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Polysaccharide Composition of Monastrell Red Wines from Four Different Spanish Terroirs: Effect of Wine-Making Techniques

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Supporting Information

ABSTRACT: Monastrell wines made from grapes grown in four different Spanish "terroirs" (Cañada Judío, Albatana, Bullas, and Montealegre) were studied. Different wine-making techniques were also used, including different refrigeration techniques (prefermentative cold maceration and dry ice addition) and the addition of two different enzymes (β-galactosidase and commercial pectinase enzyme). The results pointed to significant differences in the Monastrell wine polysaccharide fractions according to the geographical origin of the grapes. The Rhamnogalacturonan II (RG-II) concentration was 2-fold higher in the Montealegre terroir than in the Bullas terroir. The use of enzymes also modified the polysaccharide content of the wines. RG-II levels were higher in the wines from three terroirs when commercial enzymes were added. The arabinose/galactose ratio of one of the wines was modified by the use of enzymes during wine-making, and some prefermentative cold maceration samples showed high values for several polysaccharides. This study shows the great importance of the "terroir effect" in the polysaccharide composition of wines.

KEYWORDS: *wine, polysaccharides, refrigeration techniques, pectic enzymes, terroir*

INTRODUCTION

Polysaccharides, one of the main groups of wine macromolecules, have been widely isolated and characterized during the past decade.[1−3] They can be grouped into three major families: (i) polysaccharides rich in arabinose and galactose (PRAGs),[2,3] (ii) those rich in rhamnogalacturonans (RG-I and RG-II), which come from the pecto-cellulosic cell walls of grape berries,[2,3] and (iii) the mannoproteins (MPs), another group of wine macromolecules, produced by yeasts during fermentation and during the aging of wines on lees.[3,4] Vidal et al.[2] reported that the ethanol-precipitated polysaccharides from the red wine were composed of 42% arabinogalactan-proteins, 35% MP, 19% RG-II, and 4% RG-I.

Several authors have studied the properties of polysaccharides in wine, where they have been seen to act as protective colloids and to interact with aromatic compounds.[5] Their role in protein stability[4] has been demonstrated. Different polysaccharide families can specifically modify the self-aggregation of tannins in wine-like solutions.[6] When the intrinsic organoleptic properties of two wine polysaccharide fractions were investigated,[7] the "fullness" sensation was significantly increased when a mixture of arabinogalactan-proteins, mannoproteins, and rhamnogalacturonan II was added. Besides, this last compound significantly decreased the mouth-feel attributes associated with the astringency of a model wine. In short, the impact of polysaccharides on the chemical and sensorial properties of wine has been studied for several years.

The efficiency of using pectolytic enzymes to extract color has been widely studied. However, studies into the effect of enzymes on polysaccharide fractions are limited. The amount of polysaccharides released in red wines depends on the wine-making process, and they can be modified to a great extent by enzyme treatments;[3,8] such treatment may increase RG-II and decrease PRAG levels,[8,9] modify type II arabinogalactan-proteins (AGPs), and lead to a loss of their terminal arabinose residues.[8] Ducruet et al.[10] showed that the addition of commercial enzymes to musts increased the amount of total acid (49%) and neutral (5%) polysaccharides, although they did not study this effect in the different polysaccharides families.

The effect of low temperature vinification techniques has also been studied by several authors, especially in relation to wine color and aroma compounds. The low temperatures needed to achieve the required effect can be obtained in several ways. For example, cold prefermentative maceration helps extraction in an aqueous medium, because it results in the preferential solubility of water-soluble compounds, and encourages selective extraction of anthocyanins and tannins of low molecular weight.[11] Adding dry ice delays the starting time of the fermentation, and freezing causes lysis and disorganization of the skin cells, favoring the release and solubilization of pigments.[12] Freezing facilitates the release of both aromatic and phenolic compounds.[13] It has also been observed that low maceration temperatures prior to fermentation improve the extraction of pigments and tannins from the grape skins to the wine.[14]

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Although the influence of applying low temperature on the polysaccharide composition of wine has not been fully researched, all of the above results point to cell wall degradation and suggest that these techniques could also have an interesting effect on grape polysaccharide extraction.

Historically, "terroir" refers to an area or terrain, usually rather small, whose soil and microclimate impart distinctive qualities to food products. This aspect is particularly closely associated with the production of wine.[15] The true concept is not easily grasped but includes physical elements of the vineyard habitat. Beyond the measurable ecosystem, there is an additional dimension, where natural and social factors interact.[16] Terroir delineation must also take into account factors that typically do not concern ecologists, such as the current and historical geographic distribution of the human know-how or "savoir faire" associated with the product.

Monastrell is the main wine grape variety in southeastern Spain. Whereas polysaccharides have been studied in other grape varieties, information about the Monastrell cell-wall composition and its behavior during wine-making is almost limited to the studies of Ortega-Regules et al.,[17,18] Romero-Cascales et al.,[19] and Apolinar-Valiente et al.[20] On the other hand, there have been no studies on the influence of terroir on the amount and composition of wine polysaccharides. In this study, Monastrell grapes grown in four different "terroirs" (Cañada Judío, Albatana, Bullas, and Montealegre) were used for wine-making, and the resulting wines were studied. Also, four different wine-making techniques were used to obtain wines: prefermentative cold maceration or the addition of dry ice, and the addition of β-galactosidase or pectinolytic commercial enzyme.

MATERIALS AND METHODS

Grape Materials. Grapes from *Vitis vinifera* cv. Monastrell grown in four different terroirs (Cañada Judío, Albatana, Bullas, and Montealegre) located near Murcia in southeastern Spain were harvested at commercial maturity for the 2008 vintage. The geographical location of the terroirs was as follows: Cañada Judío (1°21′37.02″ W, 38°33′15.84″ N; 450 m above sea level (a.s.l.)); Albatana (1°27′49.78″ W, 38°32′28.56″ N; 693 m a.s.l.); Bullas (1°40′59.06″ W, 38°02′38.24″ N; 432 m a.s.l.); and Montealegre (1°17′42.82″ W, 38°46′39.85″ N; 771 m a.s.l.).

Cañada Judío terroir is close to Jumilla village, and the plot used is formed of limestones, dolomites, loams, and sandstorm. The Albatana terroir plot is close to Albatana village and is composed of conglomerates, gravel, sand, and slime. Bullas terroir is near Cehegín village, and the plot used contains conglomerates, sandstones, clay, limestones, evaporites, and vulcanites. Montealegre terroir is close to Montealegre del Castillo village, and the plot is formed of sand, clay, gravel, mud, and gypsum. Climate information for the different terroirs was obtained from weather stations between September 2007 and October 2008. The climatic parameters obtained were monthly mean temperature (Supporting Information Figure 1), monthly mean rainfall (Supporting Information Figure 1), monthly mean maximum and minimum temperatures (Supporting Information Figure 2), monthly mean relative humidity (Supporting Information Figure 3), monthly mean maximum and minimum relative humidity (Supporting Information Figure 4), monthly mean wind speed (Supporting Information Figure 5), and monthly mean maximum wind speed (Supporting Information Figure 6). The instruments used were HMP45AC thermohigrometer (Vaisala, Helsinki, Finland), 05103-5 wind anemometer (Young Co., MI), and different rainfall-meter models: PCP-214 (Geónica, Madrid, Spain), 4.4031.30.006 (Thies-CLima, Göttingen, Germany), and ARG-100 (Campbell Scientific Ltd., Loughborough, UK).

