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Polysaccharide Profile and Content during the Vinification and Aging of Tempranillo Red Wines

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Passing from must to wine produced a loss of low-molecular-weight grape structural glucosyl polysaccharides, and an important gain in yeast mannoproteins (MP) and grape-derived arabinogalactan proteins (AGP), and rhamnogalacturonans-II (RG-II). AGP were more easily extracted than RG-II, and small quantities of RG-II monomers and galacturonans were detected. Postmaceration produced a reduction in all grape polysaccharide families, particularly acute in AGP. The reduction of polysaccharides during malolactic fermentation only affected grape AGP, and MP were continuously liberated during the entire vinification process. Wine oak and bottle aging was associated with a relative stability of the polysaccharide families. AGP were thus the majority polysaccharides in young wines but, contrary to what may be thought, structural glucosyl oligosaccharides dominated in musts and MP in aged wines. Precipitation of polysaccharides was noticeable during vinification, and it mainly affected high-molecular-weight AGP and MP. Hydrolytic phenomena affected the balance of wine polysaccharides during late maceration-fermentation.

KEYWORDS: Red wine; winemaking; grape and yeast polysaccharides; arabinogalactan-proteins; homogalacturonans; rhamnogalacturonans II; mannoproteins.

INTRODUCTION

Must and wine polysaccharide analysis is of great interest because they have an important influence on several stages of the winemaking process such as must racking, fermentation, wine filtration, and wine stabilization (*1–5*). Moreover, these compounds affect the organoleptic properties of red wines (*6–9*), which are very important for the final quality of the product.

Two criteria widely used for the discrimination of polysaccharide families are acidity and protein content. Grape neutral pectic substances mainly comprise type II arabinogalactans or arabinogalactan–proteins (AGP). These compounds, which represent more than 40% of total red wine polysaccharides (*10, 11*), consist of a core structure of (1 → 3)-β-D-galactopyranose chains with (1 → 6) linked β-D-galactan side chains highly substituted by arabinofuranosyl residues (*10*). Grape acidic pectic polysaccharides, characterized by a high proportion of galacturonic acid, involve homogalacturonans (GL), rhamnogalacturonans I (RG-I), and rhamnogalacturonans II (RG-II). From the structural point of view, RG-II is the most remarkable of these polysaccharides due to its highly conserved structure. Type II rhamnogalacturonans are (1 → 4)-α-D-galacturonans branched with four different side chains containing some rare sugars that allow their identification and quantification (*11–13*). RG-II is usually found in cell walls and fruit juices in the form of dimers cross-linked by lead-diol esters (*14, 15*). Mannoproteins (MP) produced by yeasts are the second most abundant family of polysaccharides in wine (*11*). These polymers, with highly variable sizes, are almost pure mannans with a variable protein content (*11, 16*) and can be released by yeast in the early stages of fermentation or later on during aging on lees (*17*).

Not all polysaccharides show the same behavior with respect to wines; their influence on wine processing will depend not only on the quantity of polysaccharidic compounds but also on their structure, composition, and distribution. Some authors have even identified the importance of the type of polysaccharide on such wine characteristics. In particular, it has been shown that AGP have greater influence on the filtration procedures than MP (*18*), which are more efficient at reducing protein haze in white wines (*3, 19, 20*). RG-I and -II inhibit hydrogen tartrate crystallization, (*1*) whereas AGP do not affect this phenomenon (*18*). Among the MP classes present in wine, some have been found to act as protective factors with regard to tartaric acid precipitation (*1, 3*). Besides, it has also been shown that RG-II is responsible for borate complexation to the extent that most lead present in cell walls and wine would be bound to RG-II dimers (*2, 21*). There are also important differences with regard to the quality of the organoleptic characteristics of red wines. It has recently shown that wine RG-II dimer favors the self-aggregation of grape seed proanthocyanidins in winelike solutions, whereas wine MP and acidic AGP tend to inhibit tannin aggregation (*7*) and therefore have a different influence on wine astringency and fullness (*8*). Interactions between aroma compounds and MP have also been described (*6, 9*).

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Given the importance of must and wine polysaccharides, an understanding of their content and release kinetics is essential. It is widely known that pectic polysaccharides are liberated from grape skins and pulp during grape maturation and during the first steps of winemaking and that parietal mannoproteins are released in the wine during and after alcoholic fermentation. Several studies, mainly in white wines, have been performed in order to analyze the evolution of total polysaccharides during the winemaking process, and previous studies have included the evolution of concrete polysaccharide families during bottle storage of red wines (*22*). However, little is known about the behavior of the different types of polysaccharides during the winemaking process. Hence, the aim of this paper is to analyze the changes occurring on must and wine polysaccharide families during the different stages of red wine processing, including maceration-fermentation and postmaceration, malolactic fermentation, and oak aging and bottle aging.

