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Ultrasound treatment of crushed grapes: Effect on the must and red wine polysaccharide composition

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ABSTRACT

This paper studied the effect on the molecular weight and polysaccharide composition of musts and wines of the application of high-power ultrasound (US) at 20 and 28 kHz on crushed grapes. Two different pomace maceration times (short and mid) were tested for sonicated and control vinifications. A long pomace maceration time was also tested for non-treated wines. In must samples, US significantly increased the content of monosaccharides and polysaccharides rich in arabinose and galactose (PRAG), and the average molecular weight of smaller PRAG, mannoproteins (MP) or mannans. The 28 kHz had a major effect on most wine monosaccharides and grape polysaccharides. The wine obtained from sonicated grapes at 28 kHz and with mid maceration had higher rhamnogalacturonans type II and PRAG content than its control, and closer polysaccharide and monosaccharide content to long maceration control wines. No significant differences were obtained in the MP content between sonicated and control wines.

1. Introduction

Polysaccharides, the composition of which is closely related to pomace maceration and alcoholic fermentation, largely influence red wine organoleptic characteristics. Wine polysaccharides originate from the cell walls of the tightly packed skin cells and from the pulp tissues as well as from the cell wall of the yeasts acting during winemaking. During crushing, the red grape berry cell walls are physically broken down, and during the maceration-fermentation there is a subsequent degradation of berry tissues through chemical and enzymatic reactions, causing depectination of the cell walls and a release of the phenolic and flavor compounds located inside the skin cells, into the must (Garrido-Bañuelos et al., 2019; Gao et al., 2019; González-Neves et al., 2010). Moreover, the cell wall polysaccharides and proteins are released in significant amounts into the must during the maceration-fermentation (Gao et al., 2015; Guadalupe & Ayestarán, 2007).

Different red winemaking techniques have been developed to facilitate the degradation of the skin's cell walls, since they can increase the wine polysaccharide content to a greater or lesser extent (different pomace contact times and/or addition of enzymes, cryomaceration, flash release systems and accentuated cut edges techniques, etc.; Apolinar-Valiente et al., 2016, 2014; Romero-Cascales et al., 2012; Doco et al., 2007; Kant et al., 2020). Regarding the addition of enzymes, the extent of cell wall degradation depends on the activity and dosage of the enzymes, the enzymes optimal conditions, the maceration duration (Larsen et al., 2021), and the composition and morphology of the skin cell wall material from each cultivar (Apolinar-Valiente et al., 2016). Cryomaceration significantly affected the concentration of polysaccharides in red wines, while cold pre-fermentation maceration and flash release had no effect (Apolinar-Valiente et al., 2014; Doco et al., 2007). The polysaccharide concentration in Shiraz wines was mainly influenced by the maceration time rather than the accentuated cut edges technique (Kant et al., 2020).

The main polysaccharides present in wines are grouped in four major

Abbreviations: 2-*O*MeFuc, 2-*O*-CH_3-fucose; 2-*O*MeXyl, 2-*O*-CH_3-xylose; Api, Apiose; Ara/Gal, Arabinose to Galactose ratio; Ara, Arabinose; Fuc, Fucose; Gal, Galactose; GalA, Galacturonic acid; Glc, Glucose; GluA, Glucuronic acid; HL, Homogalacturonans; Kdo, 2-keto-3-deoxyoctonate ammonium salt; Man, Mannose; MP, Mannoproteins; PRAG, Polysaccharides Rich in Arabinose and Galactose; RG-II, Rhamnogalacturonan type II; Rha, Rhamnose; TMS, Total Monosaccharides; TPF, Total Polysaccharides Families; Xyl, Xylose.

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families: (i) polysaccharides rich in arabinose and galactose (PRAG) (arabinogalactans type I, AG-I, and arabinogalactans type II joined to protein, AGP); (ii) rhamnogalacturonans (rhamnogalacturonans type I, RG-I, and rhamnogalacturonans type II, RG-II); and iii) homogalacturonans (HL) (all of them arising from the pectocellulosic portion of the cell walls of grape berries); and (iv) mannoproteins (MP) released by yeasts during fermentation or aging on yeast lees (Martínez-Lapuente et al., 2019). In wine, polysaccharides of fungal origin, β-glucans and botricin produced by *Botrytis cinerea*, and exogenous polysaccharides, such as acacia gum and carboxymethylcellulose used as additives, may also be present.

From a sensory perspective, polysaccharides affect all aspects of wine mouthfeel, such as astringency, viscosity, and hotness. Moreover, wine polysaccharides have shown to significantly reduce the perception of palate hotness in red wines, especially in those with high pH and alcohol content, explaining differences in hotness in wines with the same or higher alcohol content (Gawel et al., 2018). Therefore, winemaking techniques to increase the concentration of polysaccharides could be used to diminish the negatively perceived ethanol-derived hotness, a very common problem in some viticulture areas associated to high temperatures. In this sense, the application of high-power ultrasound (US) during winemaking could increase the extraction of grape skin compounds, polysaccharides among them. Recently, the International Organization of Vine and Wine has approved the use of this new technology based on sonication to enhance the extraction from grape tissues (OIV, 2019). It is recommended to apply the US just after crushing of the grapes and before the beginning of the maceration-fermentation process (Bautista-Ortín et al., 2017).

US is based on the use of mechanical waves of 16 to 100 kHz (power US) to produce physical and chemical changes in the matrix where they are applied (Ferraretto et al., 2013). These mechanical and chemical changes occur due to the phenomenon of cavitation (Cacciola et al., 2013), and affect the structure and composition of the cell walls. Gonçalves et al. (2015) also observed that ultrasound affected cell wall integrity as the enzyme's pectin methylesterase and polygalacturonase were stimulated, improving the release of skin phenolic and flavor compounds into the must. Recently, it has been shown that sonication of grapes may cause a reduction of more than 50% in the maceration time without losing wine color or stability (Pérez-Porras et al., 2021). Moreover, it can produce an increase in the content of some volatile compounds of sensory relevance, obtaining red wines with an aroma quality similar or higher than those elaborated with longer maceration times (Oliver Simancas et al., 2021). The application of US seems to increase total colloids, such as proteins and polysaccharides, and reduce the colloid's particle diameter (Cacciola et al., 2013). However, there are no studies analyzing the effect of the application of US after grape crushing on the composition and molecular weight of wine polysaccharide families.

Considering that the role of the different wine polysaccharides depends not only on their quantity but also on their structure, composition and molecular size, this paper studies the changes occurring in the molecular weight and composition of polysaccharides in Monastrell musts and wines when grapes are treated with high-power US at two frequencies (20 kHz and 28 kHz) before the maceration process. The effect of US is compared with controls with no US-treatment. Different pomace maceration times were also tested for the control and sonicated wines.

2. Materials and methods

2.1. Vinification and sample collection

Red grapes from *Vitis vinifera var*. Monastrell (VIVC: 7915) were grown in Jumilla (Murcia, Spain), and were harvested on the vintage 2019 at commercial maturity when they reached 26.3 °Brix (hand refractometer ATAGO, Tokyo, Japan). Grapes were taken to the small-scale winery in plastic boxes of 20 kg that were stored refrigerated (3 °C).

