



Potential use of grape and wine polysaccharide extracts as fining agents to modulate the volatile composition of *Viura* wines

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ARTICLE INFO

Keywords:

Grape-by products
Aroma
Polysaccharides rich in arabinose and galactose
Rhamnogalacturonans
Mannoproteins
Homogalacturonans

ABSTRACT

This paper describes for the first time the use of grape derived polysaccharide extracts as potential fining agents to modulate the volatile composition of *Viura* white wines. Polysaccharide extracts were obtained from white grape pomace, red wine pomace, white must, red must, white wine, and lees from white wine.

Except for higher alcohols, the extracts from white pomace, red pomace and white lees increased the content of most volatile compounds after one and twelve months of bottle aging. They could be used to enhance fruity and floral aromas and reduce unpleasant aromas, showing as good modulators of white wine aroma. The presence of mannoproteins, glucans, non-pectic polysaccharides, and low molecular weight polysaccharides increased the content of most volatile families. Polysaccharides of medium molecular weight showed negative correlations with volatile contents. Our results support the use of winemaking by-products to obtain valuable polysaccharides, contributing to the circular economy.

1. Introduction

Fining agents are widely used in the wine industry to clarify, stabilize, or modify the wine's organoleptic properties. Fining techniques and agents are used to improve the color, odor, flavor, stability, and mouthfeel of the finished wine. Nowadays, an oenological industry challenge is the development of alternative solutions to traditional animal-derived fining agents to avoid allergenic risk or food intolerance and switch to a sustainable and vegan-friendly wine production. In this sense, there is an increasing interest in developing alternative solutions including the use of fining proteins extracted from plants (e.g., proteins from cereals, grape seeds, potatoes, legumes, etc.), and non-proteinaceous plant-based substances (e.g., cell wall material or fiber from different vegetal sources). Up to now, most of the studies have focused on the potential use of protein-based fining agents from plants (Gambutí et al., 2016; Gazzola et al., 2017; Granato et al., 2018) but little attention has been paid to other macromolecules such as plant and grape polysaccharides.

Polysaccharides in wine play important roles in the stabilization and the organoleptic wine quality properties like aroma, foaming properties,

color, and mouthfeel (Del Barrio-Galán et al., 2012; Guadalupe et al., 2014; Martínez-Lapuente et al., 2020), showing different effects depending on the concentration, structure, size, and type of polysaccharide (Guadalupe et al., 2014; Brandão et al., 2017; Martínez-Lapuente et al., 2020; Jones-Moore et al., 2022). In recent years, polysaccharide products have been developed to reduce aggressive tannins and improve wine aroma, mouthfeel, and texture quality. However, all these commercial products are produced from yeast with different techniques and purification degrees. Although grape polysaccharides have shown many positive effects on red and white sensory quality, they are not commercially available and thus it has not been possible to evaluate their potential use as fining agents. Therefore, further studies about the effectiveness of these molecules are needed, which is also of great interest because it would allow the recovery and valorization of compounds from grapes and grape by-products, improving the sustainable development of the wine industry.

Grape polysaccharides arise from the cell walls of grape berries and include pectic polysaccharides like polysaccharides rich in arabinose and galactose (PRAG), rhamnogalacturonans type I and II (RG-I and RG-II), and homogalacturonans (HG); and non-pectic polysaccharides (NPP)

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<https://doi.org/10.1016/j.foodchem.2023.137047>

Received 6 March 2023; Received in revised form 19 July 2023; Accepted 27 July 2023

Available online 1 August 2023

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like celluloses and hemicelluloses. In addition to these, wine polysaccharides also comprise mannoproteins (MP) and glucans from yeast cell walls released during wine fermentation and aging on lees (Guadalupe et al., 2012; Guadalupe et al., 2014; Guadalupe and Ayestarán, 2007; Martínez-Lapuente et al., 2020).

A recent study by our research group has recovered and characterized polysaccharide extracts from different grape and winemaking products like grape pomaces, musts, wines and wine lees (Canalejo et al., 2022). The extracts obtained had different polysaccharide composition and purity, and white pomace and lees revealed as a good source to obtain extracts rich in polysaccharides.

The use of commercial mannoproteins and yeast cell walls-based products during the winemaking, aging and fining of wines has shown to modulate the wine volatile composition and aroma (Comuzzo et al., 2006; Guadalupe & Ayestarán, 2008; Pérez-Magariño et al., 2015; Jones-Moore et al., 2022). These products are specially recommended in white wines to improve their aromatic characteristics by protecting aroma and enhancing complexity, increasing the perception of fruity aromas and their intensity, and refreshing the aromatic potential of already oxidized wines (Del Barrio-Galán et al., 2011; Pérez-Magariño et al., 2015). Other polysaccharides have also shown to affect the volatility of aroma compounds in model solutions. AGP isolated from a Carignan noir red wine enhanced the volatility of some volatile compounds while RG-II decreased the volatility of some esters (Dufour & Bayonove, 1999). A commercial arabinogalactan (AG) from larch wood increased the volatility of some volatile compounds in model systems, while at higher concentrations reduced the amount of most of volatile compounds (Mitropoulou et al., 2011). Another study points out the interaction of aroma compounds and xanthan and guar gum polysaccharides by hydrophobic interactions and hydrogen bonds, which results in the retention of some volatile compounds (Jouquand et al., 2008); and different polysaccharide concentrations seem to modulate the quantities of free water molecules to influence the release of aroma compounds (Jouquand et al., 2008). AG has also been reported to interact with other macromolecules in the wine matrix, such as tannins, forming complexes which produce extra hydrophobic regions resulting in retention of volatile compounds (Mitropoulou et al., 2011). A recent study of our workgroup used polysaccharides extracted from grape pomace and must during wine deposit storage, and observed an improvement of some wine characteristics, such as polysaccharide and volatile composition (Pérez-Magariño et al., 2023).

The present paper aims to evaluate the potential use of polysaccharide extracts as fining agents to modulate the volatile composition of *Viura* wines. It describes for the first time the use of six different polysaccharide extracts obtained from grape pomaces, musts, wine, and lees as fining agents at bottling, and analyze their effect on the volatile composition of *Viura* wines.

2. Material and methods

2.1. Chemicals

Sodium hydroxide, ACS reagent, $\geq 97.0\%$ (Merck, Darmstadt, Germany), Bromothymol blue ACS (VWR, Leicester, UK), phenolphthalein ACS reagent (Sigma, Beerse, Belgium) and potassium hydrogen phthalate $\geq 99.95\%$ (Sigma, Beerse, Belgium) were used for the analyses of standard oenological parameters. The volatile compound standards were purchased from Fluka (Buchs, Switzerland), Sigma-Aldrich (Steinheim, Germany), and Lancaster (Strasbourg, France).

2.2. Obtention and composition of polysaccharide extracts

Polysaccharide (PS) extracts were recovered from different grape and winemaking products as previously described (Canalejo et al., 2022). The extracts were obtained from: white pomace (WP) obtained from *Viura Vitis vinifera* L. variety after the pressing; red pomace (RP)

obtained from *Tempranillo Vitis vinifera* L. variety after the pressing of the solid parts after alcoholic fermentation; white concentrated must (WM) supplied by Julian Soler S.A. (Cuenca, Spain); red must (RM) obtained from *Tempranillo Vitis vinifera* L. variety after the crushing and destemming of the grapes; white wine (WW) made by traditional winemaking of *Viura Vitis vinifera* L. variety; and white lees (WL) recovered after the winemaking of *Viura Vitis vinifera* L. variety. The different PS extracts were obtained as described in Canalejo et al. (2022), and characterized in terms of monosaccharide and PS composition, PS molecular weight distribution and PS purity (mg of total polysaccharides per 100 mg of extract). As described in Canalejo et al. (2022), WP extract (55.5% purity) was mainly composed of PRAG (35.8%) and NPP (40.6%) and smaller amounts of HG (13.6%) and RG-II (9.1%); RP extract (38.6% purity) was mainly composed of PRAG (27.8%) and NPP (34.5%) and smaller amounts of HG (15.7%), RG-II (15.7%), and MP (6.2%); WM extract (87.9% purity) was mainly composed of PRAG (50.7%) and smaller amounts of NPP (21.2%), HG (2.9%) and mannans (24.4%); RM extract (45.5% purity) was mainly composed of PRAG (61.0%), and smaller amounts of NPP (10.1%), HG (6.2%), RG-II (12.2%) and MP (10.4%); WW extract (42.3% purity) was mainly composed of PRAG (58.7%) and smaller amounts of NPP (11.3%), HG (6.4%), RG-II (12.4%), and MP (11.2%); and WL extract (51.9% purity) was mainly composed of MP (72.2%) and GL (26.0%). The extracts also presented different molecular weight (Mw) distributions. WP extract was mainly composed of low Mw PS (77.4%), followed by high (19.0%) and medium (3.6%) Mw PS. RP extract presented similar Mw distributions than WP extract (79.9 % of low Mw PS). WM and RM extracts were mainly composed of low Mw (54.4% and 55.5% respectively) and high Mw PS (41.0% and 37.8% respectively). WW extract was composed of low (51.3%), medium (23.1%) and high Mw PS (25.6%). Finally, WL extract presented the major low Mw distributions (97.8%).