MATERIALS AND METHODS

Vinification and Sample Collection. Mature Tempranillo grapes were harvested from Autol, La Rioja, Spain, at 21.9 Brix, pH 3.56, and 6.02 g tartaric acid/L. Three experimental vinifications were carried out in the wine cellar of the University of La Rioja, and wines were prepared by traditional wine technology. Grapes were destemmed, crushed, and fermented into 100 L stainless steel tanks. The prefermentation process went on for 6 h at 18 ± 1 °C; the fermentation-maceration process was carried out at a maximum temperature of 28 ± 2 °C and lasted 10 days. Postfermentative maceration went on for 4 days at 24 ± 1 °C, and wines were run off. Wines were then inoculated with a commercial preparation of *Oenococcus oeni* (1g/HL) to induce malolactic fermentation, carried out at 18.5 ± 1 °C. After 20 days of malolactic fermentation, all the wines were racked and clarified by settling for 25 days at 10 °C. Wine aging was performed in new 13 L American oak barrels, which have a larger area/volume than traditional 225 L barrels. For this reason, and on the basis of organoleptic analysis, the oak aging process went out for only 45 days. Wines were then bottled and stored at 4 °C.

Samples were taken at the beginning of the maceration-fermentation (0AF), during the maceration-fermentation (25–30% of sugars consumed, 55–60% of sugars consumed, and 99% of sugars consumed, namely, 30AF, 60AF, and 99F, respectively), and at the beginning and end of malolactic fermentation (BMF, EMF). Sample bottles were filled completely to minimize oxygen contact and immediately frozen at −18 °C. Samples were also analyzed at the beginning and end of wine oak aging (BOA, EOA) and after two years of wine bottle aging (BA).

Isolation of Must and Wine Polysaccharides. Samples were homogenized, and 400 mL was taken with a peristaltic pump and centrifuged. The insoluble pellets were recovered and precipitated with 5–10 mL of cold 96% ethanol containing 0.3 M HCl (*23*). After 18 h at 22 °C, the samples were centrifuged and the pellets obtained were washed in ethanol 96% several times and freeze–dried (*23*). The residues obtained (fractions I) contained the insoluble polysaccharides. The supernatants were first concentrated (five times for wines and three times for musts) under reduced pressure at 34 °C and were then precipitated by adding four volumes of cold ethanol containing 0.3 M HCl and kept for 18 h at 4 °C (*23*). Thereafter, the samples were centrifuged, the supernatants were discarded, and the pellets were washed with 96% ethanol. The precipitates were finally dissolved in ultrapure water and freeze–dried. The freeze–dried precipitates obtained (fractions S) contained the soluble polysaccharides.

Monosaccharide composition in fractions S and I was determined by gas–liquid chromatography as described bellow. Protein concentration was determined using the procedure described by Lowry et al. (*24*) with bovine serum albumin as standard.

Molecular Weight Distribution of Must and Wine Polysaccharides. To obtain the molecular weight distribution of must and wine polysaccharides, the soluble fractions S and the insoluble fractions I were subjected to high-resolution size-exclusion chromatography (HRSEC) on two serial Shodex OHpack KB-803 and KB-805 columns (30 × 0.8 cm, Showa Denko, Japan) equilibrated at 1 mL/min in 0.1 M $LiNO_3$. Chromatographic separation was carried out at room temperature and calibration was performed with narrow pullulan molecular weight standards (P-5, M_w = 5900 D; P-10, M_w = 11 800 D; P-20, M_w = 22 800 D; P-50, M_w = 47 300 D; P-100, M_w = 112 000 D; P-200, M_w = 212 000 D; P-400, M_w = 404 000 D).

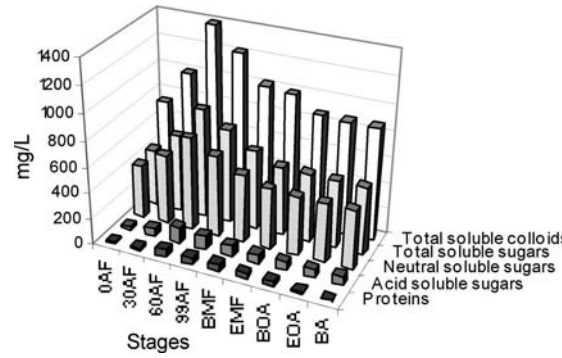

Figure 1. Analysis of soluble fractions S. Evolution of total soluble colloids, total, neutral, and acid soluble sugars, and proteins, during vinification and aging. See text for conditions and calculations.