Grapes (1400 kg) were destemmed and crushed (Nouva Zambelli, Saonara Padova, Italy), sulfited (70 mg SO_2 kg^{-1}) and divided into three batches. Two batches were treated with a pilot-scale power ultrasound system (MiniPerseo; Agrovin S.A., Alcazar de San Juan, Spain) using two different frequencies, 20 kHz (S20) and 28 kHz (S28); and one batch was not treated to be used as control. The US system was applied to the whole batch (400 kg grapes) per hour and operated at 2500 W with a power density of 8 W cm^{-2}. The crushed-destemmed and sonicated grapes were distributed in 21 50-kg stainless steel tanks maintaining the same pomace (solid)/liquid ratio, and were named as control must (C-M), 20 kHz-treated must (S20-M), and 28 kHz-treated must (S28-M). Seven microvinifications with different pomace maceration times were performed as follows: (1) control winemaking (not US-treated) with a long fermentation-maceration time of 7 days (CW-7d); (2) control winemaking (not US-treated) with a short fermentation-maceration time of 2 days (CW-2d); (3) control winemaking (not US-treated) with a mid-fermentation-maceration time of 3 days (CW-3d); (4) S20-treated vinification (grapes treated at 20 kHz) with 2 days maceration time (S20W-2d); (5) S20-treated vinification (gapes treated at 20 kHz) with 3 days maceration time (S20W-3d); (6) S28-treated vinification (grapes treated at 28 kHz) with 2 days maceration time (S28W-2d); (7) S28-treated vinification (grapes treated at 28 kHz) with 3 days maceration time (S28W-3d). Each vinification was performed in triplicate.

Total acidity was corrected to 5.5 g L^{-1} of tartaric acid, and enological nutrients Actimax Natura and Actimax Plus (0.3 g L^{-1}; Agrovin, Alcazar de San Juan, España) and commercial *Saccharomyces cerevisiae* yeasts were added to all vinifications (0.20 g kg^{-1}, Viniferm CT007, Agrovin, Alcazar de San Juan, Spain). The initial fermentation-maceration temperature was 23 °C and the maximum fermentation-maceration temperature was 27 °C. The cap was punched down twice a day during maceration, and then the wines were pressed in a 75L pneumatic press. Free-run and pressed wines were combined and stored at room temperature until the end of alcoholic fermentation. When alcoholic fermentation finished (reducing sugars content lower than 2 g L^{-1}), free sulfur was corrected to 70 mg L^{-1}. Thereafter, the wines were cold-stabilized at 2 °C for one month, racked and bottled.

Samples were taken at different stages to control the winemaking process; most of the analyses were done at the beginning of maceration and wine samples were taken at the time of bottling. Standard enological parameters of musts and wines are shown in Table 1.

2.2. Standard enological parameters

The musts and wines were characterized by measuring the alcohol content, pH, total and volatile acidity according to Commission Regulation EEC (1990). Color intensity (CI) was determined as the sum of absorbances at 620, 520 and 420 nm (Glories, 1984). Total phenol index (TPI) was calculated by measuring wine absorbance at 280 nm, according to Ribéreau-Gayon et al. (1983).

2.3. Precipitation of total soluble wine polysaccharides

Must and wine polysaccharides were recovered by precipitation after ethanolic dehydration as previously described (Guadalupe et al., 2012; Ayestarán et al., 2004). The polysaccharide extraction was performed in triplicate in each sample.

2.4. Identification and quantification of monosaccharides by GC–MS

GC was performed on an Agilent 7890A gas chromatograph (Agilent Technologies, Waldbronn, Germany) coupled to a 5975C VL quadrupole mass detector (MS), equipped with a 7653B automatic injector, and controlled by ChemStation software. Samples were injected in triplicate. The chromatographic column was a Teknokroma fused silica capillary

Table 1
Standard enological parameters[a] of must and wine samples.

Parameter[b]	Musts[c]			Wines[c]						
	C-M	S20-M	S28-M	CW-2d	S20W-2d	S28W-2d	CW-3d	S20W-3d	S28W-3d	CW-7d
Brix	26.3 ± 0.00	26.3 ± 0.00	26.3 ± 0.00							
Alcohol				15.72 ± 0.23bA	16.07 ± 0.15b B	15.67 ± 0.01b A	15.52 ± 0.59 ab α	16.23 ± 0.07b α	15.70 ± 0.03b α	14.92 ± 0.20 a
pH	3.63 ± 0.00 a	3.74 ± 0.00b	3.75 ± 0.00c	3.58 ± 0.10	3.56 ± 0.01	3.59 ± 0.01	3.63 ± 0.07	3.59 ± 0.03	3.59 ± 0.04	3.57 ± 0.05
TA	3.39 ± 0.00c	3.32 ± 0.00 a	3.35 ± 0.00b	5.17 ± 0.15 ab	5.37 ± 0.06 ab	5.25 ± 0.05 ab	5.07 ± 0.15 a	5.40 ± 0.00 ab	5.43 ± 0.31 ab	5.57 ± 0.12b
VA				0.65 ± 0.06 ab A	0.75 ± 0.04b B	0.66 ± 0.02 ab A	0.60 ± 0.06 a α	0.75 ± 0.01b β	0.64 ± 0.04 ab α	0.60 ± 0.06 a
TPI	18.29 ± 0.36 a	28.59 ± 0.33b	32.07 ± 0.32c	32.93 ± 0.96 a A	45.46 ± 1.31b C	42.60 ± 0.86b B	44.01 ± 1.50b α	54.04 ± 3.19c β	54.63 ± 0.71c β	63.83 ± 2.63 d
CI	2.92 ± 0.00 a	5.67 ± 0.00b	7.06 ± 0.00c	7.00 ± 0.81 a A	10.48 ± 0.39b B	10.54 ± 0.33b B	9.21 ± 0.87 ab α	14.66 ± 1.48c β	14.80 ± 0.27 cd β	17.73 ± 1.48 d
Tonality	1.21 ± 0.00c	1.01 ± 0.01b	0.84 ± 0.00 a	0.55 ± 0.04	0.55 ± 0.00	0.53 ± 0.01	0.54 ± 0.04	0.53 ± 0.06	0.49 ± 0.03	0.50 ± 0.02

column (30 m × 0.25 mm × 0.25 mm) of phase 5% phenyl/95% methyl polysiloxane. The oven program started at an initial temperature of 120 °C which was increased at a rate of 1 °C min^{-1} to 145 °C and then to 180 °C at a rate of 0.9 °C min^{-1} and finally to 230 °C at 40 °C min^{-1}. The GC injectors were equipped with a 3.4 mm I.D. liner and were maintained at 250 °C with a 1:20 split ratio. The carrier gas was helium (99.996%) at a flow rate of 1 mL min^{-1}. Ionization was performed by electron impact (EI) mode at 70 eV. The temperatures used were 150 °C for the MS Quad, 230 °C for the MS Source, and 250 °C for the transfer line. The monosaccharide composition was determined by GC–MS of their trimethylsilyl-ester *O*-methyl glycosyl residues obtained after acidic methanolysis and derivatization as previously described (Guadalupe et al., 2012). The total monosaccharides components of the precipitated polysaccharides were called TMS. The content of each polysaccharide family in the must and wine samples was estimated from the concentration of individual glycosyl residues which are characteristic of structurally identified must and wine polysaccharides (Ayestarán et al., 2004; Doco et al., 1999). The content of total polysaccharides families (TPF) was estimated from the sum of PRAG, MP, RG-II and HL.