2.3. Winemaking and trials

A white wine from *Viura Vitis vinifera* L. variety was used. It was made by traditional winemaking in 2019 in a winery of Rioja Qualified Denomination of Origin (D.O.Ca Rioja). Grapes were harvested at optimum maturity (22.8 °Brix, pH 3.32, 6.54 g L⁻¹ total acidity as g L⁻¹ tartaric acid), destemmed-crushed and pressed (BucherVaslin XPro 8, France) to obtain juice. The must was fermented in a stainless-steel deposit at 14 to 16 °C after inoculation with 0.15 g/L of *Saccharomyces cerevisiae* yeast (Vitilevure Chardonnay Yseo, Martin Vialate, Magenta, France). Fermentation took twelve days and thereafter the wines were cold-settled. After cold stabilization (-5 °C) and clarification with 0.8 g/L of bentonite (Laffort, Bordeaux-Cedex, France), the PS extracts were added to the wine 24 h before filtration and bottling.

Seven experiments were carried in triplicate: control wine (without the addition of any product, C); wine with the addition of PS extracted from WP; wine with the addition of PS extracted from RP; wine with the addition of PS extracted from WM; wine with the addition of PS extracted from RM; wine with the addition of PS extracted from WW; wine with the addition of PS extracted from WM; wines with the addition of WL.

The doses used for the different PS extracts were 0.10 g/L.

2.4. Standard oenological parameters

Standard oenological parameters in the wines were measured according to the official methods established by the International Organization of Vine and Wine (International Organization of Vine and Wine, 2021): pH, titratable acidity (g L⁻¹ tartaric acid), volatile acidity (g L⁻¹ acetic acid), alcohol content (% vol: mL ethanol for 100 mL wine at 20 °C), absorbance at 420 nm, free SO₂ (mg L⁻¹ free sulfur dioxide) and total SO₂ (mg L⁻¹ total sulfur dioxide). Malic acid was analyzed by the autoanalyzer BioSystems Y15 (Biosystem, Barcelona, Spain).

2.5. Analysis of volatile compounds by gas chromatography

Higher alcohols were quantified by direct injection of wine in split mode (25:1), using an Agilent 7890A gas chromatograph with a flame ionization detector (GC-FID) and the chromatographic conditions described in Pérez-Magariño et al. (2019). Calibration curves used are described in Pérez-Magariño et al. (2023).

Volatile compounds found in lower concentrations in the wine were quantified by headspace solid-phase micro-extraction (HS-SPME) (autosampler PAL RSI 120) and gas chromatography with mass spectrometer (GC-MS) (Agilent 78902B CG coupled to a 5977B MSD). 10 mL of wine was diluted (1:3 with an hydroalcoholic solution and the addition of four internal standards (IS): methyl 2-methylbutyrate, methyl

octanoate, heptanoic acid and 3,4-dimethylphenol) and placed into a 20-mL glass vial with 3.5 g/L of sodium chloride. The samples were incubated 5 min at 40 °C and after that the volatiles in the headspace of the vial were extracted with a 1-cm 50/30- μ m DVB/Carboxen/PDMS SPME fiber (Supelco) at the same temperature and with agitation speed of 500 rpm during 60 min. After extraction, the fiber was desorbed 3 min in the injector at 250 °C, using the splitless mode. Chromatographic analyses were carried out with a DB-WAX Ultra Inert capillary column (60 m length, 0.25 mm i.d., and 0.50 mm film thickness, Agilent), and with the chromatographic conditions established by Rodríguez-Bencomo et al. (2010). The identification of the volatile compounds was carried out using the retention times and mass spectra of the standard compounds and the NIST library. Quantification followed the internal

Table 1

Concentration (μ g/L)^a of volatile compounds of *Viura* wines treated with the different PS extracts after one month of bottling (T1).

Compounds	C ^b	WP ^b	RP ^b	WM ^b	RM ^b	WW ^b	WL ^b	F-value
1-propanol	33,643.0 (1,522.3)	23,291.2 (3,005.0)	26,845 (1,590.3)	29,646.7 (159.0)	27,749.5 (1,814.6)	29,326.5 (2,580.3)	23,571.7 (1,265.8)	1.084 (ns)
Isobutanol	1,259.7 (39.6)ab	2,086.7 (2.0)d	1,756.7 (320.6) c	1,514.8 (189.7) bc	1,163.4 (263.3) a	966.5 (174.1)a	2,277.0 (88.8)d	20.198**
2-Methyl-1-butanol	29,536.5 (2,762.3)c	21,791.8 (1,572.8)ab	22,456.3 (4,257.0)ab	25,919 (11.0)bc	24,973 (1540.6) bc	24,614.4 (615.0)ab	20,069.6 (3,635.6)a	4.601*
3-Methyl-1-butanol	18,933.5 (2,015.8)b	31,466.4 (1,020.0)d	26,042.7 (4,827.8)c	22,143.2 (1,284.8)bc	17,669.2 (2,504.6)ab	14,246.9 (2,514.7)a	34,288.5 (1167.1)d	26.128**
2-phenylethyl alcohol	17,197.7 (104.3) ab	27,419.1 (2,254.6)d	22,239.0 (4,039.7)c	18,541.4 (437.2)b	14,997.6 (1,458.2)ab	13,696.1 (1,322.8)a	28,020.1 (1,366.2)d	25.403**
TOTAL HIGHER ALCOHOLS	100,570.4 (15,602.4)c	106,055.2 (2,932.1)c	99,339.7 (7,770.5)bc	97,765.1 (1,379.6)bc	86,552.7 (3,759.6)ab	82,850.4 (3,838.2)a	108,226.9 (4,084.9)c	5.207*
1-Hexanol	444.9 (20.0)bc	769.1 (105.3)d	553.3 (96.6)c	489.0 (2.9)c	379.3 (35.6)ab	332.0 (45.6)a	733.9 (24.8)d	24.018**
E-3-Hexen-1-ol	57.8 (4.2)ab	90.4 (3.5)d	74.1 (4.1)c	64.9 (3.8)b	50.7 (4.1)a	51.2 (3.9)a	99.2 (7.3)d	52.243**
Z-3-Hexen-1-ol	181.2 (8.5)ab	283.2 (13.0)d	232.3 (34.4)c	203.4 (6.0)bc	158.8 (14.3)a	160.4 (27.0)a	310.9 (1.4)d	31.367**
Benzyl alcohol	198.5 (8.4)bc	184.3 (7.0)b	223.9 (9.0)d	267.3 (9.1)e	261.4 (11.07)e	102.0 (7.8)a	204.6 (14.6)c	95.193**
TOTAL C6 ALCOHOLS	882.4 (23.7)b	1,327 (106.4)d	1,083.6 (103.0) c	1,024.6 (11.9)c	850.2 (40.1)b	645.6 (53.7)a	1,348.6 (29.7)d	49.258**
Ethyl butyrate	54.0 (2.4)abc	83.6 (4.8)e	68.0 (12.5)cd	55.4 (5.0)bc	46.9 (7.6)ab	39.7 (10.9)a	79.8 (6.3)de	13.514**
Ethyl hexanoate	722.6 (88.1)b	1,004.2 (28.9)c	819.8 (152.9)b	729.2 (14.0)b	579.8 (59.5)a	561.2 (24.0)a	1,114.5 (70.7)c	21.774**
Ethyl octanoate	1,058.2 (89.8)ab	1,612.4 (143.4)c	1,360.9 (290.7) bc	1,175.8 (208.7) bc	890.4 (166.3)a	895.6 (151.3)a	1,351.5 (87.2)bc	6.967**
Ethyl decanoate	237.8 (40.5)a	360.5 (145.3)ab	460.3 (100.9)b	369.0 (58.7)ab	282.7 (59.4)a	267.4 (56.4)a	337.7 (55.3)ab	2.589*
Ethyl-2-methylbutyrate	10.6 (0.2)c	11.7 (0.1)e	10.6 (0.1)c	9.5 (0.2)b	9.7 (0.1)b	8.3 (0.1)a	11.1 (0.2)d	169.063**
Ethyl isovalerate	22.0 (2.1)abc	25.1 (1.9)d	21.5 (0.4)ab	20.3 (0.3)a	24.4 (0.9)cd	23.5 (1.1)bcd	24.3 (1.4)cd	5.385*
Ethyl lactate	1,067.1 (40.2)ab	1,657.3 (26.0)d	1,462.4 (301.9) cd	1,260.3 (94.0) bc	943.1 (124.2)a	824.7 (96.5)a	1,906.5 (100.9)e	23.75**
TOTAL ETHYL ESTERS	3,172.3 (138.2)b	4,754.8 (207.8)e	4,203.5 (457.5) d	3,619.5 (236.8) c	2,777 (224.1)ab	2,620.4 (190.0)a	4,825.4 (160.9)e	39.262**
Propyl acetate	565.2 (2.0)ab	890.0 (83.4)d	716.4 (111.9)c	594.7 (51.4)b	477.2 (51.1)ab	461.3 (36.6)a	856.1 (55.3)d	21.648**
Isobutyl acetate	367.0 (72.5)ab	489.3 (83.1)c	450.3 (78.1)bc	359.2 (12.3)ab	274.4 (31.9)a	274.7 (49.9)a	516.0 (38.0)c	8.615**
Isoamyl acetate	2,554.5 (250.1)c	3,534.1 (103.5) d	2,857.8 (359.1) c	2,463.8 (122.3) bc	2,015.9 (290.7) ab	1,741.2 (393.9)a	3,986.5 (170.2) d	27.599**
Hexyl acetate	384.2 (53.0)b	430.1 (60.0)b	408.1 (71.0)b	343.4 (6.8)ab	273.5 (35.7)a	264.4 (33.8)a	525.7 (33.3)c	11.756**
β -Phenylethyl acetate	543.3 (1.4)bc	826.3 (72.8)d	643.3 (103.8)c	564.6 (39.8)bc	462.5 (46.5)ab	396.5 (91.9)a	827.4 (64.2)d	18.262**
TOTAL ACETATES	4,414.2 (309.3)b	6,169.7 (402.8) d	5,075.8 (301.6) c	4,325.7 (230.8) b	3,503.5 (446.3) a	3,138.2 (580.0)a	6,711.7 (361.1) d	24.804**
Isovaleric acid	38.8 (0.1)ab	64.3 (4.8)c	49.8 (14.7)b	41.6 (2.6)ab	34.2 (5.4)a	29.7 (7.0)a	64.3 (0.3)c	12.528**
Hexanoic acid	1,221.7 (17.5)bc	1,988.7 (183.1)e	1,520.7 (194.7) d	1,372.7 (81.5) cd	1,097.9 (108.9) ab	912.6 (188.9)a	2,016.0 (129.6)e	26.641**
Octanoic acid	2,574.9 (12.8)ab	4,175.4 (408.7) d	3,341.3 (464.2) c	2,735.6 (208.1) b	2,306.0 (236.8) ab	1,977.4 (471.5)a	4,093.7 (249.5) d	20.39**
Decanoic acid	41.2 (10.2)a	61.0 (3.9)b	60.1 (13.3)b	42.3 (10.0)a	36.9 (5.7)a	37.1 (4.6)a	63.1 (12.4)b	4.984*
TOTAL ACIDS	3,945.6 (7.2)b	6,404.8 (602.8) d	5,066.7 (659.9) c	4,278.5 (243.1) bc	3,542.1 (345)ab	3,012.1 (658.6)a	6,361.8 (390.5) d	24.017**
4-vinylguaiacol	332.5 (46.3)ab	441.3 (76.8)cd	394.1 (60.4)bc	299.5 (25.2)ab	252.5 (20.2)a	228.8 (36.0)a	510.5 (90.5)d	9.936**
4-vinylphenol	267.5 (45.6)a	457.7 (87.0)cd	381.6 (60.4)bc	293.9 (12.5)ab	248.2 (30.2)a	257.9 (49.0)a	535.0 (96.7)d	10.133**
TOTAL PHENOLS	600.0 (79.4)ab	899.0 (163.8)cd	775.7 (120.8)bc	593.4 (37.6)ab	500.6 (45.2)a	486.7 (85.0)a	1,045.5 (179.9) d	10.462**
Linalool	37.1 (1.9)b	42.6 (1.6)cd	33.5 (2.0)a	37.9 (1.8)b	45.8 (2.1)d	42.1 (1.2)c	41.3 (2.1)c	15.017**
α -Terpineol	6.2 (0.2)b	6.7 (0.1)c	5.7 (0.1)b	5.5 (0.2)a	6.7 (0.1)c	7.0 (0.1)d	6.8 (0.1)cd	54.769**
TOTAL TERPENES	43.3 (1.9)b	49.3 (1.6)cd	39.2 (2.0)b	43.4 (1.8)b	52.5 (2.1)d	49.1 (1.2)c	48.1 (2.1)c	18.668**