Preparation of Control Trials. Three 90 kg lots of Monastrell grapes from four different terroirs (Cañada Judío, Albatana, Bullas, and Montealegre) were destemmed and crushed using a crusher/destemmer unit (Gamma 30, Zambelli Enotech, Italy), and distributed into 100 L stainless steel tanks to yield triplicate control lots named JUCO, ALCO, BUCO, and MTCO. At the same time, bisulfite (8 g/100 kg grape) was added. This was the basic wine-making process followed in all of the wines detailed below.

Prefermentative Cold Maceration Trials. The same process as above was followed except that a prefermentative cold maceration was carried out: tanks containing the crushed grapes were introduced into a cold chamber at 10 °C for 10 days. These were named JUCM, ALCM, BUCM, and MTCM.

Dry Ice Addition Trials. The same process as in the control was followed except that dry ice (−78 °C) was added directly into the tanks, mixing it with the crushed grapes, using 100 kg of dry ice per each tank. The dry ice kept the must frozen for 3 days at temperatures lower than −3 °C. The resulting wines were named JUIA, ALIA, BUIA, and MTIA.

Commercial Enzyme Addition Trials. Following the control process, a commercial enzyme was added to the tanks (5 g/100 kg), and the resulting wines were named JUCE, ALCE, BUCE, and MTCE. The company (Agrovin Company, Alcázar de San Juan, Spain) that produces the commercial enzyme (Enozym Vintage) provided the following information on the enzyme: polygalacturonase activity, 546.6 IU/g; pectinesterase activity, 7.3 IU/g; pectin lyase activity, 2.8 IU/g; and β-glucanase activity, 179.6 IU/g.

Galactosidase Enzyme Addition Trials. Instead of the commercial enzyme a mixed purified enzymatic preparation of α- and β-galactosidase (Agrovin Co., Alcázar de San Juan, Spain) was added to the tanks (1 g/100 kg). The wines were named JUGE, ALGE, BUGE, and MTGE.

Fermentation. All fermentations were started by adding commercial dry yeast (Laffort, Servian, France) at 10 g/hL and were carried out in 100 L stainless steel tanks, equipped with temperature control (25 °C), enabling the fermentation kinetics to be regulated. Each lot was fermented to completion, and, when alcoholic fermentation was finished (monitored by sugar analysis), the musts were pressed at 1.5 bar in a 75 L tank membrane press. Free-run and pressed wines of each trial were combined and stored in 50 L tanks. One month later the wines were racked. After malolactic fermentation was completed, the wines were racked again and supplied with 25 mg/L sulfur dioxide. The wines were not clarified or filtered, but cold stabilized (−3 °C) for 1 month, bottled, and stored in the experimental wine cellar at 18 °C until analysis.

Enological Analysis. Dry extract, alcohol content, total acidity, volatile acidity, and the chromatic characteristics of wines were determined according to the official methods of the European Union.[21]

Preparation of Total Soluble Polysaccharides from Wines. Wine polysaccharides were isolated as previously described.[2] Wine (2.5 mL) was evaporated in a centrifugal evaporator (EZ-2, Genevac, Ipswich, UK). The residue was dissolved in 0.5 mL of water to obtain wine concentrated 5 times. 2.66 mL of ethanol (95%) acidified by 0.5% HCL was added to obtain a final concentration of 80% ethanol. After one night at 4 °C, wine polysaccharides were precipitated, and the supernatant was eliminated after centrifugation (16 500*g*, 10 min). The pellet that corresponds to total wine colloids was dissolved in 1 mL of water (Millipore). The oligosaccharides and salts contained in the total colloids were eliminated by retention on an ion exchange column (4 mL of mixed resin: Mix Bed Resin AG 501-X8, Bio Rad, Hercules, CA). Wine polysaccharides that were not retained were eluted by 2.5 bed volumes of water. Total soluble polysaccharides were obtained after freeze-drying of the water-eluted materials.

Analysis of Polysaccharides. The molecular weight distribution of wine polysaccharides was established by high-performance size-exclusion chromatography (HPSEC) using a system composed of a 234-Gilson sampling injector (Roissy, France) and an LC-10 AS Shimadzu pump (Kyoto, Japan). HPSEC elution was performed on two serial Shodex Ohpak KB-803 and KB-805 columns (0.8 × 30 cm;

Table 1. Enological Data of Monastrell Wines from Four Different Terroirs[a]