2.5. Analysis of polysaccharides by HRSEC-RID

High-resolution size-exclusion chromatography (HRSEC) was performed using a modular 1100 Agilent liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with one G1311A quaternary pump, an on-line G1379A degasser, a G1316A column oven, a G1362 refractive index detector, a G1313A automatic injector, and controlled by the Chemstation Agilent software. Two serial Shodex OHpack KB-803 and KB-805 columns (30 × 0.8 cm; Showa Denko, Tokyo, Japan) were used. The precipitated polysaccharides were dissolved in 2.5 mL of 0.1 M $LiNO_3$, filtered through a membrane with a 0.45 mm pore size, and 100 mL were injected and eluted with a 0.1 M solution of $LiNO_3$ at a flow rate of 1 mL min^{-1}. The molecular weight distribution of the must and wine polysaccharides was determined as previously described (Guadalupe et al., 2012). Calibration was performed with narrow pullulan molecular weight standards (Shodex P-82; Waters, Barcelona, Spain): P-5, MW = 5.9 kDa; P-10, MW = 11.8 kDa; P-20, MW = 22.8 kDa; P-50, MW = 47.3 kDa; P-100, MW = 112 kDa; P-200, MW = 212 kDa. The apparent molecular weights were deduced from the calibration equation log MW = 10.835–0.3955t_R (t_R = column retention time at peak maximum, and r^2 = 0.994).

2.6. Statistical analyses

Analyses of variance and principal component analyses were performed using the SPSS v. 15.0 for Windows statistical package (SPSS Statistics, Inc., Chicago, IL) and the XLstat-Pro (Addinsoft) program.

3. Results and discussion

3.1. Glycosyl residue composition of musts and wines polysaccharides

The glycosyl residue profiles of the must and red wines (Table 2) are used to provide a rough overview of the types of polysaccharides from the grape cell walls and yeast. Glucose was the most prevalent glycosyl residue detected in must samples and there were no significant differences among samples (Table 2). Glucose content was 17.5 and 11.0 times higher than the total glycosyl residues (TMS without glucose) in the control and sonicated must, respectively. According to bibliography, glucose is the prevalent sugar in both the skin and pulp cell walls of grape berries (Vidal et al., 2001; Guadalupe & Ayestarán, 2007), because it is the main component of major structural polysaccharides from the grape cell walls such as cellulose and hemicellulosic xyloglucans, arabinoglucans and mannans. The content of glucose in the Monastrell musts was significantly higher than those obtained for Tempranillo grapes (Guadalupe & Ayestarán, 2007), indicating a major solubilization of these polysaccharides, alone or in combination with pectic polysaccharides. This difference was explained because Tempranillo grapes were harvested at 21.3 °Brix, while Monastrell grapes were harvested at 26.3 °Brix. The degree of maturity of the berries affects the composition and concentration of soluble polysaccharides (Nunan et al., 1998).

After glucose, the most prevalent glycosyl residues were arabinose, galactose and galacturonic acid (Table 2), which are components of must pectic polysaccharides (PRAG, as galacturonans, galactans, arabinogalactans, arabinogalactan proteins and arabinans; and homogalacturonans, HL) (Vidal et al., 2000). Must samples showed a high content of glucose and galactose, in accordance with a study on the composition of skin and pulp cell walls from Monastrell grapes (Ortega-Regules et al., 2008). Rhamnose, mannose, xylose, glucuronic acid, and other minor sugar constituents of must as 2-*O*-CH_3-fucose, 2-*O*-CH_3-xylose, apiose and fucose could also be detected in musts (Table 2). Rhamnosyl residue could arise from pectic polysaccharides, such as RG-II or rhamnogalacturonan type I (RG-I). The characteristic sugars of RG-II were 2-*O*-CH_3-fucose, 2-*O*-CH_3-xylose and apiose (Vidal et al., 2000).

The presence of xylose in musts (Table 2) confirmed the presence of hemicellulosic xyloglucans and arabinoxylans. The mannose residue in must was attributed to MP of endogenous yeast cell walls (Guadalupe & Ayestarán, 2007) or from mannans or xyloglucans (Arnous & Meyer, 2009; Minjares-Fuentes et al., 2016; Doco et al., 2003).

Sonicated and control musts showed important differences in the total glycosyl residues (without glucose) (Table 2), this content being

Table 2
Glycosyl composition (mg L^{-1}) and arabinose/galactose ratio of polysaccharides of must and.

Parameter[a, b]	Musts[c]			Wines[c]						
	C-M	S20-M	S28-M	CW-2d	S20W-2d	S28W-2d	CW-3d	S20W-3d	S28W-3d	CW-7d
2-*O*MeFuc	0.89 ± 0.18 a	1.23 ± 0.13b	1.14 ± 0.13 ab	4.48 ± 0.54 a	4.17 ± 1.16 a	5.36 ± 0.07 a	6.83 ± 0.69b α	7.54 ± 0.88b αβ	9.34 ± 1.10c β	16.81 ± 0.89 d
2-*O*MeXyl	0.61 ± 0.02 a	0.90 ± 0.01b	0.79 ± 0.09b	1.80 ± 0.36 a A	3.16 ± 0.36 ab B	2.77 ± 0.10 ab B	2.75 ± 1.09 ab	3.54 ± 0.89b	4.26 ± 0.67b	9.14 ± 1.35c
Api	0.64 ± 0.36b	0.28 ± 0.10 ab	0.10 ± 0.01 a	0.33 ± 0.07 a A	0.69 ± 0.22 ab B	2.00 ± 0.02c C	1.02 ± 0.39b α	2.14 ± 0.60c β	0.78 ± 0.09 ab α	4.07 ± 0.45 d
Ara	28.04 ± 4.27 a	59.93 ± 5.65b	59.65 ± 6.57b	77.02 ± 9.82 a A	85.73 ± 8.31b A	114.64 ± 21.47c B	67.43 ± 16.65 a α	79.35 ± 11.70b α	96.94 ± 15.98c β	148.31 ± 2.96 d
Rha	9.48 ± 0.82 a	19.23 ± 1.79b	17.69 ± 1.77b	15.13 ± 1.84 a A	27.16 ± 0.99 a B	36.08 ± 3.58 a C	23.05 ± 7.44 a	29.02 ± 4.29 a	35.59 ± 6.64 a	69.11 ± 33.07b
Fuc	0.82 ± 0.07 a	1.43 ± 0.21b	1.44 ± 0.10b	1.35 ± 0.42 a A	2.08 ± 0.40b B	2.25 ± 0.21b B	2.07 ± 0.40b	2.33 ± 0.29b	2.44 ± 0.17b	5.00 ± 0.38c
Xyl	3.88 ± 1.26 a	7.24 ± 1.21b	7.16 ± 0.38b	8.20 ± 0.29 bc C	3.28 ± 0.27 a A	5.34 ± 0.66 ab B	7.57 ± 3.37 bc	11.49 ± 1.57c	9.25 ± 3.01 bc	15.55 ± 3.21 d
Man	9.09 ± 0.53 a	16.61 ± 2.72b	17.06 ± 0.93b	213.10 ± 8.75 ab	203.26 ± 16.12 a	189.31 ± 23.46 a	165.27 ± 67.32 a	189.14 ± 24.68 a	218.89 ± 32.17 ab	268.50 ± 20.51b
Gal	91.12 ± 13.60 a	177.28 ± 18.93b	159.48 ± 15.71b	439.63 ± 24.91b	441.06 ± 39.00b	438.96 ± 48.10b	305.19 ± 122.84 a α	430.15 ± 47.78b αβ	490.05 ± 35.11b β	651.55 ± 27.36c
GalA	38.51 ± 10.04 a	57.07 ± 3.11b	60.84 ± 8.50b	57.62 ± 4.31b B	56.72 ± 3.55b B	16.53 ± 2.03 a A	51.20 ± 17.44b	79.02 ± 6.05c	78.28 ± 14.94c	142.50 ± 13.43 d
GluA	4.61 ± 0.39 a	7.88 ± 1.54b	8.16 ± 1.05b	7.01 ± 1.84 a A	13.03 ± 1.25 bc B	20.45 ± 2.88 d C	10.07 ± 3.75 ab	15.99 ± 2.09c	15.07 ± 3.42c	24.08 ± 0.55 d
Kdo	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.88 ± 0.14 a A	0.82 ± 0.18 a A	1.48 ± 0.12b B	1.38 ± 0.16 ab	0.98 ± 0.65 ab	1.22 ± 0.40 ab	3.53 ± 0.11c
Glc	3289.61 ± 1488.35	4134.92 ± 820.34	3715.03 ± 304.28	26.36 ± 4.47 a A	24.42 ± 1.83 a A	72.92 ± 9.09c B	19.45 ± 6.29 a α	48.07 ± 12.78b β	28.66 ± 4.64 a α	29.70 ± 3.43 a
TMS without Glc	187.71 ± 27.10 a	349.07 ± 35.17b	333.52 ± 35.20b	826.53 ± 42.68b	841.14 ± 57.10b	835.18 ± 42.35b	643.83 ± 163.02 a α	850.68 ± 92.71b αβ	962.11 ± 77.19b β	1358.17 ± 82.15c
TMS	3477.32 ± 1461.26	4483.99 ± 855.50	4048.55 ± 339.48	852.90 ± 38.69b	865.57 ± 56.16b	908.10 ± 33.27b	663.28 ± 168.88 a α	898.74 ± 79.94b β	990.77 ± 81.82b β	1387.87 ± 83.21c
Ara/Gal	0.37 ± 0.00 a	0.41 ± 0.01b	0.45 ± 0.01c	0.21 ± 0.02 a	0.23 ± 0.01 a	0.32 ± 0.09b	0.28 ± 0.04 ab	0.22 ± 0.02 a	0.24 ± 0.04 ab	0.27 ± 0.01 ab