^a Mean values and standard deviations are shown (n = 3). Different letters in the same row 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: * and ** indicates significance at $p < 0.01$ and $p < 0.001$.

^b Control wine (C) and wines treated with the different PS extracts. WP: White Pomace; RP: Red Pomace; WM: White Must; RM: Red Must; WW: White Wine; WL: White Lees.

standard quantification method, using selected quantification ions and IS chosen for each compound, and calibration curves described in Pérez-Magariño et al. (2023).

The volatile composition of the *Viura* wines was analysed after one month of bottling (T1) and after twelve months of bottle aging (T12). The Odor Activity Value (OAV) was used to evaluate the potential contribution of a chemical compound to wine aroma. We have considered that odorants with higher OAVs (>0.2) contribute more strongly to overall aroma.

2.6. Statistical analyses

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 volatile composition of the *Viura* wines, using the Duncan post-hoc testing. A multivariate analysis of variance (MANOVA) was applied at a confidence level of 95 % (*p*-value of 0.05) to determine differences among the wines by aging or by treatment. A Spearman correlation test was made to correlate the chemical composition of the extracts with the volatile composition of the wines.

3. Results and discussion

3.1. Oenological parameters

The standard oenological parameters of the *Viura* wines were analysed after one month of bottling.

The average ethanol degree was 12.3 ± 0.11 %; titratable acidity was 6.11 ± 0.09 g L⁻¹ of tartaric acid; volatile acidity was 0.22 ± 0.05 g L⁻¹ of acetic acid; malic acid was 1.59 ± 0.06 g L⁻¹; pH was 3.43 ± 0.10 ; free SO₂ was 29.6 ± 1.3 mg L⁻¹; total SO₂ was 102.3 ± 1.8 mg L⁻¹; and the absorbance at 420 nm was 0.13 ± 0.02 . The oenological parameters were like those described for this variety.

3.2. Effect of PS extracts on the volatile composition of *Viura* wines after one month of bottling

Table 1 shows the concentration of individual volatile compounds and volatile families of *Viura* wines treated with different PS extracts after one month of bottling (T1). Table 2 shows the OAV values, odor thresholds and descriptors of the volatile compounds with OAV > 0.2. A total of 29 volatile compounds were detected by gas chromatography, and were organized into seven different chemical families: higher alcohols, represented by 1-propanol, isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethyl alcohol; C6 alcohols, represented by 1-hexanol, *E*-3-hexen-1-ol, *Z*-3-hexen-1-ol, and benzyl alcohol; ethyl esters, represented by ethyl butyrate, ethyl hexanoate, ethyl octanoate,

Table 2
Odor activity values (OAV > 0.2)^a in *Viura* wines treated with the different PS treatment after one month of bottling (T1).

Compounds	Odor descriptor	Odor threshold (µg/L)	Reference	C ^b	WP ^b	RP ^b	WM ^b	RM ^b	WW ^b	WL ^b	F-value
2-Methyl-1-butanol	Alcohol	30,000	Gutiérrez-Gamboa et al., 2018	1.0c	0.7 ab	0.7 ab	0.9 bc	0.8 ab	0.8 ab	0.7 a	14.000**
3-Methyl-1-butanol	Alcohol, Banana	7,000	Etiévant, 1991	0.6b	1.0 d	0.9c	0.7 bc	0.6 ab	0.5 a	1.1 d	4.263*
2-phenylethyl alcohol	Roses, honey	14,000	Ferreira et al., 2000	1.2 ab	2.0 d	1.6c	1.3b	1.1 ab	1.0 a	2.0 d	25.400**
<i>E</i> -3-Hexen-1-ol	Green, floral	400	Guth, 1997	0.1 a	0.2b	0.2b	0.2b	0.1 a	0.1 a	0.2b	23.689**
<i>Z</i> -3-Hexen-1-ol	Green, cut grass	400	Ferreira et al., 2000	0.5 a	0.7 cd	0.6 bc	0.5 ab	0.4 a	0.4 a	0.8 d	9.333**
Ethyl butyrate	Papaya, apple, sweet	20	Vilanova et al., 2009	2.7b	4.2 e	3.4 cd	2.8 bc	2.3 ab	2.0 a	4.0 de	21.286**
Ethyl hexanoate	Apple, fruity, sweet	14	Vilanova et al., 2009	51.6b	71.7c	58.6b	52.1b	41.4 a	40.1 a	79.6c	14.375**
Ethyl octanoate	Apple, fruity	5	Vilanova et al., 2009	211.6 ab	322.5c	272.2 bc	235.2 ab	178.1 a	179.1 a	270.3 bc	21.717**
Ethyl decanoate	Grape, fruity	200	Wang et al., 2017	1.2 a	1.8 ab	2.3 ab	1.8 ab	1.4 a	1.3 a	1.7 ab	6.968**
Ethyl 2-methylbutyrate	Fruity, strawberry, apple, blackberry	2	De la Fuente-Blanco, 2020	5.3c	5.9 e	5.3c	4.8b	4.9b	4.2 a	5.6 d	2.726(ns)
Ethyl isovalerate	Fruity, strawberry, apple	0.7	De la Fuente-Blanco, 2020	31.3 ab	36.0 d	30.9 ab	29.1 a	34.7 cd	33.4 bc	34.7 cd	156.692**
Isoamyl acetate	Banana, apple	30	Ferreira et al., 2000	85.2c	117.8 d	95.3c	82.1 bc	67.2 ab	58.0 a	132.9 d	5.400**
β-Phenethyl acetate	Banana	250	Ferreira et al., 2000	2.2 bc	3.3 d	2.6c	2.3 bc	1.9 ab	1.6 a	3.3 d	27.590**
Isovaleric acid	Cheese	33	Ferreira et al., 2000	1.2 ab	1.9c	1.5b	1.2 ab	1.0 ab	0.9 a	1.9c	18.066**
Hexanoic acid	Cheese, fatty	3,000	Wang et al., 2017	0.4 ab	0.7 d	0.5 bc	0.5 bc	0.4 ab	0.3 a	0.7 d	12.139**
Octanoic acid	Cheese, fatty, rancid	1,000	Wang et al., 2017	2.6b	4.2 d	3.3c	2.7 ab	2.3 ab	2.0 a	4.1 d	12.800**
4-vinylguaiaicol	Clove, curry	40	Ferreira et al., 2000	8.3 ab	11.0 cd	9.9 bc	7.5 ab	6.3 a	5.7 a	12.8 d	22.147**
4-vinylphenol	Smoky, almond	180	Ferreira et al., 2000	1.5 a	2.5 cd	2.1 bc	1.6 ab	1.4 a	1.4 a	3.0 d	9.897**
Linalool	Floral, citrus	25	Vilanova et al., 2013	1.5b	1.7c	1.3 a	1.5b	1.8 d	1.7c	1.7c	10.485**

