://doi.org/10.20870/oeno-one.1997.31.1.1092

Morata, A., Gómez-Cordovés, M. C., Colomo, B., & Suarez, J. A. (2005). Cell wall anthocyanin adsorption by different *Saccharomyces* strains during the fermentation of *Vitis vinifera* L. cv Graciano grapes. *European Food Research and Technology*, *220*(3–4), 341–346. https://doi.org/10.1007/s00217-004-1053-8

Morata, A., Gómez-Cordovés, M. C., Suberviola, J., Bartolomé, B., Colomo, B., & Suárez, J. A. (2003). Adsorption of Anthocyanins by Yeast Cell Walls during the Fermentation of Red Wines. *Journal of Agricultural and Food Chemistry*, *51*(14), 4084–4088. https://doi.org/10.1021/jf021134u

Morel-Salmi, C., Souquet, J.-M., Bes, M., & Cheynier, V. (2006). Effect of Flash Release Treatment on Phenolic Extraction and Wine Composition. *Journal of Agricultural and Food Chemistry*, *54*(12), 4270–4276. https://doi.org/10.1021/jf053153k

Nakajima, T. & Ballou, C. E. (1974). Structure of linkage region between polysaccharide and protein parts of *Saccharomyces cerevisiae* mannan. *Journal of Biological Chemistry*, *249*, 7685-7694. https://doi.org/10.1016/S0021-9258(19)81291-7

Nigen, M., Valiente, R. A., Iturmendi, N., Williams, P., Doco, T., Moine, V., Massot, A., Jaouen, I., & Sanchez, C. (2019). The colloidal stabilization of young red wine by Acacia *senegal* gum: The involvement of the protein backbone from the protein-rich arabinogalactan-proteins. *Food Hydrocolloids*, *97*, 105176. https://doi.org/10.1016/j.foodhyd.2019.105176

Ntuli, R. G., Saltman, Y., Ponangi, R., Jeffery, D. W., Bindon, K., & Wilkinson, K. L. (2021). Impact of Fermentation Temperature and Grape Solids Content on the Chemical Composition and Sensory Profiles of Cabernet Sauvignon Wines Made from Flash Détente Treated Must Fermented Off-Skins. *Food Chemistry*, *369*, 130861. https://doi.org/10.1016/j.foodchem.2021.130861

Ntuli, R. G., Saltman, Y., Ponangi, R., Jeffery, D. W., Bindon, K., & Wilkinson, K. L. (2022). Impact of Skin Contact Time, Oak and Tannin Addition on the Chemical Composition, Color Stability and Sensory Profile of Merlot Wines made from Flash Détente Treatment. *Food Chemistry*, *405*(1):134849. https://doi.org/10.1016/j.foodchem.2022.134849

Núñez, Y. P., Carrascosa, A. V., González, R., Polo, M. C., & Martínez-Rodríguez, A. (2006). Isolation and Characterization of a Thermally Extracted Yeast Cell Wall Fraction Potentially Useful for Improving the Foaming Properties of Sparkling Wines. *Journal of Agricultural and Food Chemistry*, *54*(20), 7898–7903. https://doi.org/10.1021/jf0615496

Oguro, Y., Nakamura, A., & Kurahashi, A. (2019). Effect of temperature on saccharification and oligosaccharide production efficiency in *koji amazake*. *Journal of Bioscience and Bioengineering, 127*(5), 570-574. https://doi.org/10.1016/j.jbiosc.2018.10.007

OIV. (2016). Compendium of International Methods of Wine and Must Analysis. *International Organisation of Vine and Wine*, Press, Paris, France.

Pati, S., Liberatore, M. T., Lamacchia, C., & La Notte, E. (2010). Influence of ageing on lees on polysaccharide glycosyl-residue composition of Chardonnay wine. *Carbohydrate Polymers*, *80*, 332-336. https://doi.org/10.1016/j.carbpol.2009.11.017

Pérez-Bibbins, B., Torrado-Agrasar, A., Salgado, J. M., Oliveira, R. P. de S., & Domínguez, J. M. (2015). Potential of lees from wine, beer and cider manufacturing as a source of economic nutrients: An overview. *Waste Management*, *40*, 72–81. https://doi.org/10.1016/j.wasman.2015.03.009

Quijada-Morin, N., Williams, P., Rivas-Gonzalo, J., Doco, T., & Escribano-Bailon, M. (2014). Polyphenolic, polysaccharide and oligosaccharide composition of tempranillo red wines and their relationship with the perceived astringency. *Food Chemistry*, *154*, 44–51. https://doi.org/10.1016/j.foodchem.2013.12.101

Redgwell, R. J., Schmitt, C., Beaulieu, M., & Curti, D. (2005). Hydrocolloids from coffee: physicochemical and functional properties of an arabinogalactan–protein fraction from green beans. *Food Hydrocolloid*, *19*:1005–1015. https://doi.org/10.1016/j.foodhyd.2004.12.010

Rinaldi, A., Coppola, M., & Moio, L. (2019). Aging of Aglianico and Sangiovese wine on mannoproteins: Effect on astringency and colour. *LWT-Food Science and Technology*, *105*, 233–241. https://doi.org/10.1016/j.lwt.2019.02.034

Salgado, J. M., Rodríguez, N., Cortés, S., & Domínguez, J. M. (2010). Improving downstream processes to recover tartaric acid, tartrate and nutrients from vinasses and formulation of inexpensive fermentative broths for xylitol production: Improving recovery of tartrates and nutrients from vinasses. *Journal of the Science of Food and Agriculture*, *90*(13), 2168–2177. https://doi.org/10.1002/jsfa.4065

Samoticha, J., Wojdylo, A., Chmielewska, J., & Ozmianski, J. (2016). The Effects of Flash Release Conditions on the Phenolic Compounds and Antioxidant Activity of Pinot Noir Red Wine. *European Food Research and Technology*, *243*(6), 999–1007. https://doi.org/10.1007/s00217-016-2817-7

Vidal, S., Williams, P., Doco, T., Moutounet, M., & Pellerin, P. (2003). The Polysaccharides of Red Wine: Total Fractionation and Characterization. *Carbohydrate Polymers*, *54*(4), 439–447. https://doi.org/10.1016/S0144-8617(03)00152-8

Wang, S., Wang, X., Zhao, P., Ma, Z., Zhao, Q., Cao, X., Cheng, C., Liu, H., & Du, G. (2021). Mannoproteins interfering wine astringency by modulating the reaction between phenolic fractions and protein in a model wine system. *LWT-Food Science and Technology*, 152, 112217. https://doi.org/10.1016/j.lwt.2021.112217