almost double in the sonicated must.

US treatment significantly increased the content of sugars forming pectic polysaccharides (PRAG and rhamnogalacturonans) such as 2-*O*-CH_3-xylose, arabinose, rhamnose, galactose, fucose, and galacturonic acid. An increase in xylose and mannose was also observed. The significant increase of most of the glycosylated residues in sonicated musts proved the disruption of the grape berry cell wall polysaccharides caused by the sonomechanical effect of ultrasound at two frequencies. Ultrasound treatment weakened the crosslinking between pectic and hemicellulosic domains in plant primary cell walls (Vidal et al., 2003), facilitating the extraction of grape cell contents, such as anthocyanins and tannins, which was also confirmed by the increase of CI and TPI in sonicated must, and lower tonality values (Table 1). The arabinose/galactose ratio is characteristic of the PRAG, and is affected by the release of polysaccharides rich in arabinose-like arabinans (Belleville et al., 1993). A significant increase in the arabinose/galactose ratio was observed in sonicated must compared to control (Table 2), suggesting a greater release of arabinose or polysaccharides rich in arabinose.

Regarding wines, important differences were also observed in the content of their glycosyl residues (Table 2). The total content of glycosylated (TMS) residues in wines was 4 times lower than in their respective musts; except for CW-7d, which was 2.5 times lower. This fact was due to a significant decrease of glucose, suggesting that the solubilization of structural polysaccharides from grape cell walls was limited due to the enzymatic activity and/or ethanol content formed during the alcoholic fermentation.

When comparing the sonicated wines with their respective musts, the total content of glycosylated residues (without glucose) was 2.4 times in wines from grapes sonicated at 20 kHz, and 2.7 times in wines from grapes sonicated at 28 kHz. In the control wine made with long maceration (CW-7d), the increase was greater (7.2 times), in the CW-2d was 4.4 times, and in the CW-3d it was lower (3.4 times) compared to the C-M.

The composition profile of the glycosyl residues of wines changed with respect to musts. Galactose was the main sugar in wines, in agreement with previous studies (Apolinar-Valiente et al., 2013), followed by arabinose, mannose, and galacturonic, and distantly, rhamnose and glucuronic acid. In general, the content of 2-*O*-CH_3-fucose, 2-*O*-CH_3-xylose, apiose, fucose, xylose and 3-deoxy-*manno*-2-octulosonic acid was lower than 1% in all the wines.

Except for mannose and glucose, wines obtained with long maceration time (CW-7d) had a significant higher content of glycosyl residues from pectic and hemicellulosic polysaccharides than wines with mid and short maceration time (Table 2). This fact proved that extraction of polysaccharides from the grapes increased with the maceration time.

The total content of glycosyl residues in wines made with short maceration time did not show significant differences. However, wines from sonicated grapes obtained with mid maceration had higher total content of glycosyl residues than its control. Control wine obtained with mid maceration time (CW-3d) showed significantly lower content of glycosyl residues than the rest of the wines.

Mid pomace maceration of sonicated grapes produced an increase of galactose of 1.6 times in S28W-3d and 1.4 times in S20W-3d compared to the control wine (CW-3d). No differences were observed in the content of galactose among S20W-2d, S28W-2d and CW-2d samples. Compared to CW-7d, galactose content decreased 25% and 34%, respectively, in S28W-3d and S20W-3d wines. These results showed that sonicating crushed grapes at 28 kHz would be the most effective treatment in the disruption of galacturonans and galactans from grapes, because the wine from sonicated grapes at 28 kHz and 3 days maceration showed the closest results to the wine with long maceration (CW-7d).

Arabinose, the main component of arabinans, was the second pectic glycosyl residue, found in most wines (Table 2). After CW-7d wine, the highest arabinose values were reached in S28W-2d and S28W-3d wines, without significant differences between them. When comparing wines made with the same sonication frequencies, it was observed that the

arabinose content of S28W-2d and S28W-3d wines showed significant differences compared to the controls CW-2d and CW-3d. The results suggested that the greatest disruption of arabinans in wines was already achieved with a short pomace maceration (2 days) at the highest frequency (28 kHz), while at the same frequency, the disruption of galacturonans and galactans was achieved with a longer maceration time (3 days). This fact indicates that polysaccharides rich in arabinose were less resistant to endogenous pectolytic enzymes. Arabinose decreased 23 and 35%, respectively, in S28W-2d and S28W-3d wines compared to CW-7d. The arabinose content of the wine with grapes treated at 28 kHz and two days of maceration was the most similar to that achieved in wines with seven days of maceration.

No significant differences were observed in the arabinose/galactose ratio among wines (except for S28W-2d), but the values were lower than those found in musts (Table 2). Passing from must to wine produced a decrease in the arabinose/galactose ratio, suggesting a greater release of galactose or polysaccharides rich in galactose.

The galacturonic acid glycosyl residues are the main components of homogalacturonans (HL). S20W-3d and S28W-3d wines showed significantly greater galacturonic acid content than wines with two days of maceration. The content of galacturonic acid was similar in S20W-3d and S28W-3d, but higher than observed in the control (CW-3d). Sonication to crushed grapes with three days of maceration promoted the extraction of galacturonic acid, reaching in these wines, half of the galacturonic acid content than in wines with seven days of maceration.

In general, the content of rare monosaccharides markers of RG-II molecule (2-*O*-CH_3-xylose, 2-*O*-CH_3-fucose, apiose and 3-deoxy-*manno*-2-octulosonic acid) was significantly higher in the S28W-3d wine. This fact suggested that RG-II was more tightly bound to the cell wall matrix of grape cell walls, needing longer maceration times (Guadalupe & Ayestarán, 2007) and higher sonication frequencies for extraction.

The time of skin maceration did not affect to the rhamnose content when sonication at 28 kHz was used. In fact, S28W-2d and S28W-3d wines did not show significant differences in the rhamnose content. However, S28W-2d showed Rha values significantly higher than S20W-2d and CW-2d. Xylose content showed the highest value in the S28W-2d, but it was not significant.