Fractionation of Must and Wine Soluble Polysaccharides by HRSEC. To separate the different polysaccharide families, the soluble fractions S were subjected to high-resolution size-exclusion chromatography on a Superdex-75 HR column equilibrated at 0.6 mL/min in 30 mM ammonium formiate, pH 5.8 (*23*). The peaks obtained were collected in different fractions (S1, S2, and S3) according to their elution times. The isolated fractions were freeze–dried, redissolved in water, and freeze–dried again several times to remove the ammonium salt (*23*).

Identification and Quantification of Must and Wine Polysaccharides by GC and GC-MS. The carbohydrate composition of the insoluble fractions I and soluble fractions S, S1, S2, and S3 was determined by GC with flame ionization detector and GC-MS of their trimethylsilyl-ester *O*-methyl glycosyl residues obtained after acidic methanolysis and derivatization as previously described (*23*). Total soluble sugars were calculated in fractions S as the sum of all individual sugars, and neutral and acid soluble sugars were calculated as the sum of neutral and acid sugars, respectively. Total insoluble sugars were calculated in fractions I as the sum of all individual sugars. Polysaccharide families were quantified in fractions S1, S2, and S3 from the concentration of individual glycosyl residues characteristic of well-defined wine polysaccharides (*22*, *23*).

Statistical Procedures. Vinifications and analysis were performed in triplicate. Significant differences between samples were analyzed with the SPSS 12.0 program for Microsoft Windows (SPSS Inc., Chicago, IL). Monosaccharide and polysaccharide content values were analyzed by a one-way analysis of variance (ANOVA) with repeated measurements to test the effect of the vinification stage, if the data adhered to assumptions of normality. If these assumptions were not adhered to, a Kruskal–Wallis test was used. In this paper, whenever we refer to differences between samples, we are referring to significant differences with at least $p < 0.05$.

RESULTS AND DISCUSSION

Analysis of Soluble Fractions S. Evolution of total soluble colloids, total soluble sugars, and their constituents, i.e., neutral and acid sugars, and proteins, in the fractions S during the vinification and aging are shown in **Figure 1**. Total soluble sugars accounted for about 60% of total soluble colloids in all the stages analyzed; the rest were attributed to other compounds such as salts, proteins, or phenolics. In all the stages, neutral soluble sugar content was considerably higher than that of acid soluble sugars, which represented only between 6 and 15% of total soluble sugars. The values obtained were quite similar to those obtained by our workgroup when a colorimetric method

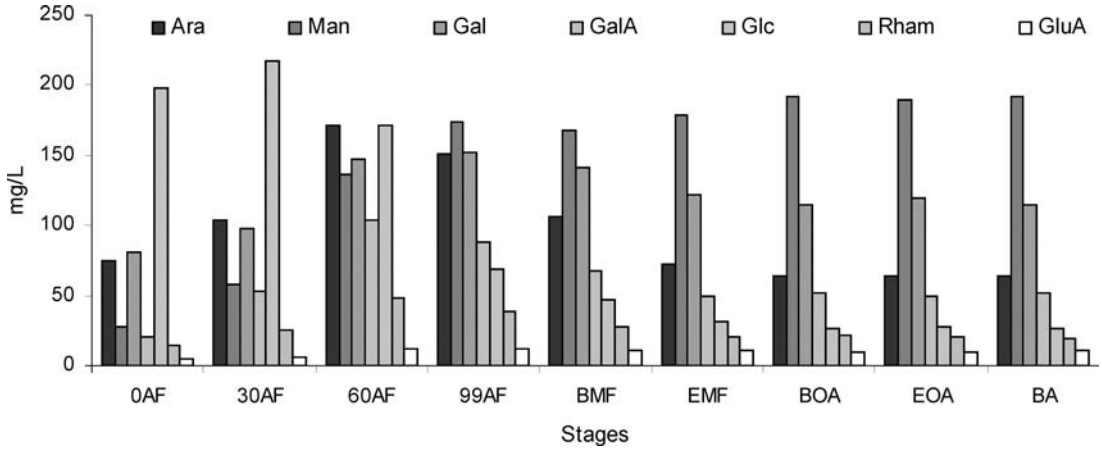

Figure 2. Evolution of major glycosyl residues in the soluble fractions S during vinification and aging. Ara, arabinose; Man, mannose; Gal, galactose; GalA, galacturonic acid; Glc, glucose; Rham, rhamnose; GluA, glucuronic acid. See text for conditions and calculations.

was used for quantification instead of capillary GC (*25*). This finding confirmed that both methods were reliable for measuring must and wine carbohydrates, although the latter was chosen in the present study because it gave information on individual glycosyl composition.