	experimental nomenclature	DE[b]	alcohol[c]	pH	TA[d]	VA[e]	TPI[f]	CI[g]	Hue[h]
Cañada Judio									
control	JUCO	30.5 ± 1.5 a	14.6 ± 0.2 ab	3.31 ± 0.05 a	6.8 ± 0.1 a	0.23 ± 0.02 a	56.6 ± 3.2 a	16.2 ± 0.6 a	0.42 ± 0.00 b
commercial enzyme addition	JUCE	29.4 ± 1.2 a	14.3 ± 0.2 a	3.34 ± 0.02 a	6.9 ± 0.1 a	0.22 ± 0.01 a	55.1 ± 3.0 a	15.9 ± 0.8 a	0.42 ± 0.01 b
galactosidase enzyme addition	JUGE	32.7 ± 1.0 a	14.4 ± 0.2 a	3.32 ± 0.02 a	7.1 ± 0.1 a	0.24 ± 0.03 a	57.6 ± 0.7 a	17.1 ± 0.7 a	0.41 ± 0.00 ab
prefermentative cold maceration	JUCM	29.8 ± 0.3 a	14.9 ± 0.1 a	3.35 ± 0.01 a	6.8 ± 0.0 a	0.21 ± 0.01 a	55.9 ± 1.4 a	17.2 ± 0.9 a	0.42 ± 0.00 b
dry ice addition	JUIA	31.1 ± 1.9 a	14.2 ± 0.3 b	3.33 ± 0.03 a	6.9 ± 0.2 a	0.26 ± 0.03 a	55.9 ± 1.5 a	17.0 ± 0.4 a	0.40 ± 0.01 a
Albatana									
control	ALCO	28.9 ± 0.6 ab	14.4 ± 0.4 a	3.29 ± 0.02 a	6.8 ± 0.1 a	0.25 ± 0.04 a	54.4 ± 1.7 ab	14.7 ± 0.3 ab	0.46 ± 0.01 a
commercial enzyme addition	ALCE	30.5 ± 0.5 c	14.5 ± 0.1 a	3.28 ± 0.04 a	6.8 ± 0.2 a	0.27 ± 0.04 a	56.1 ± 0.2 ab	15.8 ± 0.6 b	0.46 ± 0.01 a
galactosidase enzyme addition	ALGE	30.8 ± 0.6 c	14.5 ± 0.3 a	3.34 ± 0.07 a	6.6 ± 0.4 a	0.26 ± 0.02 a	56.6 ± 1.1 b	14.7 ± 1.4 ab	0.48 ± 0.03 a
prefermentative cold maceration	ALCM	29.9 ± 0.5 b	14.5 ± 0.2 a	3.31 ± 0.00 a	6.7 ± 0.0 a	0.30 ± 0.01 a	59.1 ± 1.6 c	16.2 ± 0.5 c	0.45 ± 0.00 a
dry ice addition	ALIA	28.2 ± 0.7 a	14.6 ± 0.2 a	3.35 ± 0.01 a	6.6 ± 0.1 a	0.30 ± 0.01 a	53.7 ± 2.1 a	14.2 ± 0.3 a	0.46 ± 0.01 a
Bullas									
control	BUCO	26.3 ± 0.8 a	14.2 ± 0.4 a	3.54 ± 0.08 a	5.8 ± 0.2 a	0.23 ± 0.04 a	34.1 ± 1.0 a	8.6 ± 0.2 a	0.62 ± 0.05 ab
commercial enzyme addition	BUCE	27.2 ± 0.5 ab	14.3 ± 0.2 a	3.58 ± 0.08 a	5.7 ± 0.3 a	0.25 ± 0.02 a	35.0 ± 1.4 a	9.0 ± 1.1 a	0.64 ± 0.03 b
galactosidase enzyme addition	BUGE	28.1 ± 0.5 b	14.2 ± 0.2 a	3.55 ± 0.03 a	5.8 ± 0.2 a	0.27 ± 0.02 a	35.2 ± 1.9 a	8.8 ± 0.1 a	0.64 ± 0.06 b
prefermentative cold maceration	BUCM	28.7 ± 0.7 c	14.3 ± 0.2 a	3.54 ± 0.05 a	6.1 ± 0.1 a	0.29 ± 0.02 a	36.7 ± 1.5 a	8.5 ± 0.6 a	0.56 ± 0.02 a
dry ice addition	BUIA	27.4 ± 0.5 ab	14.1 ± 0.1 a	3.58 ± 0.04 a	5.8 ± 0.1 a	0.23 ± 0.02 a	36.5 ± 2.3 a	9.1 ± 0.6 a	0.62 ± 0.02 ab
Montealegre									
control	MTCO	26.4 ± 0.6 a	11.9 ± 0.1 a	3.37 ± 0.03 a	6.3 ± 0.1 a	0.13 ± 0.00 b	36.4 ± 2.1 a	10.0 ± 0.5 b	0.47 ± 0.01 a
commercial enzyme addition	MTCE	26.6 ± 1.0 a	12.2 ± 0.3 a	3.41 ± 0.03 a	6.2 ± 0.1 a	0.14 ± 0.00 b	35.2 ± 1.1 a	9.3 ± 0.1 b	0.49 ± 0.01 a
galactosidase enzyme addition	MTGE	27.3 ± 0.8 a	12.3 ± 0.5 a	3.42 ± 0.04 a	6.2 ± 0.1 a	0.14 ± 0.00 b	36.5 ± 3.4 a	9.3 ± 0.6 b	0.49 ± 0.01 a
prefermentative cold maceration	MTCM	27.4 ± 0.2 a	12.3 ± 0.3 a	3.41 ± 0.04 a	6.4 ± 0.3 a	0.13 ± 0.02 b	36.2 ± 1.0 a	7.9 ± 0.4 a	0.48 ± 0.02 a
dry ice addition	MTIA	27.2 ± 0.8 a	12.3 ± 0.3 a	3.43 ± 0.01 a	6.1 ± 0.1 a	0.11 ± 0.01 a	37.5 ± 1.7 a	9.3 ± 0.7 b	0.49 ± 0.01 a

[a]Different letters within the same terroir column represent significant differences according to an LSD test ($p < 0.05$). [b]DE, dry extract g/L. [c]Alcohol content (% vol). [d]Total acidity (g/L tartaric acid). [e]Volatile acidity (g/L acetic acid). [f]Total polyphenols index at 280 nm. [g]Colour intensity (Abs 420 nm + Abs 520 nm + Abs 620 nm). [h]Abs 420 nm/Abs 520 nm.

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Showa Denko, Japan) connected to an ERC-7512 refractometer (Erma, Japan), at a 1 mL/min flow rate in 0.1 M $LiNO_3$. The apparent molecular weights were calculated from the calibration curve established with a Pullulan calibration kit (P-400, MW = 380 000 Daltons (Da); P-200, MW = 186 000 Da; P-100, MW = 100 000 Da; P-50, MW = 48 000 Da; P-20, MW = 23 700 Da; P-10, MW = 12 200 Da; P-5, MW = 5800 Da; Showa Denko, Japan). The calibration equation was log MW = 28.321−1.04 × tR (tR = column retention-time at peak maximum, and r^2 = 0.997).

Neutral monosaccharides were released after hydrolysis of the wine polysaccharides by treatment with 2 M trifluoroacetic acid (75 min at 120 °C).[22] They were then converted to the corresponding alditol acetate derivatives by reduction and acetylation and quantified by GLC analysis using a fused silica DB-225 (210 °C) capillary column (30 m × 0.32 mm i.d., 0.25 μm film), with hydrogen as the carrier gas, on a Hewlett-Packard model 5890 gas chromatograph. The different alditol acetates were identified from their retention times by comparison with standard monosaccharides. Neutral sugar amounts were calculated relative to the internal standard (myo-inositol).

Polysaccharide Concentration. The polysaccharide composition of each wine was estimated from the concentration of individual glycosyl residues, determined by GLC after hydrolysis, reduction, and acetylation, and did not differ from previously described wine polysaccharide compositions.[23] The calculation of wine polysaccharide concentrations took into account the composition of characteristic monosaccharides as well as the hydrolysis yield.[23] The concentrations of MPs, PRAGs, and RG-II were estimated from the concentration of individual glycosyl residues, as determined by GLC after hydrolysis, reduction, and acetylation. All of the mannose was attributed to yeast MPs.[4] The PRAGs in wines, mainly arabinogalactan-proteins, arabinogalactans, and arabinans, were estimated from the sum of galactose and arabinose residues. RG-II was calculated from the concentration of 2-*O*-methyl-fucose and 2-*O*-methyl-xylose.