Mannose was one of the prevalent sugars detected in wines. Mannose content in CW-7d wine did not show significant differences compared to S28W-3d and CW-2d wines. S28W-3d and CW-2d wines did not present significantly higher contents of mannose than the other wines studied. A previous study demonstrated that MP concentration in wines increased in the last stages of maceration-fermentation (Guadalupe & Ayestarán, 2007). Considering that the sonication was applied prior to yeast inoculation, and that the dose and the yeast strain was the same in all the trials, the differences observed in the mannose content of the wines were attributed to slight variations in the metabolic phase of the alcoholic fermentation process.

S28W-2d wine had significantly higher concentration of glucose than the other wines. Glucose in wines can arise from cell wall of yeasts (glucans), anthocyanins (Doco et al., 2007) or structural polysaccharides from grape cell walls.

3.2. Polysaccharides families of must and wine

The concentrations of rhamnogalacturonans type II (RG-II), mannoproteins (MP) or mannans, polysaccharides rich in arabinose and galactose (PRAG), and homogalacturonans (HL) in musts are shown in Fig. 1A.

RG-II represented about 29% of total soluble polysaccharides in the control must (C-M) and 23% in the musts obtained after sonication (S20-M and S28-M). However, MP or mannans represented only a small percent (4% in the C-M and S20-M and 5% in the S28-M). PRAG was the most prevalent polysaccharide family in all must samples, indicating that it was easily released into the must by endogenous enzymes, as it is localized in soluble form within grape cell walls (Vidal et al., 2001). In fact, PRAG represented about 55% in the control must, and 62 and 61% in musts obtained after sonication at 20 and 28 kHz, respectively. The proportion of HL ranged from 10% in S20-M to 12% in C-M and S28-M samples.

RG-II concentration showed significant higher values in sonicated musts than in controls (~40%). The content of MP increased ~ 83% in sonicated musts (S20-M and S28-M) compared to their respective control, confirming the disruptive effect of ultrasound on endogenous yeast cellular structure, or on mannans (Nunan et al., 1998; Arnous & Meyer, 2009), or on xyloglucans (Doco et al., 2003) of the grape cell walls. In the grape pericarp, mannans is basically composed of chains of mannose, linear chains made up of β-1,4-linked mannose units. Mannose is not a constituent of any other plant cell wall polysaccharide as it is assumed that mannose is not a side-chain substituent of rhamnogalacturonan I (RG-I) (Nunan, et al., 1998; Arnous & Meyer, 2009). The PRAG content was significantly lower in the control must compared to crushed grapes sonicated at 20 kHz (increased by ~ 100%) and 28 kHz (increased by ~ 84%). Finally, no significant differences were observed in the content of HL between control and treated musts.

Fig. 1B shows the concentrations of rhamnogalacturonans type II (RG-II), mannoproteins (MP), polysaccharides rich in arabinose and galactose (PRAG), and homogalacturonans (HL) in the wines.

In general, the content of RG-II and PRAG was higher than that previously reported in Monastrell wines (Apolinar-Valiente et al., 2013, 2014). Differences in PRAG and RG-II concentration could be attributed to different ripening states at harvest (Doco et al., 2007) and to the different geographical origin of the grapes (Apolinar-Valiente et al., 2013). The contents of MP and HL in wines were quite similar to those previously reported in Tempranillo red wines (Guadalupe & Ayestarán, 2007). CW-7d wine showed the highest content of RG-II and PRAG, which may be due to a higher extraction of polysaccharides from grapes during the longer pomace maceration time. Control wines obtained from short and mid pomace maceration showed much lower amounts of grape polysaccharides families than wine from long pomace maceration, proving that extraction of polysaccharides, especially RG-II, requires skin contact during alcoholic fermentation (Doco et al., 2007). The maceration time increased the extraction of PRAG, RG-II and the chromatic characteristics of wines, such as CI and TPI (Table 1). When the same skin-maceration time was used, sonicated wines showed significantly higher TPI and CI values than their control (Table 1). Moreover, the TPI and CI values of sonicated wines with short maceration (S20W-2d and S28W-2d) were similar to the control wine with medium maceration (CW-3d). Therefore, sonication of grapes may allow a reduction of more than 50% in the maceration time without losing wine chromatic characteristics or stability. Pérez-Porras et al. (2021) observed the highest percentage of galloylation in wines with long maceration (7 days) and in wines with short and medium maceration made from grapes sonicated at 20 kHz, indicating that sonication, especially at the lower frequency, may also affect seed tannin extraction, which could ensure a long-term wine aging stability.

With regards to the MP content, since the yeast strain was the same in all the wines, no significant differences were observed, indicating that sonication did not affect the MP content. Therefore, the possible disruptive effect on endogenous grape yeast cell walls in sonicated must was not observed in the following stages.

HL were present in wines in low amounts, suggesting that they could be fragmented by polygalacturonases either from the grapes or the yeast used for the fermentation during winemaking (Vidal et al., 2001). S28W-2d and S28W-3d wines showed significantly higher contents of RG-II than their respective controls. However, no significant effect of sonication at 20 kHz was observed in the content of RG-II. S28W-3d wines showed significantly higher contents of PRAG than their respective control, although S20W-2d and S28W-2d wines did not significantly differ from control wine with short pomace maceration time. Results confirmed that sonication at 28 kHz had a positive effect on the