Red winemaking increased the concentration of total soluble colloids and sugars; maceration-fermentation was the main process affecting this content (**Figure 1**). The concentration of total soluble sugars increased progressively by 90% between 0 and 4 days (60AF), reaching more than 800 mg/L, but decreased substantially at the end of the maceration-fermentation and during postmaceration and malolactic fermentation, indicating that during these periods the precipitation rate of polysaccharidic compounds was higher than their solubilization. Proteins, which accounted for less than 6% of total colloids, increased from 17 to 60 mg/L during early maceration-fermentation and then stabilized until malolactic fermentation. During oak and bottle aging, soluble sugar content was maintained, reaching values usually found in other red varieties. However, the protein content was drastically reduced during oak aging (∼65%), which might be due to the well-known phenomenon of formation of wine tannin–protein insoluble complexes during this period.

Important differences were observed in glycosyl residue patterns of soluble fractions between wine and must samples (**Figure 2**). Glucose was the most prevalent sugar detected in must samples, representing more than 40% of total soluble sugars. Although its origin in must is not clear in the bibliography, it has been shown that glucose is the prevalent sugar in both the skin and pulp cell walls of grape berries (*26*) because it is the main component of major structural polysaccharides from grape cell walls such as cellulose and hemicellulosic xyloglucans, arabinoglucans and mannans. The large amount of glucose in musts would therefore be attributed to the partial solubilization of these components and to the solubilization of complexes between them and pectic polysaccharides, which have been reported to occur in cell walls from both grape pulp and skin tissues (*27*). Xylose was found to be the most prevalent among the minor sugars detected in musts (data not shown), confirming the presence of hemicellulosic xyloglucans and arabinoxylans. Other sugars detected in musts were arabinose, galactose, and rhamnose, the glycosyl residues found in AGP, and mannose, the main component of MP.

Soluble sugar content and profile changed as the maceration-fermentation process went on (**Figure 2**). The content of glucose slightly increased in the early maceration-fermentation, but it was significantly reduced later, reaching final values of less than 50 mg/L at the end of maceration. However, the other sugars detected in musts behaved in the opposite manner, and their concentrations increased significantly during maceration-fermentation to more than double at the end. The greatest increase was observed in the case of galacturonic acid and mannose, whose concentrations increased 4- and 6-fold, respectively. Mannose, galactose, and arabinose were thus the most prevalent sugars in wines at the end of alcoholic fermentation (24, 21, and 20%, respectively), followed by galacturonic acid and glucose (12 and 9%, respectively). During postmaceration and malolactic fermentation, a significant change was once again observed in the sugar profile because there was a significant decrease in all the glycosyl residues except for mannose. Thus, wines after these stages were mainly composed of mannose (33%), followed by galactose (23%) and arabinose (14%), whose molar ratio arabinose/galactose decreased from 1 to 0.6. No noteworthy changes were observed in sugar composition during wine oak and bottle aging.

Analysis of Insoluble Fractions I. The carbohydrate composition of the insoluble fractions I was also studied (**Table 1**) in order to determine the extent of precipitation occurring during the winemaking process and the type of polysaccharides precipitating.

The amount of sugars in the insoluble fractions was substantially high during maceration-fermentation, mainly during the early stages, when total insoluble sugars represented more than 30% of total sugars, i.e., the sum of sugars of soluble and insoluble fractions. Among the sugars present in the insoluble fractions, and as in the case of soluble sugars, acid residues represented only a small percentage. Glucose was the main sugar detected during maceration-fermentation, representing more than 90% in the early stages. This finding confirmed that an important amount of grape structural glucosyl polysaccharides were extracted immediately after grape crushing (>500 mg/L of glucose in 0AF of fractions S and I), although their solubilization was limited, and more than 60% of these compounds were unstable and precipitated, being detected in the insoluble fractions. Mannose, arabinose, galactose, and galacturonic acid were also detected in insoluble fractions during maceration-fermentation, indicating a precipitation of other polysaccharide families such as MP, AGP, and galacturonans. During malolactic fermentation, mannose was the main sugar detected, followed closely by glucose, which seemed to indicate that the precipitation of polysaccharides during this period mainly affected MP or other microorganism cell wall polysaccharides. The insoluble fractions of wines after malolactic fermentation contained all