^a Mean values are shown (*n* = 3). Different letters in the same row 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: * and ** indicates significance at *p* < 0.05 and *p* < 0.001.

^b Control wine (C) and wines treated with the different PS extracts. WP: White Pomace; RP: Red Pomace; WM: White Must; RM: Red Must; WW: White Wine; WL: White Lees.

ethyl decanoate, ethyl-2-methylbutyrate, ethyl isovalerate and ethyl lactate; acetates, represented by propyl acetate, isobutyl acetate, isoamyl acetate, hexyl acetate, and β -phenethyl acetate; volatile acids, represented by isovaleric acid, hexanoic acid, octanoic acid, and decanoic acid; phenol volatiles, represented by, 4-vinylguaicol and 4-vinylphenol; terpenes, represented by linalool and α -terpineol. Most of the volatile compounds were detected in similar amounts than previous research (Pérez-Magariño et al., 2013).

A one-way ANOVA was applied to analyze the effect of the different PS extracts on the individual volatile compounds and volatile families. Samples showed differences in all volatile compounds except 1-propanol, which indicated that the use of PS extracts significantly affected the volatile composition of the *Viura* wines.

Higher alcohols were quantitatively the largest chemical family in all *Viura* wines (Table 1). The content of total higher alcohols, expressed as the sum of the individual compounds, did not show significant differences among control wines (C) and wines treated with PS extracted from white and red pomace (WP and RP), white must (WM) and white lees (WL), in agreement with previous results of our work group, which found that the use of PS from must, and pomace did not modify the content of total higher alcohols in *Albillo* and *Verdejo* white wines (Pérez-Magariño et al., 2022). On the contrary, wines treated with PS extracted from red must (RM) and white wines (WW) showed lower contents of total higher alcohols than the rest of the wines. Only 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethyl alcohol showed OAV values higher than 0.5 in all the wines. Most higher alcohols are mostly related to herbaceous notes with strong and pungent tastes and smells. However, in this study the use of PS extracts had positive effects on the content of higher alcohols of the wines. Hence, wines treated the PS extracts showed lower contents of 2-methyl-1-butanol, characterized by alcoholic notes, than the control wines. Moreover, the use WP, RP and WL extracts produced wines with higher contents of 2-phenylethyl alcohol, which is characterized by positive floral aromas (Ferreira et al., 2000), and 3-methyl-1-butanol, characterized by banana notes (Etiévant, 1991). A previous study (Mitropoulou et al., 2011) analyzed the effect of different doses of a commercial arabinogalactan (AG) from larch wood on the relative headspace concentration of some volatile compounds in model solutions and observed different results depending on the AG concentration used and the specific volatile compound. They concluded that the volatility of 2-methyl-1-butanol was weakly affected upon addition of AG in a 0–5 g/L range. On the contrary, as it was observed in our study with the addition of WP, RP, WM and WL extracts, the addition of AG up to 1 g/L in the model media induced an increase in the release of isobutanol into the headspace attributable to a salting out effect. Arabinogalactan is a neutral high molecular weight polysaccharide consisting of a β -D-(1 \rightarrow 3)-galactopyranan main chain and, according to bibliography (Rinaudo, 2001), it probably has a relatively low solubility because it has many hydrogen bonds, which stabilize intrachain and interchain interactions. However, at higher AG addition levels, the volatility of isobutanol was reduced suggesting intermolecular binding of the volatiles to the macromolecule. It is important to notice that the addition of PS extracts in the *Viura* wines did not affect the content of 1-propanol, and isobutanol showed OAV values lower than 0.2 in all the wines (Table 2).

Wines treated with WP, RP, WM and WL extracts showed higher contents of total C6 alcohols than control wines, in agreement with the results previously reported (Pérez-Magariño et al., 2022), which observed an increment of C6 alcohols in white wines treated with PS extracted from must and pomace. Mitropoulou et al. (2011) did not observe any effect for hexanol volatility up to 1 g/L AG while higher doses of AG decreased its volatility. C6 alcohols are related with herbaceous and floral notes (Table 2). 1-Hexanol was quantitatively the major C6 alcohol detected in all samples, but it showed OAV values lower than 0.2 in all the wines. Only (*Z*)-3-Hexen-1-ol showed remarkable OAV values (Table 2). Wines treated with WP, RP, WM and WL extracts showed higher concentrations of 1-hexanol, (*E*)-3-Hexen-1-

ol and (*Z*)-3-Hexen-1-ol than control wines. On the contrary, the addition of RM and WW extracts did not show any effect on the concentration of these compounds.

Ethyl esters are usually found in high concentrations in wines and present low detection thresholds (Table 2), so they are especially important contributors to fruity wine aroma (Vilanova et al., 2009; Wang et al., 2017; De la Fuente-Blanco et al., 2020). As observed with C6 alcohols, the addition of WP, RP, WM, and WL extracts increased the content of total ethyl esters due to increase of most volatile individual compounds. WP and WL extracts produced the largest increase in concentration of ethyl esters in *Viura* wines since their total ethyl ester content were 1.5 times higher than the content of control wines, in agreement with data previously reported by our work group when using PS extracts during wine deposit storage (Pérez-Magariño et al., 2022). Other studies made in model solutions also observed an effect of PS on the volatility of some ethyl ester compounds but only when using very high concentrations of PS (Dufour & Bayonove, 1999; Mitropoulou et al., 2011). Hence, Dufour & Bayonove concluded that doses of purified wine polysaccharides in the range 5–20 g/L did not affect the volatility of these compounds (Dufour & Bayonove, 1999); and Mitropoulou et al. (2011) concluded that ethyl esters were weakly affected upon addition of commercial AG in a 0–5 g/L range. On the contrary, the present study demonstrates that low doses of PS extracts obtained from grape and winemaking products (0.10 g/L) caused a significant effect on most of volatile compounds. Ethyl butyrate, ethyl hexanoate, ethyl octanoate and ethyl decanoate showed high OAV values in all the wines (Table 2), being ethyl octanoate and ethyl hexanoate the compounds with the highest OAV. WP and WL extracts produced the greatest increase in the OAV values of these compounds. WP extracts were mainly composed of PRAG and NPP while WL extracts were mainly composed of MP (Canalejo et al., 2022). A previous research (Dufour & Bayonove, 1999) stated that the protein part of arabinogalactan proteins may play a role in their interactions with lipophilic volatile compounds as ethyl esters. The retention of the aroma compounds by MP has also been described to be dependent of the physico-chemical and hydrophobic nature of the volatile compounds governing their binding affinity for the protein content of the PS (Lubbers et al., 1994; Landy et al., 1995). Moreover, the addition of yeast derivatives rich in MP has been shown to enhance the fruity aromas of white wines since MP increases the levels of fruity esters (Del Barrio-Galán et al., 2011). However, different results on the retention of the aroma compounds have been reported (Chalier et al., 2007) since compounds such as ethyl hexanoate seems to have higher affinity for glycosidic parts of MP (of lower molecular weight) rather than the protein parts. Therefore, esters could present affinity for both, glycosidic and protein part of PS, being also important the PS molecular weight.