Statistical Data. Average values, standard deviation, and statistical significance were calculated and performed with the package Statgraphics Plus 5.1.

RESULTS AND DISCUSSION

Enological Parameters for Monastrell Wines. The enological parameters for Monastrell wines from different terroirs are presented in Table 1. Alcohol, pH, total acidity and volatile acidity, total polyphenols, and color indexes reflect the course of the wine-making process and provide information on wine quality. No treatment (enzymes, dry ice addition, or cold maceration) had a marked effect on the dry extract, alcohol level, pH, total acidity, and volatile acidity in the different wine samples. In the case of Montealegre, the alcohol level was lower, but its maturation parameters (traditional and phenolic maturity measures) were optimal. The total phenol and color indices reflected the values considered normal for dry young wines, although differences between terroirs were evident.

Effect of Terroir on Monastrell Wine Polysaccharide Content. The molecular weight distribution of Monastrell wine polysaccharides was studied by size exclusion chromatography, as shown in Figure 1. The polysaccharides isolated from the wines were distributed as described in the literature for Carignan,[2,8,24] Tempranillo,[3] Grenache,[8] and Merlot[9] wines. The population eluting between 14.5 and 15.7 min corresponds to the mannoproteins (MPs) released from the yeast during fermentation.[4] A second population, eluting between 15.7 and 17.8 min, corresponds to a complex mixture of PRAGs (arabinogalactan-proteins (AGPs) and arabinans) from the grape berries, and MPs of low molecular weight.[2,3,8,9,24] The third peak, eluting between 17.8 and 20 min, corresponds mainly to RG-II, a complex pectic fragment, with PRAGs and MPs of lower molecular weight.[1−3,8,24]

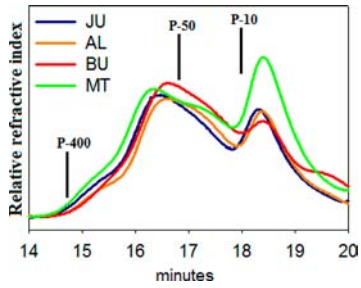

Figure 1. Molecular weight distribution in Cañada Judío (JU), Albatana (AL), Bullas (BU), and Montealegre (MT) terroir control wines. Relative refractive index versus retention time (min).

There were evident differences between the profiles of the four terroir control samples (Figure 1). A slightly higher peak corresponding to the second population was observed in the case of Bullas terroir, while the Montealegre profile showed little difference from the other profiles, except a marked increase in the second population peak. Besides its height, the position of the second peak pointed to differences between the apparent molecular weights of the four studied terroir wines. When the calibration equation was applied (log M_w = 28.321 − 1.04 × tR), the molecular weight of the second population was highest in the Montealegre terroir wine (M_w = 80 910 Da), whereas the lowest molecular weight of the PRAG peak was observed in Bullas terroir wine (M_w = 63 005 Da). In the case of Cañada Judío and Albatana wines, the molecular weights of the second population had intermediate values (M_w = 72 051 Da and M_w = 66 686 Da, respectively). A markedly higher peak corresponding to the third population could be observed in the case of Montealegre terroir. Because the grapes and the wine-making process used to obtain wines were similar, the results suggest a possible terroir effect on the polysaccharide composition of grapes and, consequently, on the resulting wines.

Table 2 represents the glycosyl residue composition analysis of wine polysaccharides. The presence of neutral sugars that commonly form part of wine polysaccharides can be observed.[1,2] These include all known neutral sugars (mannose, arabinose, galactose, glucose, rhamnose, fucose, and xylose), and several rare sugars like apiose, 2-*O*-methyl-fucose, and 2-*O*-methyl-xylose, which are known to be markers of the presence of RG-II.[1] Glucose is not known as a component of pectic polysaccharides, but may arise from microbial or bacterial polysaccharides or anthocyanins.[25] Mannose, galactose, and arabinose are sugars of the major polysaccharides, as shown by Ducasse et al.[9] Our results pointed to significantly higher quantities of xylose and mannose in Montealegre terroir wines than in the wines from Cañada Judío, Albatana, and Bullas terroirs. It is interesting that Ortega-Regules et al.[17] found differences in Monastrell grape skin cell walls coming from three different terroirs.

Figure 2 presents the concentration of MPs, PRAGs, and RG-II in mg/L in wines. There was a higher MP concentration in Montealegre terroir wines (MTCO: 185 mg/L) than in Cañada Judío, Albatana, and Bullas wines (JUCO, 132 mg/L; ALCO, 139 mg/L; and BUCO, 126 mg/L). Taking into account that all of the mannose was attributed to yeast MPs[4] and that the yeast strains were similar in every wine studied, this higher amount of MPs in Montealegre wines might be attributed to the different alcoholic degrees of the wines, which means changes in yeast conditions. The MPs released by yeasts

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Table 2. Glycosyl Residue Composition (mg/L) of Polysaccharides of Monastrell Wines from Four Different Terroirs[a]