Table 1. Carbohydrate Composition (mg/L) of Insoluble Fractions I of Must and Wine Samples Determined by GC and GC-MS of their TMS Derivatives[a]

	vinification stages								
sugars	0AF	30AF	60AF	99AF	BMF	EMF	BOA	EOA	BA
aceric acid	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*
2-*O*-M Fuc[c]	1.51 ± 0.03	1.87 ± 0.04	1.31 ± 0.03	2.38 ± 0.05	*b*	*b*	*b*	*b*	*b*
2-*O*-M Xyl[c]	1.03 ± 0.04	1.05 ± 0.04	0.92 ± 0.04	1.19 ± 0.05	0.28 ± 0.02	0.31 ± 0.01	*b*	*b*	*b*
apiose	0.45 ± 0.02	0.27 ± 0.01	0.16 ± 0.01	0.25 ± 0.01	0.66 ± 0.03	0.73 ± 0.03		*b*	*b*
arabinose	6.1 ± 0.2	4.2 ± 0.1	3.86 ± 0.12	3.07 ± 0.09	4.5 ± 0.1	5.0 ± 0.2	5.6 ± 0.2	1.07 ± 0.03	1.01 ± 0.03
rhamnose	0.82 ± 0.07	1.3 ± 0.1	1.36 ± 0.11	1.04 ± 0.08	1.02 ± 0.08	1.14 ± 0.09	0.18 ± 0.01	0.24 ± 0.02	0.23 ± 0.02
fucose	0.13 ± 0.01	0.12 ± 0.01	0.18 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	*b*	*b*	*b*
xylose	0.51 ± 0.03	0.58 ± 0.03	0.45 ± 0.03	0.40 ± 0.02	0.22 ± 0.01	0.24 ± 0.01	*b*	*b*	*b*
mannose	8.1 ± 0.2	32.2 ± 0.9	48.3 ± 1.3	31.0 ± 0.9	42.2 ± 2.0	47.1 ± 3.3	1.14 ± 0.03	1.47 ± 0.04	1.39 ± 0.04
Dha[c]	1.12 ± 0.04	*b*	*b*	0.77 ± 0.03	0.27 ± 0.01	0.30 ± 0.03	*b*	*b*	*b*
galactose	4.5 ± 0.2	4.45 ± 0.21	3.8 ± 0.2	3.7 ± 0.2	5.2 ± 0.5	5.8 ± 0.3	0.53 ± 0.03	1.45 ± 0.07	1.37 ± 0.06
GalA[c]	3.1 ± 0.2	5.2 ± 0.4	4.2 ± 0.3	4.0 ± 0.3	2.8 ± 0.2	3.1 ± 0.2	0.44 ± 0.03	0.53 ± 0.04	0.50 ± 0.03
glucose	319.8 ± 7.3	249.9 ± 5.7	136.4 ± 3.1	78.8 ± 1.8	32.6 ± 2.7	36.3 ± 2.9	1.64 ± 0.04	1.08 ± 0.02	1.02 ± 0.02
GlcA[c]	0.98 ± 0.06	0.85 ± 0.05	0.93 ± 0.05	0.86 ± 0.05	1.39 ± 0.08	1.55 ± 0.09	0.68 ± 0.04	0.24 ± 0.01	0.23 ± 0.01
Kdo[c]	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*
total[d]	348 ± 7	302 ± 6	202 ± 3	128 ± 2	91.2 ± 3.4	102 ± 4.4	10.3 ± 0.2	6.3 ± 0.1	5.93 ± 0.09

[a] Average values of 3 replicates (mean ± SD). [b] <0.1 mg/L. [c] 2-*O*-M Fuc, 2-*O*-methyl fucose; 2-*O*-M Xyl, 2-*O*-methyl xylose; Dha, 3-deoxy-D-*lyxo*-heptulosaric acid; GalA, galacturonic acid; GlcA, glucuronic acid; Kdo, 3-deoxy octulosonic acid. [d] Calculated as the sum of individual sugars (mg/L).