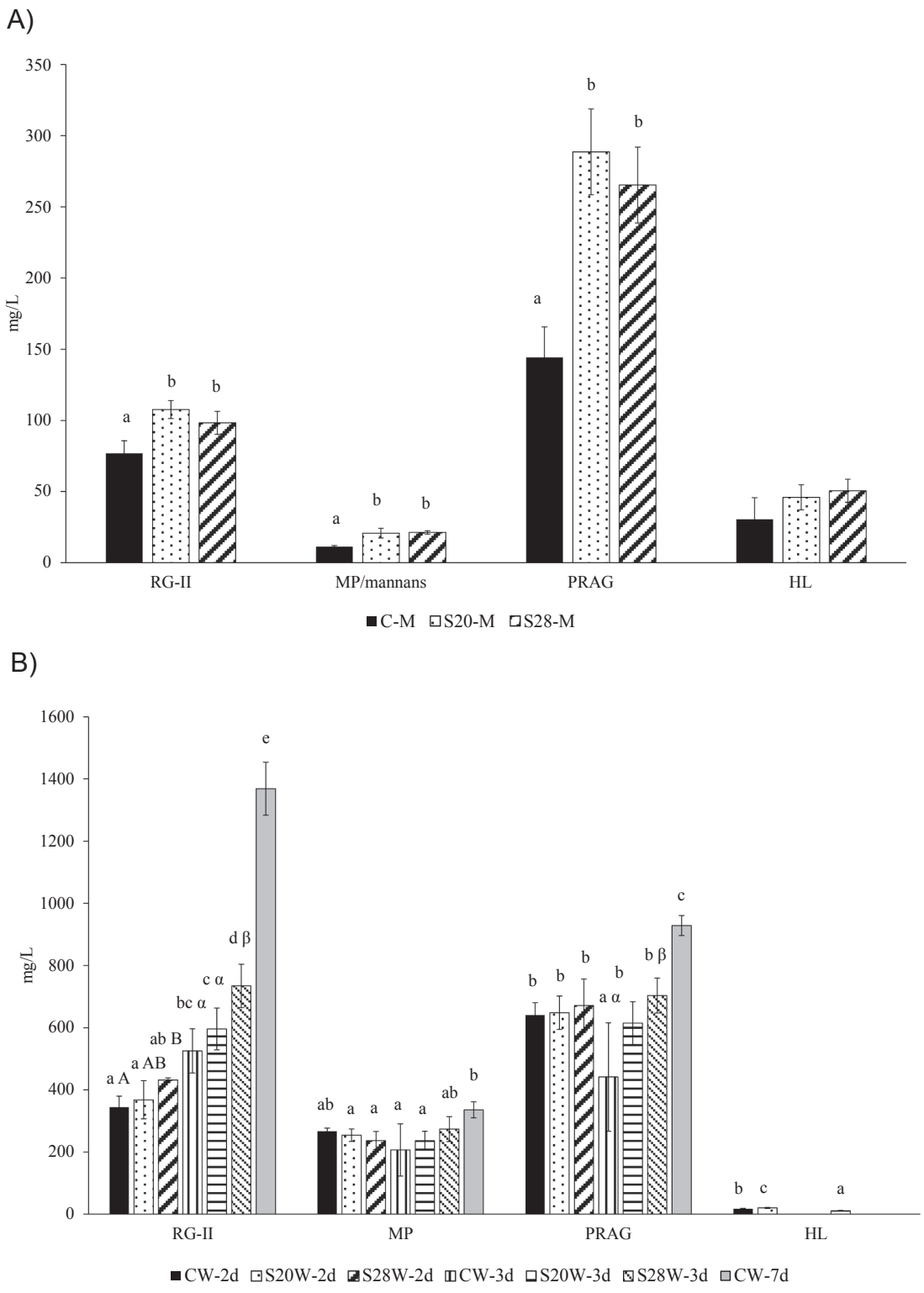

Fig. 1. Concentration of rhamnogalacturonan type II (RG-II), mannoproteins (MP) or mannans, polysaccharides rich in arabinose and galactose (PRAG), and homogalacturonans (HL) in musts (A). Concentration of rhamnogalacturonan type II (RG-II), mannoproteins (MP), polysaccharides rich in arabinose and galactose (PRAG), and homogalacturonans (HL) in wines (B). Average of the three measurements. Different letters indicate statistical differences ($p < 0.05$), using the Duncan post-hoc testing was used. Lower case letters compare separately musts and wines. Upper case letters compare wines with two days maceration time. Greek alphabet letters compare wines with three days maceration time. C-M, control must; S20-M, must with sonicated grapes at 20 kHz; S28-M, must with sonicated grapes at 28 kHz; CW-2d, control wine with two days maceration, S20W-2d, 20 kHz-treated wine with two days maceration; S28W-2d, 28 kHz-treated wine with two days maceration; CW-3d, control wine with three days maceration, S20W-3d, 20 kHz-treated wine with three days maceration; S28W-3d, 28 kHz-treated wine with three days maceration; CW-7d, control wine with seven days maceration.

extraction of polysaccharides families, especially of those more tightly bound to cell walls such as RG-II. In fact, S28W-3d wine showed higher content of RG-II and PRAG than its control, and a similar IC value than CW-7d wine (Table 1).

3.3. Principal factors of variability of the content of wine monosaccharides and polysaccharides families

A multivariate analysis of variance (MANOVA) was conducted in wine samples to analyze the effect of maceration time (short and mid) and sonication frequency (20 or 28 kHz) on wine monosaccharides and polysaccharides families (Table 3).

The sonication frequencies effect was the dominant factor of variation for the majority of monosaccharide and polysaccharide concentration, whereas the maceration time and maceration time × sonication frequencies accounted for a small fraction of the observed variation (Table 3). Except for 2-*O*-CH_3-xylose, xylose, mannose, arabinose to galactose ratio, mannoproteins and galacturonic acid, sonication frequencies had a great effect on the average concentration of monosaccharides and polysaccharides, confirming the higher extraction in US samples. 28 kHz had a greater effect on glycosyl and polysaccharide families than 20 kHz (29% of total compounds from 20 kHz versus 67% of total compounds from 28 kHz).

Regarding maceration time, wines obtained after mid pomace maceration time presented significantly higher content of 2-*O*-CH_3-fucose, 2-*O*-CH_3-xylose, xylose, galacturonic acid and rhamnogalacturonan type II than those with a short pomace maceration time. However, the maceration time decreased the homogalacturonan content. When grapes were sonicated 28 kHz presented significantly higher amounts of 2-*O*-CH_3-fucose, arabinose, rhamnose, fucose, glucose, and rhamnogalacturonan type II.

The effect of the maceration time × sonication frequencies interaction was significant for apiose, xylose, galacturonic acid, glucuronic acid, total monosaccharides (without glucose), glucose, total monosaccharides, and homogalacturonans, and it was the dominant factor in the variation of apiose, galacturonic acid and glucose content. In general, the content of wine monosaccharides and polysaccharides could be explained by the factors studied. However, 8 of 21 compounds showed high weight of the residual factor and were more poorly explained (Table 3).

Multivariate statistics was used to evaluate the similarity among wines. Fig. 2 shows the results of the principal component analysis (PCA) using all the data of the wines, which were distributed in the plane created by principal components 1 and 2.

Principal component (PC1) explained 72.3% of the variance, and PC2 explained 14.4% of the variance. PC1 was strongly correlated with

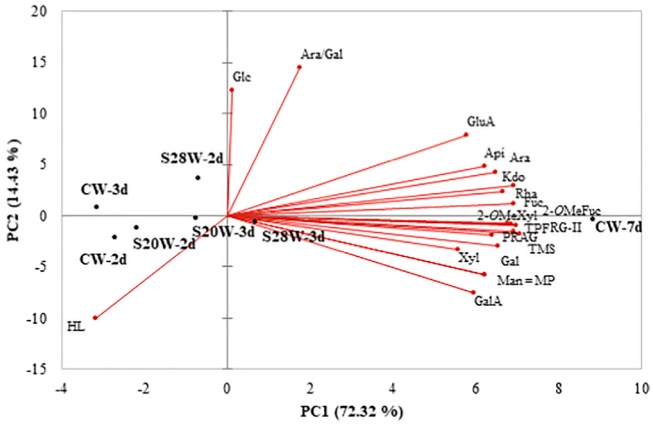

Fig. 2. Principal component analysis (PCA) of the wines performed with monosaccharides and polysaccharides families concentration. *See abbreviations*: CW-2d, control wine with two days maceration, S20W-2d, 20 kHz-treated wine with two days maceration; S28W-2d, 28 kHz-treated wine with two days maceration; CW-3d, control wine with three days maceration, S20W-3d, 20 kHz-treated wine with three days maceration; S28W-3d, 28 kHz-treated wine with three days maceration; CW-7d, control wine with seven days maceration.

Table 3
MANOVA statistical analysis and percentage of variance attributable (%) of the independent effect of maceration time (M) and sonication frequencies (S) and the interaction of both of them (M × S) of wine samples.