	2-OMeFuc[b]	Rha[b]	Fuc[b]	2-OMeXyl[b]	Ara[b]	Api[b]	Xyl[b]	Man[b]	Gal[b]	Glc[b]
Cañada Judio										
JUCO	**2.8 ± 0.7 a**	**20.5 ± 3.3 a**	**2.4 ± 0.0 a**	**1.6 ± 0.0 a**	**104.9 ± 8.7 a**	**2.1 ± 0.5 a**	**2.8 ± 1.1 a**	**105.7 ± 25.7 a**	**126.1 ± 31.0 a**	**24.9 ± 9.7 a**
JUCE	4.5 ± 0.5 b	36.3 ± 8.2 b	3.5 ± 0.9 a	2.9 ± 0.5 b	146.4 ± 30.4 b	2.1 ± 0.5 a	4.8 ± 0.8 b	117.3 ± 6.9 a	162.9 ± 29.2 a	26.9 ± 11.6 a
JUGE	2.7 ± 0.5 a	20.4 ± 5.7 a	2.1 ± 0.9 a	1.6 ± 0.0 a	104.4 ± 12.2 a	1.9 ± 0.5 a	2.9 ± 0.5 a	97.6 ± 20.2 a	124.8 ± 7.6 a	23.9 ± 12.1 a
JUCM	2.4 ± 0.0 a	23.2 ± 1.4 a	1.9 ± 0.5 a	2.0 ± 0.7 a	122.8 ± 11.1 ab	2.3 ± 0.2 a	4.0 ± 0.8 ab	116.5 ± 10.7 a	139.9 ± 6.4 a	26.3 ± 9.8 a
JUIA	2.8 ± 0.7 a	19.1 ± 0.6 a	1.9 ± 0.5 a	1.5 ± 0.2 a	102.4 ± 11.3 a	1.9 ± 0.5 a	3.1 ± 0.2 a	98.0 ± 16.0 a	117.7 ± 11.7 a	20.5 ± 8.1 a
Albatana										
ALCO	**2.4 ± 0.8 a**	**22.4 ± 3.7 b**	**2.7 ± 1.2 a**	**1.6 ± 0.0 a**	**119.2 ± 14.1 a**	**2.1 ± 0.5 a**	**2.9 ± 0.5 a**	**111.5 ± 6.7 a**	**137.6 ± 5.6 a**	**19.7 ± 12.8 a**
ALCE	4.3 ± 0.9 b	29.9 ± 2.0 c	2.4 ± 0.8 a	2.9 ± 0.9 a	148.5 ± 8.8 b	2.1 ± 0.5 a	7.2 ± 2.9 b	101.1 ± 12.5 a	172.8 ± 18.6 a	23.2 ± 18.7 a
ALGE	2.9 ± 0.5 a	18.4 ± 2.9 ab	1.9 ± 0.5 a	2.1 ± 0.9 a	109.3 ± 15.7 a	2.1 ± 0.9 a	4.0 ± 1.6 a	103.0 ± 24.9 a	142.9 ± 25.9 a	24.3 ± 16.8 a
ALCM	2.9 ± 0.5 a	26.1 ± 2.8 bc	2.1 ± 0.5 a	2.1 ± 0.5 a	143.7 ± 14.5 b	2.9 ± 1.2 a	4.3 ± 0.9 ab	109.1 ± 15.9 a	160.0 ± 30.7 a	17.1 ± 9.8 a
ALIA	1.9 ± 0.5 a	15.7 ± 4.1 a	1.3 ± 1.2 a	2.1 ± 0.9 a	105.9 ± 5.7 a	1.3 ± 1.2 a	1.9 ± 0.2 a	94.1 ± 21.1 a	132.8 ± 17.7 a	19.5 ± 10.9 a
Bullas										
BUCO	**1.9 ± 0.5 a**	**21.1 ± 1.8 a**	**1.3 ± 1.2 a**	**1.1 ± 0.5 a**	**120.3 ± 17.7 a**	**2.7 ± 1.2 a**	**2.1 ± 1.2 ab**	**101.1 ± 16.4 a**	**136.0 ± 22.8 a**	**16.8 ± 9.1 a**
BUCE	4.8 ± 0.8 c	42.7 ± 11.4 b	3.7 ± 1.2 a	2.9 ± 1.8 a	160.3 ± 34.0 a	3.2 ± 1.4 a	6.4 ± 2.4 c	126.4 ± 32.6 a	202.4 ± 41.6 a	25.9 ± 17.6 a
BUGE	2.4 ± 0.8 a	19.7 ± 5.1 a	1.9 ± 0.2 a	0.8 ± 0.8 a	128.0 ± 34.8 a	4.8 ± 3.7 a	1.1 ± 0.1 a	121.1 ± 21.7 a	164.8 ± 34.2 a	31.5 ± 21.0 a
BUCM	3.5 ± 0.5 b	29.1 ± 2.3 a	2.4 ± 0.0 a	2.7 ± 0.5 a	158.9 ± 2.8 a	2.7 ± 1.2 a	4.3 ± 2.0 c	136.2 ± 16.9 a	188.0 ± 14.4 a	28.8 ± 18.9 a
BUIA	2.4 ± 0.0 a	24.0 ± 2.1 a	2.4 ± 0.8 a	1.3 ± 0.5 a	126.1 ± 4.4 a	3.2 ± 2.1 a	2.4 ± 0.2 b	113.1 ± 9.4 a	152.5 ± 17.2 a	28.0 ± 16.9 a
Montealegre										
MTCO	**3.5 ± 1.2 a**	**21.9 ± 3.2 a**	**2.1 ± 0.9 a**	**2.4 ± 0.8 a**	**95.2 ± 23.1 a**	**1.3 ± 0.5 a**	**5.1 ± 0.9 a**	**148.0 ± 17.7 a**	**140.5 ± 29.7 a**	**21.1 ± 5.9 a**
MTCE	5.1 ± 0.5 a	27.5 ± 2.4 a	3.2 ± 0.8 a	3.7 ± 1.2 a	110.9 ± 23.1 a	1.9 ± 0.9 a	5.9 ± 2.0 a	163.2 ± 20.9 a	182.1 ± 26.8 a	28.3 ± 15.7 a
MTGE	3.7 ± 1.2 a	24.5 ± 1.7 a	2.1 ± 0.5 a	2.9 ± 0.5 a	102.9 ± 25.2 a	1.6 ± 0.0 a	5.9 ± 0.5 a	140.5 ± 3.7 a	161.3 ± 42.3 a	24.8 ± 3.7 a
MTCM	4.0 ± 0.0 a	25.6 ± 4.5 a	2.9 ± 0.2 a	3.5 ± 0.5 a	104.8 ± 18.7 a	1.9 ± 0.9 a	5.9 ± 0.9 a	130.0 ± 33.6 a	169.6 ± 33.5 a	22.4 ± 10.4 a
MTIA	3.2 ± 0.0 a	22.9 ± 2.4 a	1.3 ± 0.1 a	2.9 ± 0.5 a	97.1 ± 20.4 a	2.7 ± 0.3 a	3.5 ± 0.3 a	148.1 ± 23.3 a	145.3 ± 28.2 a	24.5 ± 0.9 a

[a]Different letters within the same terroir column represent significant differences according to an LSD test ($p < 0.05$). [b]Average of three measurements and standard deviation. 2-OMeFuc, 2-*O*-CH_3-fucose; Rha, rhamnose; Fuc, fucose; 2-OMeXyl, 2-*O*-CH_3-xylose; Ara, arabinose; Api, apiose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose.

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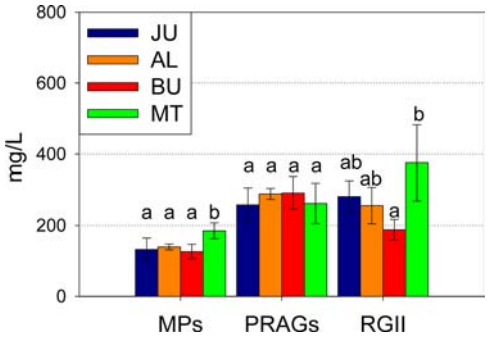

Figure 2. Concentration (mg/L) of MPs, PRAGs, and RG-II in Cañada Judío (JU), Albatana (AL), Bullas (BU), and Montealegre (MT) terroir control wines.

depend not just on the strain,[26] but also on wine-making conditions[27] and the initial colloid content of the must.[28]

PRAG concentrations were statistically similar in Cañada Judío (JUCO: 258 mg/L), Albatana (ALCO: 289 mg/L), Bullas (BUCO: 291 mg/L), and Montealegre (MTCO: 262 mg/L) terroir wines, meaning that arabinogalactans-proteins (AGPs), which may be found free or linked to pectins, were released in the same quantity in the four wines.