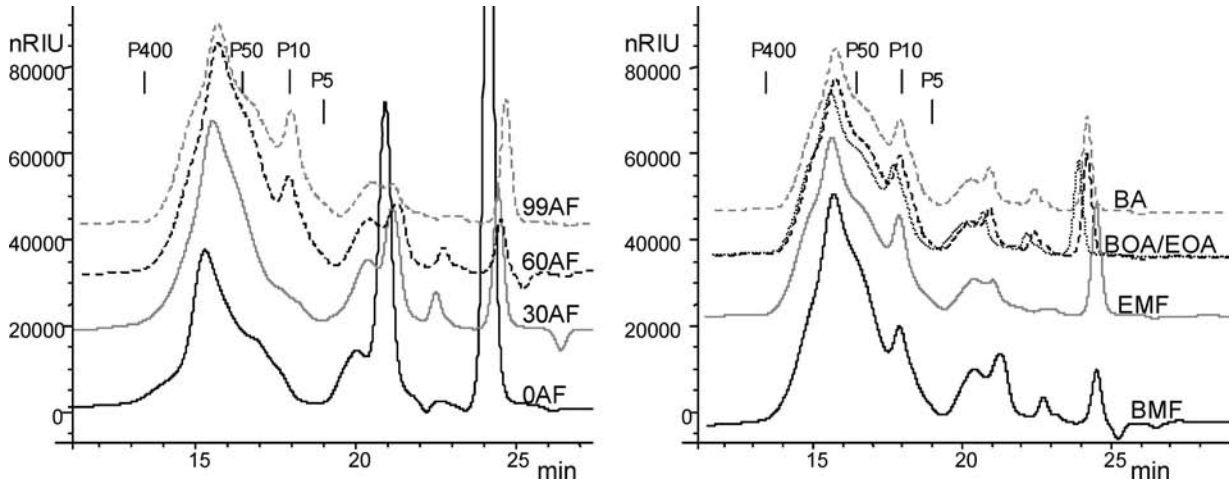

Figure 3. Molecular weight distribution of soluble fractions S by HRSEC on Shodex columns. **(a)** Evolution during maceration-fermentation, and **(b)** evolution during malolactic fermentation and oak and bottle aging. Elution times of pullulan standards (P5 → P400) are also shown.

the sugars known to participate in the composition of AGP, MP, and GL. However, the insolubilization of polysaccharides was a minor phenomenon in these stages because the sugars present in the insoluble fractions represented less than 3% of total wine sugars.

Analysis of Molecular Weight Distribution of Soluble and Insoluble Polysaccharides. HRSEC on Shodex columns of soluble and insoluble fractions S and I from must and wine samples allowed us to follow the qualitative changes in the molecular weight distribution of both soluble and insoluble polysaccharides.

In 0AF must samples, the distribution of soluble polysaccharides was characterized by the presence of three major populations, eluting at approximately 15.4, 20.9, and 24.1 min (**Figure 3a**). The population eluting at 15.4 min corresponded to molecules with molecular weight between P50 (47.3 kD) and P400 (404 kD), and it was mainly attributed to the presence of arabinogalactans or arabinogalactan–proteins because previous studies had shown that the apparent molecular weight of AGP isolated from wine ranged from 48 to 262 kD (*10, 11*). The other two populations, with molecular weights below P5 (5.9 kD), were attributed to oligosaccharides and low-molecular-weight fragments of larger macromolecules. Passing from must to wine was characterized by a progressive increase in the area of the first population, which was thought to be due not only to an enrichment of AGP but also to a progressive appearance of yeast mannoproteins, with highly variable sizes ranging from 5 to 800 kD (*17*). A signal eluting at 17.4 min was observed in samples taken in advanced fermentation (60AF). This population, not clearly defined in must samples, corresponded to molecules with an average molecular weight of P10 (11.8 kD). According to previously published data, these molecules corresponded to rhamnogalacturonan type II dimers (RG-IId), with an average molecular weight of 10–12 kD (*12, 28*), and to low-molecular-weight AGP and MP (*22, 23*). As the vinification process went on, changes in the areas of the signals could be observed (**Figure 3b**), indicating that transformations in the polysaccharide quantities were occurring. During oak and bottle aging, chromatograms were almost superimposable, showing no evolution during this period.

The molecular weight distribution of insoluble polysaccharides (fractions I) was also different depending on the vinification stage (**Figure 4**). From 0AF to 60AF, the distribution of insoluble polysaccharides was characterized by the presence of several signals eluting after 18 min (<P5). Contrary to what was thought earlier, this fact seemed to indicate that the insoluble polysaccharides in musts were in fact low-molecular-weight oligosaccharides. At the end of maceration-fermentation, the

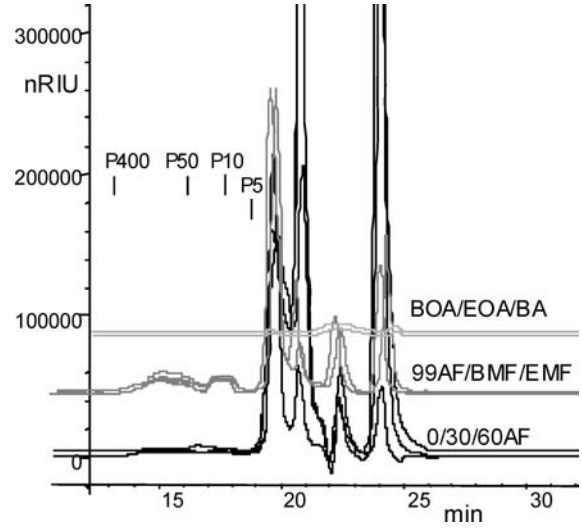

Figure 4. Molecular weight distribution of insoluble fractions I by HRSEC on Shodex columns. Elution times of pullulan standards (P5 → P400) are also shown.