Parameter[a, b]	Maceration time			Sonication frequencies				Interactions		
	2 days	3 days	M (%)	C[c]	S20[c]	S28[c]	S (%)	*p*	M × S (%)	Residual (%)
2-*O*MeFuc	4.67 ± 0.84 a	7.91 ± 1.37b	69.59	5.65 ± 1.41 a	5.85 ± 2.06 a	7.35 ± 2.29b	15.24	0.268	2.99	12.18
2-*O*MeXyl	2.58 ± 0.66 a	3.52 ± 1.01b	25.23	2.27 ± 0.89 a	3.35 ± 0.64b	3.52 ± 0.92b	34.66	0.385	5.90	34.21
Api	1.01 ± 0.77	1.31 ± 0.72	4.50	0.67 ± 0.46 a	1.41 ± 0.89b	1.39 ± 0.67b	22.70	0.000	60.70	12.10
Ara	92.47 ± 21.16	81.24 ± 18.24	8.33	72.22 ± 13.31 a	82.54 ± 9.72 a	105.79 ± 19.51b	52.10	0.793	1.50	38.07
Rha	26.12 ± 9.34	29.22 ± 7.68	3.57	19.09 ± 6.51 a	28.09 ± 2.97b	35.84 ± 4.78c	69.49	0.320	4.66	22.28
Fuc	1.89 ± 0.52	2.28 ± 0.31	18.80	1.71 ± 0.53 a	2.21 ± 0.34b	2.35 ± 0.20c	37.88	0.345	7.04	36.28
Xyl	5.60 ± 2.18 a	9.44 ± 2.94b	38.25	7.88 ± 2.17	7.39 ± 4.61	7.30 ± 2.90	0.70	0.008	33.91	27.14
Man	201.89 ± 18.13	191.10 ± 45.67	2.64	189.18 ± 50.29	196.20 ± 20.18	204.10 ± 29.94	3.37	0.189	22.78	71.21
Gal	439.88 ± 33.39	408.46 ± 106.41	4.27	372.41 ± 108.20 a	435.60 ± 39.46 ab	464.50 ± 46.92b	25.61	0.064	25.75	44.37
GalA	43.62 ± 20.54 a	69.50 ± 18.15b	33.39	54.41 ± 11.89	67.87 ± 13.00	47.40 ± 35.14	14.39	0.000	38.94	13.28
GluA	13.50 ± 6.11	13.71 ± 3.88	0.05	8.54 ± 3.13 a	14.51 ± 2.24b	17.76 ± 4.09b	62.50	0.028	16.80	20.65
Kdo	1.06 ± 0.34	1.19 ± 0.42	3.22	1.13 ± 0.31 ab	0.90 ± 0.43 a	1.35 ± 0.30b	24.54	0.181	17.91	54.33
Glc	41.24 ± 24.33	32.06 ± 14.70	5.54	22.91 ± 6.18 a	36.25 ± 15.31b	50.79 ± 25.09c	34.10	0.000	50.71	9.65
TMS without Glc	834.29 ± 41.94	818.87 ± 172.76	0.42	735.18 ± 146.20 a	845.91 ± 69.06 ab	898.65 ± 89.07b	32.90	0.033	28.87	37.81
TMS	875.52 ± 45.46	850.93 ± 178.31	0.99	758.09 ± 150.97 a	882.15 ± 64.40 ab	949.44 ± 71.90b	41.32	0.047	23.07	34.62
Ara/Gal	0.25 ± 0.07	0.24 ± 0.04	0.82	0.24 ± 0.05	0.23 ± 0.01	0.28 ± 0.08	16.45	0.056	31.52	51.21
RG-II	381.23 ± 53.06 a	618.47 ± 109.93b	68.00	434.66 ± 111.25 a	481.85 ± 137.38 a	583.05 ± 171.59b	18.52	0.218	3.02	10.46
MP	252.36 ± 22.95	238.87 ± 48.89	2.64	236.48 ± 54.00	245.25 ± 21.00	255.13 ± 35.56	3.37	0.189	22.78	71.21
PRAG	653.38 ± 70.09	586.45 ± 151.27	8.31	541.13 ± 157.14 a	631.24 ± 58.40 ab	687.39 ± 85.64b	26.94	0.118	17.61	47.14
HL	12.18 ± 9.23b	3.72 ± 5.61 a	25.64	8.67 ± 9.54b	15.19 ± 4.56b	0.00 ± 0.00 a	55.49	0.000	17.98	0.90
TPF	1299.16 ± 105.94	1447.52 ± 308.90	10.40	1220.93 ± 211.37 a	1373.53 ± 165.41 ab	1525.56 ± 241.38b	29.24	0.132	17.26	43.10

almost all compounds determined in the samples, except for arabinose to galactose ratio, homogalacturonans and glucose, which were explained by PC2. Both PCs allowed differentiation between treatments. Therefore, CW-7d wine was widely separated from the other wines and it was located in the most positive part of PC1 due to its highest monosaccharide and polysaccharide content compared to wines elaborated with short and mid pomace maceration. CW-2d y CW-3d wines were located in the negative part of PC1 and when sonication was applied, they moved towards the positive part of PC1. Comparing CW-2d and CW-3d and their respective sonicated wines, the latter present higher values in PC1. S28W-3d showed the most similar contents of polysaccharides and glucosyl residues to CW-7d wine.

3.4. Distribution of the molecular weights of polysaccharides

The qualitative changes in the molecular weight distribution of musts and wine polysaccharides are shown in Fig. 3. The molecular weight distributions of polysaccharides in must samples are shown in Fig. 3A. Control must and musts made with ultrasound-treated crushed grapes (S-20 M and S-28 M) shared similar profiles and agreed with those reported for white musts (Vidal et al., 2000). The distribution of polysaccharides from musts was characterized by the presence of two major populations. The first population, eluting between 13.0 and 15.4 min, corresponded to a fraction with molar mass between P200 and P50 and with an average molecular weight around 63 kDa. According to previous work (Guadalupe & Ayestarán, 2007), it corresponded to a complex mixture of high-molecular-weight PRAG from grape berries and high-molecular-weight MP or mannans. The second population, eluting between 15.4 and 18.2 min, corresponded to a fraction with molar mass between P50 and P5 and with an average molecular weight around 13 kDa. According to the literature, it could be attributed to a mixture of PRAG, MP or mannans of medium and lower molecular weight, and RG-II (Ducasse et al., 2010). Signals eluting after P5 corresponded to a molecular weight of less than 5.9 kDa, and it was