On the other hand, the concentration of RG-II in the Montealegre terroir wine was statistically higher (MTCO: 378 mg/L) than in the Bullas terroir wine (BUCO: 192 mg/L), while there were no significant differences with the wines from Cañada Judío (JUCO: 282 mg/L) and Albatana (ALCO: 256 mg/L). Although a concentration of RG-II of 150 mg/L has been previously reported for red wine,[23] other authors determined a RG-II concentration higher than 200 mg/L.[8,9] This higher RG-II concentration in the Montealegre terroir wine as compared to the Bullas terroir wine could be explained by possible differences in the pectin composition and natural enzymatic activities present in grape skin. The presence of RG-II is due in large part to the easiness with which this compound is solubilized by enzymes from the cell wall. During ripening, cell wall disassembly via wall-modifying enzymes plays a major role in fruit softening. It seems evident that to obtain a release of RG-II levels similar to those showed in literature, a good maturation level must be obtained, as in the case of our wines. Ortega-Regules et al.[17] found differences in the skin cell-wall material of Monastrell grapes from two different areas. However, very few of the genes required for RG-II biosynthesis have been identified,[29] which makes it difficult to monitor the RG-II biosynthesis pathways. Ortega-Regules et al.[18] detected differences in the extractability index in Monastrell grape skin from two different terroirs. Using the different cell wall compounds from grape skin as independent variables, Ortega-Regules et al.[17] provided a model, which explained 78% of the extractability index variability.

Table 3 shows the arabinose/galactose ratio, which is characteristic of PRAGs.[9] The ratio was lower in Montealegre terroir wines than in the other wines, suggesting a greater release of galactose or galactose-rich polysaccharides (Table 2). These results agree with those of Ortega-Regules et al.,[17] who observed differences in galactose, although the concentration of arabinose remained unaltered when they compared Monastrell grapes from two different terroirs.

Effect of Wine-Making Treatments on Monastrell Wine Polysaccharide Content. Figure 3 shows the molecular weight distribution obtained by HPSEC of

Table 3. Glycosyl Ratio Ara/Gal of PRAGs of Monastrell Wines from Four Different Terroirs[a]

	Ara/Gal[b]
Cañada Judío	
JUCO	**0.85 ± 0.13 a**
JUCE	0.91 ± 0.11 a
JUGE	0.88 ± 0.15 a
JUCM	0.88 ± 0.10 a
JUIA	0.88 ± 0.15 a
Albatana	
ALCO	**0.87 ± 0.10 a**
ALCE	0.87 ± 0.14 a
ALGE	0.77 ± 0.05 a
ALCM	0.91 ± 0.12 a
ALIA	0.80 ± 0.08 a
Bullas	
BUCO	**0.89 ± 0.02 a**
BUCE	0.79 ± 0.04 a
BUGE	0.77 ± 0.06 a
BUCM	0.85 ± 0.07 a
BUIA	0.84 ± 0.11 a
Montealegre	
MTCO	**0.67 ± 0.04 a**
MTCE	0.61 ± 0.01 a
MTGE	0.64 ± 0.06 a
MTCM	0.62 ± 0.02 a
MTIA	0.67 ± 0.05 a

[a]Different letters within the same terroir column represent significant differences according to an LSD test ($p < 0.05$). [b]Average of three measurements and standard deviation. Ara, arabinose; Gal, galactose.

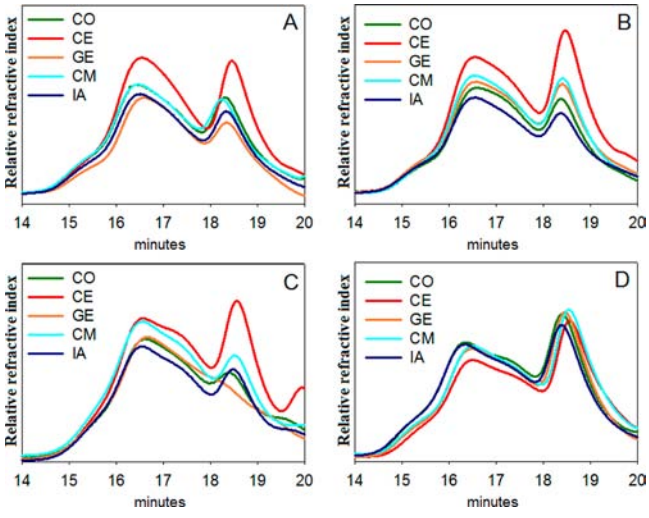

Figure 3. Molecular weight distribution in Cañada Judío (A), Albatana (B), Bullas (C), and Montealegre (D) terroir wines made using different wine-making techniques. Relative refractive index versus retention time (min). CO, control; CE, commercial enzyme; GE, galactosidase enzyme; CM, prefermentative cold maceration; IA, dry ice addition.

polysaccharides of wines made using the prefermentative cold maceration, dry ice, galactosidase, and commercial enzyme treatments for Cañada Judío, Albatana, Bullas, and Montealegre terroirs. In the case of the refrigeration techniques, there were no evident differences in the polysaccharide profiles between control samples (CO) and prefermentative cold maceration (CM) and ice addition (IA). Second and third populations

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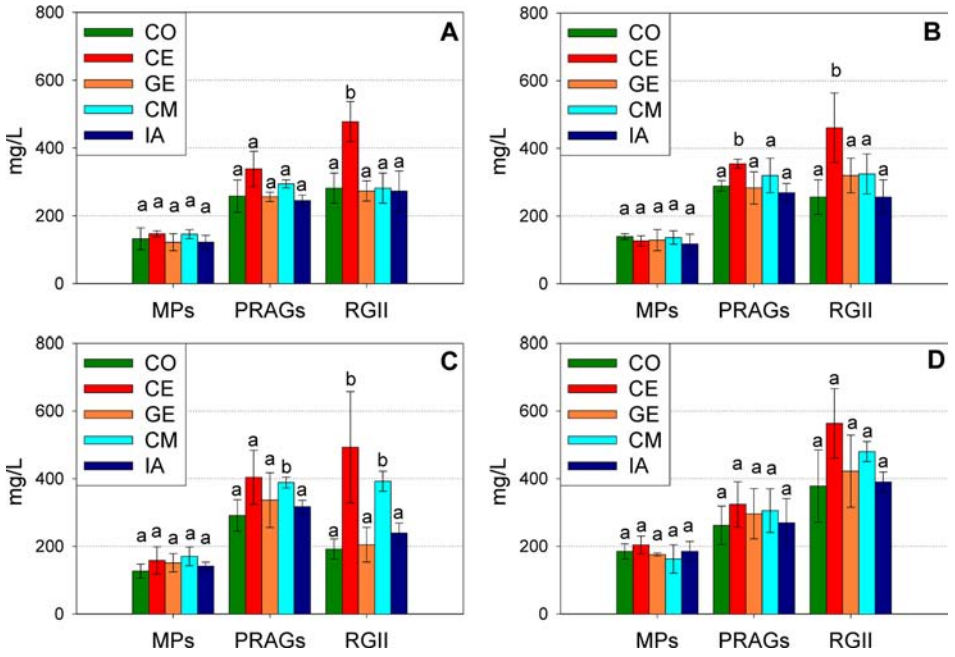