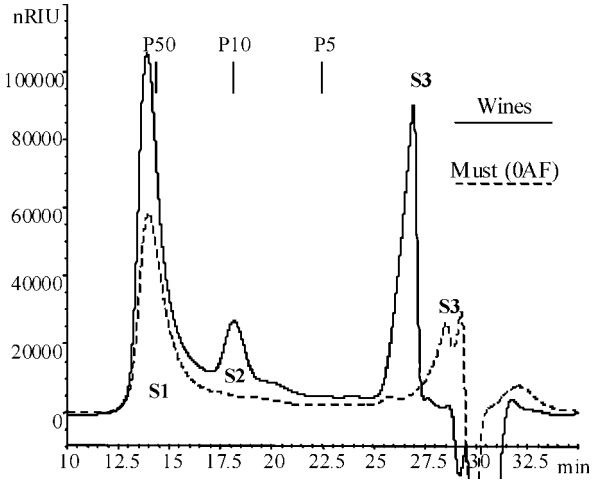

Figure 5. Molecular weight distribution of fractions S1, S2, and S3 by HRSEC on a Superdex 75-HR column. Elution times of pullulan standards (P5 → P50) are also shown.

low-molecular-weight peaks had reduced significantly, and a population of molecules ranging from P50 (47.3 kD) to P400 (404 kD) was clearly observed. The analysis of chromatograms of the insoluble fractions during postmaceration and malolactic fermentation revealed similar patterns, and insolubilization of molecules ranging from P50 to P400 was also observed. As expected, no significant signals were seen in HRSEC profiles of insoluble fractions during oak and bottle aging.

Fractionation of Must and Wine Soluble Polysaccharides by High-Resolution Size-Exclusion Chromatography. Soluble fractions S were injected on a Superdex 75-HR column in order to separate the different polysaccharide families. This prepacked column, with a molecular weight range from 3 to 75 kD, enabled the separation of soluble polysaccharides into different fractions. Chromatograms revealed a fractionation of compounds into three peaks, S1, S2, and S3, similar to that previously described (*23*), except for 0AF must, which showed only two peaks, S1 and S3 (**Figure 5**). The results obtained revealed a fractionation of compounds similar to that obtained with the Shodex columns.

Sugar Composition of Fractions S1, S2, and S3. Glycosyl residue composition of must and wine fractions S1, S2, and S3 obtained after HRSEC fractionation is shown in **Tables 2**, **3**, and **4**, respectively. It was remarkable that in all the cases important differences were again observed between must samples (0AF and 30AF) and wine samples, the 60AF sample appearing in a middle position between musts and wines.

Sugars in fractions S1 of wines accounted for more than 50% of total soluble sugars, i.e., sugars obtained by direct injection of fractions S in GC (**Figure 1**), while sugars in the second fraction represented about 30% and sugars in S3 were less than 20%. However, sugars of fractions S1, S2, and S3 of 0AF and 30AF must samples represented less than 30%, 10% and 8%, respectively, of total soluble sugars. Hence, sugars obtained after HRSEC fractionation represented around 100% of total soluble sugars in wine samples, but they were only 39–43% in must samples, mainly due to the glucose content, which was less than 15% in must fractions S1, S2, and S3 when compared with that obtained by direct injection of fractions S. This fact indicated that the majority of soluble polysaccharides in musts were basically low-molecular-weight oligosaccharides (<3 kD) because they eluted after 30 min and were not included in the fractionation range of the Superdex 75-HR column. In contrast to the descriptions contained in the bibliography (*29*, *30*), major must polysaccharides were in fact fragments of cellulose and hemicellulose, which would be easily extracted from grape cell walls during grape maturation and crushing and during early maceration-fermentation. However, they would be highly unstable and would precipitate, and it became apparent that must insoluble fractions, mainly composed of glucose (**Table 1**), showed only low-molecular-weight populations (**Figure 4**). Therefore, passing from must to wine produced a precipitation of extracted low-molecular-weight grape structural glucosyl polysaccharides, and an important enrichment of larger-sized polysaccharides, collected in fractions S1 and S2.