Five acetates were detected (Table 1), usually related with fruity descriptors as strawberry, banana, and apple notes (Table 2). Except for ethyl butyrate, all acetates were detected in much higher quantities than its odor threshold, and isoamyl acetate showed the highest OAV value (Table 2). Acetates were significantly affected by the addition of PS extracts (Table 1). Hence, the addition of WP, RP and WL extracts significantly increased the content of total acetates and most individual compounds, being again WP and WL the extracts that produced the largest increase (1.5 and 1.4 times, respectively). On the contrary, the addition of RM and WW extracts decreased the content of total acetates since reduced the content of the acetates with longer aliphatic chains as isoamyl and hexyl acetate. A previous research (Dufour & Bayonove, 1999) showed that the volatility of isoamyl acetate was weakly affected upon addition of AG in a range of 0–5 g/L, and higher concentrations reduced its volatility. It is important to notice that RM and WW extracts contained larger proportions of high Mw PS while WP, RP and WL extracts contained larger proportions of low Mw PS (see section 2.2). Low Mw PS could interact with the hydrophobic parts of esters and acetates reducing the hydrolysis and esterification processes that led to alcohols and acids formations.

Although C₆-C₁₀ fatty acids are usually related to pungent, fatty, rancid, or cheesy odors (Wang et al., 2017; Ferreira et al., 2000), they can contribute to the aromatic equilibrium of wine as they prevent the hydrolysis of esters when they are found in concentrations of 4 to 10 mg L⁻¹, providing pleasant aromas, while levels above 20 mg L⁻¹ have negative effects (Avram et al., 2015; Malićanin et al., 2022). The mean concentrations of C₆-C₁₀ fatty acids in the studied wines were between 3.0 and 6.4 mg L⁻¹, providing positive effects onto the global aroma quality of wines. Four fatty acids were detected in the samples (Table 1). Isovaleric acid and octanoic acid were detected above their odor thresholds (OAV > 1) (Table 2). The effect of the PS extracts on the fatty acids concentrations was like that observed in the rest of volatile families. WP, RP and WL extracts increased the content of total acetates while WM and RM extracts did not show significant effects, and WW extracts reduced the concentration of most fatty acids. A previous research (Pérez-Magariño et al., 2022) also reported an increase in fatty acids contents in Verdejo wines treated with must and pomace PS

extracts during wine aging in deposits; however, fatty acids content was not affected in Albillo wines, as also observed in model solutions (Mitropoulou et al., 2011).

Volatile phenols provide sensory characteristics generally classified among the “off flavors”, and described as curry, smoky, almond, and clove (Table 2). Two volatile phenols were detected in this study, 4-vinylguaiaicol and 4-vinylphenol, and both were detected above their odor thresholds (Tables 1 and 2). The addition of WP and WL extracts increased again the content of volatile phenols with respect to control wines while the rest did not show significant differences. On the contrary, our previous study (Pérez-Magariño et al., 2022) observed similar contents or slight reductions of volatile phenols when using must and pomace PS extracts, but in this study the PS extracts were not added in the fining stage but were added and maintained two months during the wine storage in stainless steel deposits. Volatile phenols are decomposed during wine aging in white wines by reaction with other wine macromolecules such as procyanidins, proteins or flavor compounds

Table 3

Concentration (µg/L)^a of volatile compounds of Viura wines treated with the different PS extracts after twelve months of bottling (T12).

Compounds	C ^b	WP ^b	RP ^b	WM ^b	RM ^b	WW ^b	WL ^b	F-value
1-Propanol	32,796.0 (1,171.0)d	22,197.5 (2,311.5)a	26,026.0 (1,223.3)b	28,581.5 (122.3)c	26,990.0 (1,395.8)bc	28,106.5 (1,984.8)c	22,320.5 (973.7)a	38.42***
Isobutanol	22,424.0 (1,125.7)d	16,354.0 (1,480.7)a	17,654.5 (133.6) b	19,833.0 (230.5)c	19,620.0 (1,036.6)c	19,199.5 (65.8) c	15,682.0 (188.1)a	48.15***
2-Methyl-1-butanol	27,447.5 (2,124.9)e	20,563.5 (1,209.9)ab	21,766.5 (3,274.6)bc	24,409.0 (800.5)d	23,860.0 (1,185.1)cd	23,500.5 (473.1)cd	1,8709.5 (2,796.6)a	12.994***
3-Methyl-1-butanol	158,137.5 (8,747.6)e	118,617.0 (9,134.4)ab	126,501.5 (13,745.4)bc	141,854.0 (667.5)d	139,166.5 (6,883.7)d	134,615 (2,804.4)cd	112,557.0 (9,545.9)a	20.022***
2-phenylethyl alcohol	23,143.9 (1,357.6)a	22,613.7 (1,065.4)a	27,315.6 (1,083.4)b	27,052.6 (323.6)b	23,504.8 (1,242.4)a	23,578.2 (821.7)a	23,174.2 (1,264.3)a	26.364***
TOTAL HIGHER ALCOHOLS	263,948.9 (14,526.7)d	200,345.7 (15,201.9)a	219,264.1 (17,013.8)b	241,730.1 (460.6)c	233,141.3 (9,258.8)bc	228,999.7 (536.7)bc	192,443.2 (9,916.4)a	28.48***
1-Hexanol	960.9 (2.2)cd	989.6 (27.7)de	922.8 (39.7)b	856.9 (28.5)a	929.2 (9.3)bc	933.3 (37.1)bc	1,021.0 (23.8)e	20.559***
E-3-Hexen-1-ol	95.6 (6.9)a	90.9 (17.6)a	193.8 (10.7)d	180.3 (4.8)c	81.3 (10.0)a	88.5 (0.2)a	124.4 (15.4)b	109.326***
Z-3-Hexen-1-ol	485.8 (7.8)ab	494.8 (14.1)bc	500.0 (21.3)bc	466.9 (11.8)a	474.8 (5.2)ab	481.8 (40.5)ab	516.5 (14.5)c	4.272*
Benzyl alcohol	153.2 (4.3)a	164.2 (6.6)c	193.0 (10.2)d	237.3 (2.0)e	236.9 (1.3)e	161.7 (2.5)ab	162.6 (5.8)bc	254.265***
TOTAL C6 ALCOHOLS	1,695.5 (18.8)a	1,739.44 (52.8) b	1,809.6 (61.6)c	1,741.3 (37.5)b	1,722.2 (25.8) ab	1,665.3 (79.8)a	1,824.5 (17.1)d	9.922***
Ethyl butyrate	213.9 (6.7)b	274.1 (4.6)d	283.3 (2.9)e	251.3 (1.2)c	251.8 (0.1)c	177.5 (4.2)a	279.8 (1.4)e	674.6***
Ethyl hexanoate	353.4 (10.2)b	381.6 (2.3)d	435.0 (4.0)f	441.1 (2.2)g	369.9 (1.1)c	347.2 (3.9)a	394.5 (2.4)e	384.005***
Ethyl octanoate	266.5 (4.1)b	284.4 (5.3)c	298.9 (2.6)d	334.1 (1.0)f	266.0 (3.0)b	254.2 (0.7)a	289.1 (4.5)e	366.163***
Ethyl decanoate	32.2 (2.9)c	34.5 (2.6)c	83.7 (3.6)e	110.4 (0.5)f	28.9 (2.1)b	26.0 (0.4)a	45.2 (2.0)d	1193.071***
ethyl 2-methylbutyrate	7.2 (0.2)d	9.2 (0.1)f	7.8 (0.3)e	6.6 (0.1)b	7.0 (0.3)c	5.8 (0.1)a	9.3 (0.1)f	549.92***
Ethyl isovalerate	17.8 (2.3)	20.7 (0.4)	18.5 (2.2)	17.1 (1.3)	19.4 (0.5)	17.1 (0.5)	19.7 (2.7)	1.83(ns)
Ethyl lactate	21,806.8 (211.9)a	24,919.6 (607.7)bc	26,969.3 (2,342.4)c	24,940.2 (1,484.9)bc	21,814.6 (764.5)a	22,535.4 (1,025.9)ab	24,760.1 (1,862.8)bc	6.59**
TOTAL ETHYL ESTERS	22,697.7 (212.3)a	25,924.1 (607.7)b	28,096.6 (2,342.4)b	26,100.8 (1,484.9)b	27,757.6 (764.515)a	23,363.1 (1,025.9)a	25,797.6 (1862.8)b	7.219***
Propyl acetate	34.9 (1.6)b	37.5 (1.1)c	42.1 (0.8)e	37.3 (0.6)c	35.0 (1.9)b	23.9 (1.0)a	39.1 (0.5)d	146.373***
Isobutyl acetate	27.4 (2.8)b	33.9 (0.8)e	31.2 (0.5)d	29.0 (0.7)c	29.8 (0.3)c	14.4 (0.3)a	34.8 (0.3)e	223.133***
Isoamyl acetate	965.1 (31.5)b	1,051.7 (23.2)c	1,322.8 (79.6)e	1,162.5 (67.1)d	960.6 (41.0)b	385.7 (10.1)a	1,085.3 (77.6)c	178.831***
Hexyl acetate	37.3 (2.7)c	41.5 (0.8)d	41.6 (1.2)d	38.9 (0.7)c	32.9 (0.2)b	19.9 (0.1)a	44.5 (1.62)e	229.538***
β-Phenethyl acetate	66.5 (4.6)a	76.8 (0.8)cd	67.4 (0.1)a	65.3 (0.1)a	74.6 (2.0)bc	72.7 (1.4)c	78.7 (0.8)d	42.245***
TOTAL ACETATES	1,131.2 (19.9)b	1,241.4 (21.9)c	1,505.1 (79.8)d	1,333.0 (66.2)c	1,132.8 (41.1)b	516.5 (12.6)a	1,282.4 (77.5)c	208.068***
Isovaleric acid	365.3 (7.0)bc	367.2 (10.1)bc	424.2 (18.9)d	429.1 (8.4)d	296.6 (17.6)a	356.8 (35.0)b	383.3 (13.5)c	32.406***
Hexanoic acid	4,384.4 (24.2)b	4,549.9 (67.2)c	4,460.4 (7.9)bc	4,408.5 (10.6)b	4,481.7 (131.7) bc	4,260.0 (163.6) a	4,511.3 (11.4) bc	5.833**
Octanoic acid	3,685.4 (14.1)c	4,566.9 (5.2)e	3,424.7 (27.7)a	3,554.7 (3.0)b	3,950.8 (93.4)d	3,722.0 (67.2)c	5,198.5 (0.9)f	751.826***
Decanoic acid	301.7 (17.2)c	322.7 (3.5)c	995.7 (19.3)e	1,227.9 (24.2)f	203.7 (27.1)a	261.6 (3.4)b	350.2 (2.2)d	2571.969***
TOTAL ACIDS	8,736.7 (34.3)b	9,806.7 (55.9)e	9,305 (24.8)c	9,620.1 (20.8)d	8,932.7 (54.4)b	8,600.4 (185.6) a	10,443.2 (19.8) f	210.25***
4-vinylguaiaicol	138.3 (6.8)b	196.0 (1.5)c	237.2 (8.8)d	197.7 (2.9)c	114.3 (11.7)a	118.2 (7.6)a	172.3 (1.0)c	24.681***
4-vinylphenol	47.0 (0.6)a	54.1 (2.5)c	58.2 (0.8)d	51.4 (1.3)b	45.0 (1.3)a	45.5 (0.3)a	52.9 (0.6)bc	187.567***
TOTAL PHENOLS	185.2 (4.4)b	250.1 (4.1)c	295.4 (5.7)d	249.1 (2.9)c	159.3 (9.2)a	163.6 (5.5)a	225.2 (1.0)c	31.825***
Linalool	27.5 (2.3)b	32.2 (2.7)c	23.1 (0.1)a	27.2 (0.2)b	35.7 (3.4)d	32.9 (2.4)c	31.9 (1.4)c	271.883***
α-Terpineol	2.8 (0.1)b	3.9 (0.2)e	2.0 (0.1)a	1.9 (0.2)a	3.4 (0.1)d	3.1 (0.2)c	3.7 (0.1)e	60.451***
TOTAL TERPENOIDS	30.3 (2.3)b	36.0 (2.5)c	25.1 (0.1)a	29.1 (0.2)b	39.1 (3.5)d	36.0 (2.6)c	35.6 (1.3)c	291.659***