Figure 4. Concentration (mg/L) of MPs, PRAGs, and RG-II in Cañada Judío (A), Albatana (B), Bullas (C), and Montealegre (D) terroir wines made using different wine-making techniques. CO, control; CE, commercial enzyme; GE, galactosidase enzyme; CM, prefermentative cold maceration; IA, dry ice addition.

were higher in the Cañada Judío, Albatana, and Bullas terroirs wine polysaccharide profiles when commercial enzyme was added (CE), perhaps reflecting a release of PRAGs (second peak) and RG-II (third peak) due to extensive cell wall degradation by the pectinolytic enzymes present in the commercial preparation. A previous study demonstrated that the effect of enzyme on PRAG peaks seems to be dependent on the vintage,[9] but also on the grape variety (Monastrell versus Merlot) in which the enzymatic preparation is used. Our results suggest an enzyme effect, which differed according to the type of wine. On the other hand, there were no evident differences between control wines (CO) and galactosidase enzyme treated wines (GE) in any of the studied terroirs. These findings suggest an effect linked to the commercial enzyme treatment, but they must be corroborated by quantitative chemical analysis.

Table 2 shows the glycosyl residue composition analysis of polysaccharides. Mannose, galactose, and arabinose were the major polysaccharide sugars, which is coherent with results obtained by other authors.[9,30]

When commercial enzyme was added, there were statistically higher concentrations of 2-*O*-methyl-fucose in Cañada Judío terroir wines, of 2-*O*-methyl xylose in Cañada Judío and Bullas terroirs wines, of rhamnose in Cañada Judío, Bullas, and Albatana terroirs wines, and of arabinose in Albatana terroir wines, as compared to the control wines. The Montealegre terroir wines showed no significant differences between the commercial enzyme-treated wines and the corresponding control wine for any sugar. There have been no studies on the polysaccharide composition of enzymatically treated wines made from the same variety of grapes (e.g., Monastrell) grown in different terroirs. However, several studies have been published on the effect of adding enzymes on these macromolecules,[3,8−10,31] and also on anthocyanins,[11,32] color intensity,[9,33] and the total polyphenol index[9] in wine. Any changes in these parameters reflect skin cell wall degradation.

Several authors have observed close linear correlations between the phenolic compounds released in fruit juice and skin cell wall carbohydrate degradation caused by high enzyme doses, high temperatures, or long enzymatic treatments.[34,35] The release of phenolic compounds (anthocyanins and tannins) from grape skin after enzyme addition could be the consequence of the progressive degradation of cell wall polysaccharides.[36] However, our results (Table 1) point to no such linear correlation, and no differences were detected in TPI, CI, or Hue between enzyme-treated samples and control wines. This could be due to the properties of Monastrell grape skin itself, because their cell walls are genetically characterized by a very rigid structure[18] and a high extractability index.[17,18,37] It therefore seems that in some cases commercial enzyme is able to degrade grape skin cell walls, but not sufficiently to release phenolic compounds.

In the galactosidase enzyme treatment, there were no differences between the treated wines and the control wines in any of the four studied terroirs (Table 2). Nunan et al.[38] demonstrated that β-galactosidase decreased the cell wall galactans content, while the fact that we observed no differences could be due to the lower doses used. Whatever the case, Monastrell has been seen[17,18,37] to have a higher extractability index and higher weight of cell wall material as compared to other varieties, such as Cabernet Sauvignon, Syrah, and Merlot, which suggests a much firmer structure. Taking into consideration the enzyme dose used and the specific properties and composition of Monastrell skin cell walls, the effect of galactosidase enzyme on this grape cultivar seems to be less pronounced than in other varieties.

With regard to the cold-related techniques, Table 2 shows that there were no differences in polysaccharide sugars between the treated and control wines from Cañada Judío and Montealegre. In the Albatana terroir wine, the arabinose content increased over the control level when prefermentative cold maceration was applied. Similarly, the 2-*O*-methyl-fucose,

2-*O*-methyl-xylose, rhamnose, arabinose, and galactose levels were higher in Bullas terroir wines after prefermentative cold maceration (BUCM) than in the corresponding control wines (BUCO). Also, the Bullas terroir wines had a higher galactose concentration after dry ice addition (BUIA), again in comparison with the control wines. As for the rest of the sugars, there were no differences between cold treated and control wines.

Several authors[14,39] have observed improved phenolic compound extraction after prefermentative cold maceration, probably as a result of a more intense cell wall skin degradation. In the same way, some studies have detected an increase in phenolic compound extraction when dry ice was added.[13,40] However, other authors found no differences in phenolic extraction between prefermentative cold maceration and control wines.[41,42]

Figure 4 presents the concentration of MPs, PRAGs, and RG-II in mg/L in control and treated wines made from grapes from Cañada Judío, Albatana, Montealegre, and Bullas terroirs.

Following pectinolytic commercial enzyme treatment, RG-II concentration was higher in Cañada Judío, Albatana, and Bullas terroir wines (JUCE, 474 mg/L; ALCE, 461 mg/L; and BUCE, 493 mg/L) than in their corresponding control wines (JUCO, 282 mg/L; ALCO, 256 mg/L; and BUCO, 192 mg/L, respectively). In contrast, RG-II concentration in Montealegre terroir wines showed no significant differences after the same treatment (MTCE: 563 mg/L) as compared to the control wine (MTCO: 378 mg/L). These results agree with those of other authors, who also detected an RG-II increase when enzymes were added during wine-making.[2,8,9]

The same treatment led to differences in the PRAG concentration in Albatana terroir wine (ALCE: 354 mg/L) with respect to the control wine (ALCO: 289 mg/L). However, PRAG concentration was similar in commercial enzyme-treated wines from Cañada Judío (JUCE: 338 mg/L), Bullas (BUCE: 404 mg/L), and Montealegre (MTCE: 324 mg/L) and in the corresponding control wines (JUCO, 258 mg/L; BUCO, 291 mg/L; and MTCO, 262 mg/L). Doco et al.[8] observed a decrease and a modification (less arabinose) in PRAGs of Carignan wine after pectolytic enzyme treatment, whereas Ayestarán et al.[3] found that arabinogalactans and arabinogalactan-proteins increased after enzymatic treatment in a Tempranillo wine. On the other hand, Ducasse et al.[9] showed that the effect of enzymes on PRAGs depended on the vintage factor in Merlot wines. Climate, which represents one of the most important vintage and terroir factors, can result in differences between grapes and the resulting wines.[43]

Concerning MPs, there were no differences whether commercial enzyme was used in wine-making (Cañada Judío, 147 mg/L; Albatana, 126 mg/L; Bullas, 158 mg/L; and Montealegre, 204 mg/L) or not (JUCO, 132 mg/L; ALCO, 139 mg/L; BUCO, 126 mg/L; and MTCO, 185 mg/L).