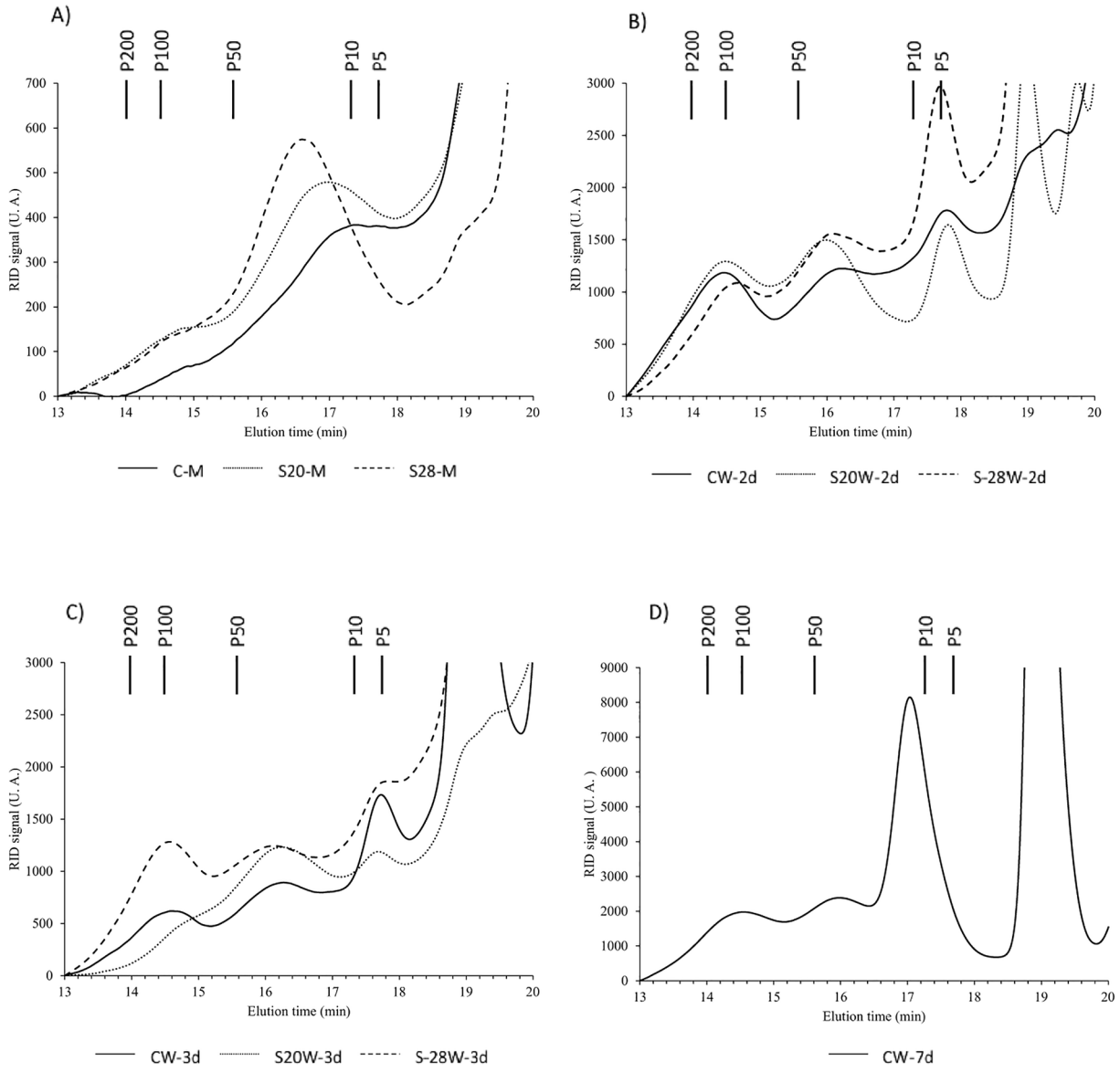

Fig. 3. HRSEC–RID chromatograms of total soluble polysaccharides in musts (A), in wines with two days maceration (B), in wines with three days maceration (C), in wines with seven days maceration (D). Chromatograms obtained using two serial Shodex OHpack KB-803 and KB-805 columns. C-M, control must; S20-M, must with sonicated grapes at 20 kHz; S28-M, must with sonicated grapes at 28 kHz; CW-2d, control wine with two days maceration, S20W-2d, 20 kHz-treated wine with two days maceration; S28W-2d, 28 kHz-treated wine with two days maceration; CW-3d, control wine with three days maceration, S20W-3d, 20 kHz-treated wine with three days maceration; S28W-3d, 28 kHz-treated wine with three days maceration; CW-7d, control wine with seven days maceration.

attributed to oligosaccharides and small fragments of PRAG, MP or mannans and RG-II (Guadalupe et al., 2012).

An important shift from lower to higher molecular weight polysaccharides in the second peak was observed in US-treated musts. In fact, the average molecular weights of the second population were 9, 13 and 19 kDa in C-M, S20-M and S-28 M, respectively. This fact suggested a release of higher molecular weight polysaccharides due to the cell wall degradation caused by the sonomechanical effect of ultrasound treatment.

Maximum higher peak heights in peaks 1 and 2 were observed in must from sonicated grapes compared to control must. This fact indicated that US treatment broke the grape cell wall more intensely than the crushing of grapes (control must).

Passing from must to wines with short and mid pomace maceration time (Fig. 3B y 3C), was characterized by a change in the peak profile in the same elution time (13.0 to 18.2 min). Wines elaborated with short and mid skin maceration time showed three populations with an average molecular weight around 111, 28 and 7 kDa. According to previously published data, the third population (Ayestarán et al., 2004; Guadalupe & Ayestarán, 2007), corresponded to a complex mixture of mainly RG-II dimers, with an average molecular weight of 10–12 kDa (Pellerin et al., 1996; Doco et al., 1997), as well as oligosaccharides and small fragments of PRAG, HL, MP, and RG-II (Guadalupe et al., 2012; Doco et al., 2007). A progressive increase in the peak height of populations was also observed, which was attributed to an increase of PRAG and a progressive enrichment of yeast mannoproteins, with highly variable sizes ranging from 5 to 800 kDa (Doco et al., 2003). Maximum higher peak heights were observed in wines elaborated with long pomace maceration time (CW-7d) compared to wines made with short and mid pomace maceration times (Fig. 3D). Moreover, the position of the third peak in CW-7d wines showed a shift towards higher molecular weights (average molecular weight of 13 kDa) compared to wines elaborated with short and mid pomace maceration times.

Important differences were observed between the peak heights of control wines with short and mid pomace maceration compared to their corresponding sonicated wines (Fig. 3B and 3C). Sonication at 28 kHz led to an increase in the height of the third peak, indicating that US treatment improved the release of RG-II dimers, oligosaccharides, and low molecular weight HL, PRAG and MP in wines with short pomace maceration time (Fig. 3B). Similarly, an increase in the maximum peak heights in the first and second population peaks was observed in S28W-3d with respect to CW-3d (Fig. 3C). In the present paper, the polysaccharides isolated from the wines showed a similar distribution to that described in the literature (Ducasse et al., 2010; Fanzone et al., 2012; Guadalupe et al., 2012; Apolinar-Valiente et al., 2014). The release of polysaccharides with an average molecular weight around 7 kDa (RG-II dimmers, oligosaccharides, and low molecular weight HL, PRAG and MP), 111 and 28 kDa (PRAG and MP) produced by the sonication of the grape at 28 kHz with medium maceration, was probably sufficient to stabilize the wine color. This wine showed CI values similar to those obtained with longer maceration times (Table 1). It should be highlighted that, in some cases, the technical resources in the wineries and the availability of maceration tanks may be limited, making necessary the reduction of the maceration time. This can be detrimental to the quality of the final product, although it could be relieved by the use of pectolytic enzymes or ultrasound treatments.

4. Conclusions

The sonomechanical effect of the application of ultrasound at two frequencies on the disruption of grape berry cell wall polysaccharides has been proved due to the significant increase in must samples of the content of glycosyl residues, forming pectic and hemicellulosic xyloglucans and arabinoxylans, and PRAG. Moreover, an increase in the average molecular weights of fragments of PRAG, MP or mannans of lower molecular mass has been observed.

Glucose was the major glycosyl residue in musts. Passing from must to wine caused a change in the distribution of the molecular weights of polysaccharides, and a significant reduction in the glucose content. Glucose reduction was produced by the limited solubility and stability of grape structural polysaccharides due to the enzymatic activity and/or ethanol increase in the stages prior to bottling. This decrease caused a reduction in the content of total of monosaccharides as well as a change in the profile of the glycosyl residue composition of wines, galactose being the major sugar detected.

Longer pomace maceration time significantly increased the extraction of glycosyl residues of pectic and hemicellulosic polysaccharides, as well as PRAG and RG-II, but it did not affect the concentration of MP. Sonication to crushed grapes did not affect the content of MP in the wines obtained with short and mid pomace maceration times, suggesting that the application of high-power ultrasound doid not affect the disruption of yeast cell wall in the following stages of maceration-fermentation and wine stabilization.

28 kHz had a greater effect on the glycosyl and polysaccharide families than 20 kHz. The wine made with sonicated grapes at 28 kHz and mid pomace maceration time had higher RG-II and PRAG content than its corresponding control wine. In fact, S28W-3d presented the most similar contents of polysaccharides and glucosyl residues to long pomace maceration control wine. Hence, results indicated that sonication at 28 kHz could be a useful technology to increase the content of polysaccharides from grapes, allowing a reduction of the maceration time. Both the conclusions of the present paper and those obtained by Pérez-Porras et al. (2021) indicate that grape sonication facilitates the release of polysaccharides, anthocyanins and tannins from the skins and the extraction of tannins from the seeds.

Declaration of Competing Interest

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

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