^a Mean values and standard deviations are shown (n = 3). Different letters in the same row 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: *, ** and *** indicates significance at p < 0.05, p < 0.01 p < 0.001.

^b Control wine (C) and wines treated with the different PS extracts. WP: White Pomace; RP: Red Pomace; WM: White Must; RM: Red Must; WW: White Wine; WL: White Lees.

(Nikfardjam et al., 2009), so the higher concentrations of WP and WL wines could be related to interactions between PS and other wine macromolecules avoiding the degradation of volatile phenols.

Terpenes play a significant role in the varietal odorants of wines. Linalool and α -terpineol were detected in the wine samples (Table 1), and linalool showed OAV values higher than 1 (Table 2), contributing to the aroma of the *Viura* wines with floral and citrus notes (Vilanova et al., 2013). As in the rest of volatile families, the addition of WP and WL extracts increased the content of terpenes with respect to control wines, and so did the addition of RM and WW extracts. RP and WM extracts did not produce any effect. Our previous work (Pérez-Magariño et al., 2022) observed that must PS extracts were the ones that increased or maintained high concentrations of terpenes. However, since terpenes are varietal aroma compounds, their content and interactions may be dependent on the white wine variety.

In conclusion, our study demonstrated that PS extracts from white pomace, red must, white wine, and white lees increased the content of most volatile compounds (except for higher alcohols) after one month of bottling, contributing to floral and fruit aromas.

3.3. Effect of PS extracts on the volatile composition of *Viura* wines after twelve months of bottling

This section aims to analyze if the changes in the volatile composition were maintained after 12 months of bottle aging or different results were obtained. Table 3 shows the concentration of individual volatile compounds and volatile families of *Viura* wines treated with different PS extracts after twelve months of bottling (T12). Table 4 shows the OAV values, odor thresholds and descriptors of the volatile compounds with OAV > 0.2.

Table 4

Odor activity values (OAV > 0.2)^a in *Viura* wines treated with the different PS treatment after twelve months of bottling (T12).

Compounds	Odor descriptor	Odor threshold ($\mu\text{g/L}$)	Reference	C ^b	WP ^b	RP ^b	WM ^b	RM ^b	WW ^b	WL ^b	F-value
Isobutanol	Fusel	40,000	Gutiérrez-Gamboa et al., 2018	0.6b	0.4 a	0.4 a	0.6b	0.6b	0.6b	0.4 a	28.000**
2-Methyl-1-butanol	Alcohol	30,000	Gutiérrez-Gamboa et al., 2018	0.9c	0.7 a	0.7 a	0.8b	0.8b	0.8b	0.6 a	12.133**
3-Methyl-1-butanol	Alcohol, Banana	7,000	Etiévant, 1991	5.3 e	4.0 ab	4.2b	4.7 e	4.6 d	4.5 cd	3.8 a	19.738**
2-phenylethyl alcohol	Roses, honey	14,000	Ferreira et al., 2000	1.7 a	1.6 a	2.0b	1.9b	1.7 a	1.7 a	1.7 a	25.867**
E-3-Hexen-1-ol	Green, floral	400	Guth, 1997	0.2 a	0.2 a	0.5c	0.5c	0.2 a	0.2 a	0.3b	33.778**
Z-3-Hexen-1-ol	Green, cut grass	400	Ferreira et al., 2000	1.2	1.2	1.2	1.2	1.2	1.2	1.3	1.889(ns)
Ethyl butyrate	Papaya, apple, sweet	20	Vilanova et al., 2009	10.7b	13.7	14.2f	12.6c	12.6c	8.9 a	14.0 e	665.611**
Ethyl hexanoate	Apple, fruity, sweet	14	Vilanova et al., 2009	25.2	27.3c	31.1	31.5	26.4b	24.8	28.2	345.762**
Ethyl octanoate	Apple, fruity, sweet	5	Vilanova et al., 2009	53.3b	56.9c	59.8	66.8f	53.2b	50.8	57.8	375.265**
ethyl 2-methylbutyrate	Fruity, strawberry, apple, blackberry	2	De la Fuente-Blanco, 2020	3.6c	4.6 e	3.9 d	3.3b	3.5c	2.9 a	4.6 e	197.000**
Ethyl isovalerate	Fruity	0.7	De la Fuente-Blanco, 2020	25.3	29.6	26.6	24.4	27.9	24.4	28.3	1.853(ns)
Isoamyl acetate	Banana, apple	30	Ferreira et al., 2000	32.2b	35.1b	44.1	38.7	32.0b	12.9	36.2c	175.260 **
β -Phenethyl acetate	Banana	250	Ferreira et al., 2000	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1.021(ns)
Isovaleric acid	Cheese	33	Ferreira et al., 2000	10.9b	11.0b	12.7c	12.8c	9.0 a	10.7b	11.5b	177.785**
Hexanoic acid	Cheese, fatty	3,000	Wang et al., 2017	1.5b	1.5b	1.5b	1.5b	1.5b	1.4 a	1.5b	31.098**
Octanoic acid	Cheese, fatty, rancid	1,000	Wang et al., 2017	3.7c	4.6 e	3.4 a	3.6b	4.0 d	3.7c	5.2f	4.000*
4-vinylguaiaicol	Clove, curry	40	Ferreira et al., 2000	3.5	4.9c	5.9 d	4.9c	2.9 a	3.0 a	4.3 bc	6.804**
4-vinylphenol	Smoky, almond	180	Ferreira et al., 2000	0.3b	0.3b	0.3b	0.3b	0.2 a	0.3b	0.3b	14.077**
Linalool	Floral, citrus	25	Vilanova et al., 2013	1.1b	1.3c	0.9 a	1.1b	1.4c	1.3c	1.3c	245.373**

^a Mean values are shown (n = 3). Different letters in the same row 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: * and ** indicates significance at $p < 0.05$ and $p < 0.001$.