The obtained results when commercial enzyme was added were similar, in general, to the results obtained by other authors in wines made from different grape varieties.[3,8,9] Hellín et al.[44] observed that enzyme addition led to a release of homogalacturonans. Our commercial enzyme had, among others, polygalacturonase and pectin methylesterase activities. Using endopolygalacturonase in combination (or not) with pectin methylesterase, polysaccharides (RG-I) have been directly isolated from purified vegetal cell walls.[45] Hilz et al.[46] have isolated RG-II with endopolygalacturonase from purified vegetal cell walls. In the same way, Arnous and Meyer[31] observed that commercial enzymes, with polygalacturonase as their principal activity, increase the amount of monosaccharides and galacturonic acid released from Cabernet Sauvignon and Merlot grape skins. The presence of polygalacturonase involves a weakening of cell wall structures into parallel sheets, probably as a result of its attack on homogalacturonans. Its action seems to more strongly affect the primary cell wall, although the middle lamella is also attacked when polygalacturonase is used in combination with pectin methylesterase.[47] This could contribute to grape cell wall degradation in some of our terroir samples. The precise way of acting might depend on the original composition of grapes, and terroir has been seen to influence this, as demonstrated by several authors. For example, Ortega-Regules et al.[18] observed differences in sugar composition and the amount of natural enzymatic activities in grapes skins when they compared Cabernet Sauvignon, Syrah, and Monastrell grapes. Carey et al.[48] found that aroma and the fullness of mouth-feel of Cabernet Sauvignon wines, among other factors, were affected by soil and climate parameters.

RG-II concentrations did not differ when galactosidase was added (JUGE, 275 mg/L; ALGE, 320 mg/L; BUGE, 205 mg/L; and MTGE, 422 mg/L) as compared to control wine levels, and neither did PRAGs (JUGE, 256 mg/L; ALGE, 283 mg/L; BUGE, 337 mg/L; and MTGE, 296 mg/L) nor MPs (JUGE, 122 mg/L; ALGE, 129 mg/L; BUGE, 151 mg/L; and MTGE, 176 mg/L).

Figure 4 shows that there were no significant differences in any of the wine polysaccharide family members arising from the pecto-cellulosic cell walls of grape berries, regardless of terroir. Neither did galactosidase enzyme addition change the optimal conditions for fermentative yeast, as can be deduced by the fact that MPs did not change between treated and control samples. Although Lazan et al.[49] have suggested that galactosidase is more responsible for fruit softening than polygalacturonase or pectin methylesterase, our results suggested that such enzymatic activity has no effect on polysaccharide extraction.

With regards to the cold technologies used, there were no significant differences in MP concentrations in prefermentative cold maceration treated wines (JUCM, 146 mg/L; ALCM, 136 mg/L; BUCM, 170 mg/L; and MTCM, 163 mg/L) or those with dry ice added (JUIA, 123 mg/L; ALIA, 118 mg/L; BUIA, 141 mg/L; and MTIA, 185 mg/L) with respect to control wines (JUCO, 132 mg/L; ALCO, 139 mg/L; BUCO, 126 mg/L; and MTCO, 185 mg/L).

Likewise, no statistically different RG-II concentrations were detected in Cañada Judío, Albanata, and Montealegre terroir wines after prefermentative cold maceration (JUCM, 282 mg/L; ALCM, 320 mg/L; and MTCM, 480 mg/L) or after dry ice treatment (JUIA, 275 mg/L; ALIA, 256 mg/L; and MTIA, 393 mg/L), with respect to control wines (JUCO, 282 mg/L; ALCO, 256 mg/L; and MTCO, 378 mg/L).

The same may be said for PRAG concentrations after the prefermentative cold maceration treatment (JUCM, 294 mg/L; ALCM, 340 mg/L; and MTCM, 306 mg/L) or dry ice addition (JUIA, 245 mg/L; ALIA, 256 mg/L; and MTIA, 270 mg/L), in comparison with the control wines (JUCO, 258 mg/L; ALCO, 289 mg/L; and MTCO, 262 mg/L).

On the other hand, while Bullas terroir wine elaborated with prefermentative cold maceration showed a significant increase in its RG-II (397 mg/L) and PRAG (388 mg/L) concentration with respect to the control wines (RG-II, 192 mg/L; and PRAGs, 291 mg/L), no differences in RG-II (237 mg/L) or PRAGs (317 mg/L) with respect to the control wine were

observed after the addition of dry ice. Differences in the extractability index have been found in Monastrell grapes from two different terroirs,[18] and 78% of the index was found to be explained by different grape skin cell wall compositions when used as independent variables.[17]

Table 3 shows the arabinose/galactose ratio in treated and control wines. This ratio is characteristic of wine PRAGs.[9] There were no significant differences between treated and control wines in any studied treatment, suggesting that there was no effect on the composition of released polysaccharides, regardless of applied treatment or grape origin. This contrasts with the results obtained by Doco et al.,[8] who observed a decrease in arabinose in PRAGs when enzymes were added. Ducasse[50] also observed an increase in MP and PRAG concentrations and a decrease in RG-II in Merlot wine after a 24 h prefermentative cold maceration step, as compared to a 12 h prefermentative cold maceration. This different behavior could be explained by the different composition of grapes of the different cultivars used.

A terroir effect on polysaccharide concentrations has been demonstrated, in particular with regards to the RG-II concentration. The addition of commercial enzyme increased the amount of released polysaccharides in wines, changing their composition with respect to control wines. These modifications were also linked with grape origin. The use of galactosidase enzyme did not change the quantity of polysaccharides released in wine, although there were some composition differences due to grape origin. Cold prefermentative maceration increased the amount of released polysaccharides in wine, changing its composition with respect to the control wine. On the other hand, dry ice addition had no effect on the quantity or composition of the polysaccharides released. It would therefore seem advisable to adapt the wine-making treatment, not only according to the grape variety used but also according to the origin of the grapes.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Monthly mean temperature (°C) and rainfall (mm), monthly mean maximum (max) and minimum (min) temperature (°C), monthly mean relative humidity (%), monthly mean maximum (max) and minimum (min) relative humidity (RH) (%), monthly mean wind speed (m/s), and monthly mean maximum wind speed (m/s) in Cañada Judío, Albatana, Bullas, and Montealegre terroirs, between September 2007 and October 2008. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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