^b Control wine (C) and wines treated with the different PS extracts. WP: White Pomace; RP: Red Pomace; WM: White Must; RM: Red Must; WW: White Wine; WL: White Lees.

wines maintained higher contents of these volatile compounds than controls; however, the wines treated with RP and WM extracts showed the highest concentrations. As in T1, the addition of WP, RM, WW and WL extracts increased the content of terpenes with respect to control wines, being RM treated wines the ones with the highest concentrations.

Except for higher alcohols, low doses of PS extracts obtained from WP, RM, WW, and WL caused a significant increase on most of volatile compounds both after one and twelve months of bottling, opening a very promising way for their use as potential modulators of white wine aroma compounds. Therefore, these PS extracts could be used to enhance pleasant fruity and floral aromas in *Viura* wines and reduce unpleasant aromas associated with higher alcohols. Future studies are clearly needed to study the effect of the PS dosage, and the effect of these extracts on other wine compounds and wine sensory properties.

3.4. Effect of bottle aging and PS extracts on the volatile composition of *Viura* wines

A multifactor analysis of variance (MANOVA) was carried out to evaluate the contributions of the effect of the PS extract addition, the bottle aging time, and their interactions in the volatile composition of *Viura* wines (Table 5).

The volatile composition of *Viura* wines was significantly affected by both factors: PS extract addition and bottle aging time. Most of the studied variables showed higher values of variance attributable (%) to the aging effect, showing values above 30% in most compounds. The volatile compounds 2-methyl-1-butanol, 2-phenylethanol, E-3-hexenol, benzyl alcohol, ethyl-2-methylbutyrate and octanoic acid showed a

variance attributable over 30% to the PS extract factor.

3.5. Correlation between the PS composition of the extracts and the volatile composition of *Viura* wines

Once it was verified that the use of the different PS extracts as fining agents significantly affected the volatile composition of the *Viura* wines, it was essential to analyze if there was any correlation between the chemical composition of the extracts and the volatile composition of the wines. Therefore, a Spearman correlation test was performed between the content of total volatile families at T1 and T12 and the chemical composition of the PS extracts (Table 6).

The Spearman test revealed more significant correlations between volatile families and extract composition in T1 wines than in T12 wines, indicating that these correlations may be reduced with the bottle aging time.

Significant correlations were found between the content of the different volatile families and the composition of the different polysaccharide families in the PS extracts. Moreover, the purity and the molecular weight distribution of the PS extracts also showed high correlations with the volatile concentrations, indicating that both the PS composition of the extracts and their molecular weight distribution affected the volatile composition of the wines.

At T1, glucans (GL) and non-pectic polysaccharides (NPP) showed a positive contribution to the content of all volatile families except total phenols. Mannoproteins (MP) were also positively correlated with all volatile families except total phenols and higher alcohols. On the contrary, polysaccharides rich in arabinose and galactose (PRAG) showed

Table 5

Multivariate analysis of variance (MANOVA) of volatile compounds of *Viura* wines after one month (T1) and twelve months of bottling (T12). Percentage of variance attributable (%) of the independent effect of Aging time and PS extract, and the interaction of both (Aging × PS extract).

Compounds	Aging time			PS extract			Aging × PS extract			error (%)
	F-ratio	p-value	% Aging	F-ratio	p-value	% PS extract	F-ratio	p-value	% Interaction	
1-propanol	0.6	0.457	1.0%	4.3	0.003	47.5%	0.0	1.000	0.0%	51.4%
Isobutanol	160,735	0.000	96.7%	30.7	0.000	1.1%	60.3	0.000	2.1%	0.2%
2-Methyl-1-butanol	4.3	0.046	3.9%	13.0	0.000	70.4%	0.1	0.999	0.4%	25.3%
3-Methyl-1-butanol	60,041	0.000	95.6%	8.2	0.000	0.8%	33.6	0.000	3.2%	0.4%
2-Phenylethanol	78.2	0.000	19.0%	23.9	0.000	34.8%	27.1	0.000	39.4%	6.8%
TOTAL ALCOHOLS	30,032	0.000	92.6%	12.8	0.000	2.3%	23.2	0.000	4.2%	0.9%
1-Hexanol	920.9	0.000	74.7%	31.9	0.000	15.5%	15.4	0.000	7.5%	2.3%
E-3-Hexenol	715.8	0.000	38.1%	109.0	0.000	34.8%	79.9	0.000	25.5%	1.5%
Z-3-Hexenol	20,851	0.000	90.9%	30.5	0.000	5.8%	12.6	0.000	2.4%	0.9%
Benzyl alcohol	72.2	0.000	4.8%	205.3	0.000	81.3%	30.6	0.000	12.1%	1.8%
TOTAL C6 ALCOHOL	20,151	0.000	80.5%	55.2	0.000	12.4%	27.0	0.000	6.1%	1.0%
Ethyl butyrate	100,780	0.000	91.7%	125.3	0.000	6.4%	33.5	0.000	1.7%	0.2%
Ethyl hexanoate	572.2	0.000	65.8%	24.9	0.000	17.2%	20.0	0.000	13.8%	3.2%
Ethyl octanoate	563.3	0.000	83.4%	7.7	0.000	6.8%	6.4	0.000	5.7%	4.1%
Ethyl decanoate	248.3	0.000	79.3%	4.6	0.002	8.9%	1.5	0.215	2.9%	8.9%
Ethyl-2-methylbutyrate	40,658	0.000	58.0%	535.7	0.000	40.0%	22.0	0.000	1.6%	0.3%
Ethyl isovalerate	82.7	0.000	57.7%	5.2	0.001	21.6%	0.3	0.941	1.2%	19.5%
Ethyl lactate	50,454	0.000	98.0%	7.9	0.000	0.9%	5.6	0.001	0.6%	0.5%
TOTAL ESTERS	40,674	0.000	97.4%	11.0	0.000	1.4%	5.6	0.001	0.7%	0.6%
Propyl acetate	10,914	0.000	86.9%	22.6	0.000	6.2%	20.7	0.000	5.7%	1.3%
Isobutyl acetate	822.0	0.000	86.2%	9.6	0.000	6.0%	7.8	0.000	4.9%	2.9%
Isoamyl acetate	913.8	0.000	69.8%	41.0	0.000	18.8%	20.3	0.000	9.3%	2.1%
Hexyl acetate	10,124	0.000	86.9%	13.6	0.000	6.3%	10.1	0.000	4.7%	2.2%
β-Phenylethyl acetate	10,310	0.000	84.1%	18.8	0.000	7.2%	17.7	0.000	6.8%	1.8%
TOTAL ACETATES	10,295	0.000	79.2%	32.5	0.000	11.9%	19.5	0.000	7.2%	1.7%
Isovaleric acid	100,101	0.000	98.3%	12.9	0.000	0.8%	12.4	0.000	0.7%	0.3%
Hexanoic acid	70,458	0.000	96.1%	29.2	0.000	2.3%	16.4	0.000	1.3%	0.4%
Octanoic acid	189.2	0.000	32.0%	51.7	0.000	52.4%	10.7	0.000	10.9%	4.7%
Decanoic acid	220,612	0.000	46.7%	20,166	0.000	26.8%	20,138	0.000	26.5%	0.1%
TOTAL ACIDS	20,063	0.000	84.8%	47.0	0.000	11.6%	10.2	0.000	2.5%	1.2%
4-vinylguaiaicol	682.3	0.000	82.2%	9.8	0.000	7.1%	10.1	0.000	7.3%	3.4%
4-vinylphenol	673.3	0.000	81.8%	10.2	0.000	7.4%	10.1	0.000	7.3%	3.4%
TOTAL PHENOLS	720.5	0.000	82.4%	10.4	0.000	7.2%	10.5	0.000	7.2%	3.2%
Linalool	9331.2	0.000	76.2%	205.0	0.000	10.0%	276.3	0.000	13.5%	0.2%
α-Terpineol	940,890	0.000	98.0%	127.7	0.000	0.8%	186.4	0.000	1.2%	0.0%
TOTAL TERPENES	170,107	0.000	83.7%	231.9	0.000	6.8%	317.4	0.000	9.3%	0.1%

Values in bold showed statistically significant differences in each compound and factor considered (p -values < 0.05).

Table 6
Spearman correlation data of volatile families of *Viura* wines and chemical composition of the PS extracts after one month (T1) and twelve months of bottling (T12).

Time	Volatile families ^a	Correlation parameters ^b	%PRAG ^c	%RG-II ^c	%HG ^c	%MP ^c	%NPP ^c	%MN ^c	%GL ^c	TP ^c	%High Mw PS ^d	%Medium MwPS ^d	%Low Mw ^d	%Purity ^e	% Protein ^e
T1	Total Higher Alcohols	ρ Spearman	-0.623**	-0.457*	-0.082	0.311	0.516*	-0.162	0.459*	0.298	-0.371	-0.635**	0.531*	0.315	0.024
		<i>p</i> -value	0.003	0.038	0.723	0.169	0.017	0.482	0.037	0.190	0.098	0.002	0.013	0.165	0.918
	Total C6 Alcohols	ρ Spearman	-0.464*	-0.310	0.088	0.423*	0.769**	0.074	0.521*	0.594**	-0.169	-0.548*	0.736**	0.605**	0.113
		<i>p</i> -value	0.034	0.171	0.704	0.046	0.000	0.749	0.015	0.004	0.465	0.001	0.000	0.004	0.627
	Total Ethyl Esters	ρ Spearman	-0.547*	-0.324	0.070	0.427*	0.787**	-0.027	0.546*	0.519*	-0.235	-0.573**	0.762**	0.531*	0.187
		<i>p</i> -value	0.010	0.152	0.762	0.044	0.000	0.907	0.010	0.016	0.305	0.007	0.000	0.013	0.418
	Total Acetates	ρ Spearman	-0.672**	-0.421	-0.039	0.523*	0.668**	-0.185	0.589**	0.378	-0.409	-0.661**	0.691**	0.379*	0.122
		<i>p</i> -value	0.001	0.057	0.868	0.015	0.001	0.421	0.005	0.091	0.065	0.001	0.001	0.041	0.599
	Total Acids	ρ Spearman	-0.518*	-0.309	0.103	0.435*	0.796**	-0.001	0.499*	0.523*	-0.221	-0.571**	0.739**	0.542*	0.145
		<i>p</i> -value	0.016	0.172	0.656	0.049	0.000	0.995	0.021	0.015	0.337	0.007	0.000	0.011	0.530
	Total Phenols	ρ Spearman	0.523*	0.039	0.061	0.205	-0.078	-0.041	0.147	0.256	0.339	0.365	0.165	0.280	0.240
		<i>p</i> -value	0.015	0.866	0.791	0.373	0.737	0.861	0.524	0.263	0.133	0.103	0.476	0.219	0.295
	Total Terpenes	ρ Spearman	-0.595**	-0.296	0.096	0.574**	0.748**	-0.149	0.526*	0.365	-0.331	-0.544*	0.764**	0.387	0.19
		<i>p</i> -value	0.004	0.193	0.679	0.007	0.000	0.520	0.014	0.103	0.143	0.011	0.000	0.083	0.409
T12	Total Higher Alcohols	ρ Spearman	0.166	-0.120	-0.307	-0.574**	-0.251	0.212	-0.564**	-0.345	0.097	0.056	-0.805**	-0.334	-0.596**
		<i>p</i> -value	0.473	0.606	0.175	0.007	0.273	0.355	0.008	0.126	0.676	0.810	0.000	0.139	0.004
	Total C6 Alcohols	ρ Spearman	-0.267	0.014	0.153	0.451*	0.456*	0.241	0.535*	0.516*	-0.08	-0.210	0.739**	0.472*	0.230
		<i>p</i> -value	0.241	0.953	0.508	0.040	0.050	0.293	0.012	0.017	0.73	0.360	0.000	0.031	0.317
	Total Ethyl Esters	ρ Spearman	-0.187	0.066	0.095	0.185*	0.424*	0.260	0.305	0.389*	0.081	-0.072	0.500*	0.355	0.304
		<i>p</i> -value	0.416	0.775	0.681	0.042	0.029	0.256	0.179	0.042	0.728	0.758	0.021	0.114	0.181
	Total Acetates	ρ Spearman	-0.299	0.090	0.203	0.000	0.326	0.441*	0.253	0.443*	-0.033	-0.276	0.500*	0.416	0.036
		<i>p</i> -value	0.188	0.698	0.378	0.999	0.149	0.045	0.269	0.044	0.886	0.226	0.021	0.061	0.875
	Total Acids	ρ Spearman	-0.250	-0.272	0.029	0.636**	0.217	0.187	0.603**	0.795**	0.021	-0.428*	0.711**	0.768**	0.103
		<i>p</i> -value	0.275	0.233	0.901	0.002	0.345	0.418	0.004	0.000	0.926	0.043	0.000	0.000	0.656
	Total Phenols	ρ Spearman	-0.408	-0.016	0.162	-0.234	0.395	0.315	-0.014	0.182	-0.087	-0.352	0.282	0.211	0.048
		<i>p</i> -value	0.066	0.944	0.482	0.308	0.077	0.164	0.952	0.43	0.709	0.17	0.216	0.358	0.836
	Total Terpenes	ρ Spearman	0.395	0.029	-0.043	0.276	-0.217	-0.139	0.186	0.151	0.159	0.227	0.003	0.124	-0.008
		<i>p</i> -value	0.076	0.901	0.852	0.225	0.345	0.547	0.421	0.514	0.492	0.321	0.991	0.592	0.971

^a Volatile families of *Viura* wines at T1 (n = 21) and T12 (n = 21).

^b Correlation parameters. Level of significance * and ** indicates significance at $p < 0.05$ and $p < 0.01$ respectively.

^c PS composition of the extracts. %PRAG: % of polysaccharides rich in arabinose and galactose; % RG-II: % of rhamnogalacturonans type II; % HG: % of homogalacturonans; % MP: % of mannoproteins; % NPP: % of non-pectic polysaccharides; % MN: % of mannans; % GL: % of glucans; TP: total polysaccharides (mg PS per g of extract).

^d % of High, medium, and low molecular weight (Mw) PS.

^e % purity (mg of TP per 100 mg of extract); % protein (mg BSA per 100 mg of extract).

high negative correlations (ρ Spearman > 0.5) with all volatile families except total phenols, which would indicate that the content of most volatile compounds was reduced by the presence of PRAG. Previous studies (Dufour & Bayonove, 1999; Mitropoulou et al., 2011) have described the uronic acid parts of PRAG structures as responsible of the volatility reduction of aroma compounds. However, the content of total phenols was only correlated with PRAG (ρ Spearman = 0.523), which indicated that the content of these volatile compounds was increased by the presence of PRAG. Mitropoulou et al. (2011) also observed different effects of commercial PS extracts in the volatility of the aroma compounds depending on the specific volatile compound. RG-II molecule had a negative contribution to the content of higher alcohols, indicating a clear effect in the reduction of these compounds, but it did not show any correlation with the rest of volatile families. Homogalacturonans (HG), whose content was low in all extracts (see section 2.2), was not correlated with any volatile family. The highest correlations (ρ Spearman > 0.7 in most volatile families) were obtained for NPP, attributed to grape cell-wall celluloses, hemicelluloses, and xyloglucans. The presence of these polysaccharides would thus increase the concentration of most volatile compounds, probably due to hydrophobic interactions with branched and esterified aroma compounds related to their neutral structure (Dufour & Bayonove, 1999; Mitropoulou et al., 2011).

Both PS purity and total PS content of the extracts also showed significant correlations with wine volatile contents. Both parameters showed a positive contribution to the content of total C6 alcohols, esters, acetates, and acids. On the contrary, the protein content of the extracts did not show any correlation with the volatile composition of the wines, which could be explained by the low protein content of the PS extracts (Canalejo et al., 2022).

Our results revealed that the molecular weight distribution of the PS extracts significantly affected the volatile composition of the *Viura* wines. Low molecular weight (Mw) PS showed high positive correlations (ρ Spearman = 0.53–0.76) with all volatile families except total phenols. On the contrary, medium Mw PS showed high negative correlations (ρ Spearman = 0.54–0.63) with these volatile families, and high Mw PS did show any correlation. These results seemed to indicate that low and medium Mw PS would interact better with aroma compounds, and high Mw PS would not interact with aroma compounds. As described in literature for MP (Chalier et al., 2007; Gambetta et al., 2014), higher Mw distributions could lead to the formation of aggregates with other wine macromolecules, decreasing their access to bind with volatile compounds. Medium Mw PS, which showed negative correlations at T1, could interact with volatile compounds through hydrophobic interactions (Mitropoulou et al., 2011) and form aggregates with less volatility. Further research is needed to study the interactions of low and medium Mw PS with volatile compounds and explain their apparent opposite effect.

After 12 months of aging (T12), fewer correlations were observed between the volatile composition of the wines and the chemical composition of the extracts. MP, NPP and GL showed again positive correlations with some volatile families. MP were positively correlated with total C6 alcohols, esters, and acids; NPP with total C6 alcohols and esters; and GL with total C6 alcohols and acids. The content of total polysaccharides showed a positive contribution to the content of total C6 alcohols, acetates, and esters, just like the PS of low molecular weight.

In conclusion, the correlations between volatile families and PS composition of the extracts were higher after one month of bottling. At T1, the presence of mannoproteins, glucans and non-pectic polysaccharides increased the content of most volatile families, while polysaccharides rich in arabinose and galactose showed a negative contribution. High positive correlations were found between low molecular weight polysaccharides and volatile contents while polysaccharides of medium molecular weight showed negative correlations. To our knowledge, this is the first time to describe all these correlations

in wine samples.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Zenaida Guadalupe reports financial support was provided by Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) and the Ministerio de Ciencia e Innovación through the project RTA2017-00005-C02-02.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank the *Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria* (INIA) and the *Ministerio de Ciencia e Innovación* for the funding provided for this study through the project RTA2017-00005-C02-02.

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