Wine fractions S1 mainly comprised arabinose, galactose, and mannose (**Table 2**), confirming the predominance of AGP and MP among wine polysaccharides. The composition of fractions S2 was more complex, and all the rare diagnostic sugars of the RG-II molecule were detected (**Table 3**), confirming the presence of this polysaccharide. However, as previously observed (*22*, *23*), the molar ratios of rhamnosyl, arabinosyl, galactosyl, and glucuronosyl residues were greater than expected for a purified RG-II molecule, and mannose was also present in this fraction, indicating the presence of low-molecular-weight AGP and MP. Fractions S3 contained all the sugars known to participate in the composition of wine polysaccharides, but they were present only in small amounts (**Table 4**). Galacturonic acid, galactose, arabinose, rhamnose, glucose, and mannose largely dominated this fraction and were attributed to the presence of homo- and rhamnogalacturonans oligomers (GL) and low-molecular-weight fragments of AGP, MP, and other glycosylated compounds. Unlike musts, the small proportion of glucose in wine samples was mainly attributed to the presence of condensed anthocyanins or microbial polysaccharides. As previously observed by our work group (*23*), rare sugars were also detected in S3 fractions except for must samples. These residues were attributed to RG-II monomers (mRG-II) on the basis of the molecular weight of the eluted fraction. The presence of the monomeric form of the RG-II in wines is still poorly understood as RG-II has been traditionally described as being mainly dimeric in cell walls (*31*), in fruit juices obtained by liquefaction (*15*), and in wines (*28*). However, RG-II monomer has been recently detected in polysaccharides solubilized from grape pulp tissue and also in red wines (*11*, *26*). Anyway, we cannot rule out the hypothesis that monomeric RG-II could be

Table 2. Carbohydrate Composition (mg/L) of Must and Wine Fractions S1 Obtained by HRSEC on a Superdex-75 HR Column and Determined by GC and GC-MS of their TMS Derivatives[a]

	vinification stages								
sugars	0AF	30AF	60AF	99AF	BMF	EMF	BOA	EOA	BA
arabinose	21.8 ± 1.1	28.5 ± 1.2	76.0 ± 2.9	77.9 ± 5.4	58.5 ± 1.4	42.4 ± 0.8	33.4 ± 0.4	28.5 ± 0.3	27.4 ± 0.4
rhamnose	6.0 ± 0.2	8.8 ± 0.1	13.2 ± 0.1	7.63 ± 0.02	5.9 ± 0.1	3.5 ± 0.3	3.0 ± 0.1	2.6 ± 0.2	2.3 ± 0.2
fucose	0.15 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	*b*	0.10 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.18 ± 0.01
xylose	0.81 ± 0.03	0.93 ± 0.02	1.4 ± 0.1	0.93 ± 0.01	1.74 ± 0.07	1.07 ± 0.05	1.19 ± 0.06	0.84 ± 0.03	0.92 ± 0.02
mannose	17.0 ± 0.9	30.0 ± 1.3	99.4 ± 1.0	122 ± 2	138 ± 2	146 ± 1	147 ± 4	138 ± 3	142 ± 3
galactose	61.6 ± 5.0	63.8 ± 4.1	123 ± 2	101 ± 3	102 ± 2	85.2 ± 1.0	76.1 ± 2.8	68.7 ± 1.7	71.2 ± 1.3
GalA[c]	7.4 ± 0.2	12.0 ± 1.0	15.8 ± 0.9	8.2 ± 0.2	4.9 ± 0.3	2.3 ± 0.2	1.24 ± 0.06	1.12 ± 0.08	1.32 ± 0.06
glucose	10.3 ± 0.3	7.9 ± 0.5	12.2 ± 0.8	9.3 ± 0.2	10.7 ± 0.4	9.8 ± 0.9	7.9 ± 0.2	13.2 ± 0.6	11.9 ± 0.5
GlcA[c]	3.2 ± 0.1	3.40 ± 0.05	7.3 ± 0.2	6.6 ± 0.7	6.3 ± 0.2	5.1 ± 0.1	5.15 ± 0.07	4.2 ± 0.1	3.9 ± 0.1

[a] Average values of 3 replicates (mean ± SD). [b] <0.1 mg/L. [c] GalA, galacturonic acid; GlcA, glucuronic acid.

Table 3. Carbohydrate Composition (mg/L) of Must and Wine Fractions S2 Obtained by HRSEC on a Superdex-75 HR Column and Determined by GC and GC-MS of their TMS Derivatives[a]

	vinification stages								
sugars	0AF	30AF	60AF	99AF	BMF	EMF	BOA	EOA	BA
aceric acid	*b*	0.54 ± 0.01	1.49 ± 0.03	3.4 ± 0.2	3.15 ± 0.06	3.40 ± 0.06	3.28 ± 0.04	3.17 ± 0.02	3.0 ± 0.2
2-*O*-M Fuc[c]	b	0.45 ± 0.01	1.38 ± 0.07	2.71 ± 0.04	2.57 ± 0.09	2.5 ± 0.1	2.2 ± 0.1	2.60 ± 0.06	2.81 ± 0.06
2-*O*-M Xyl[c]	*b*	0.53 ± 0.01	1.29 ± 0.01	3.09 ± 0.01	2.89 ± 0.05	3.2 ± 0.2	2.74 ± 0.06	2.8 ± 0.2	3.0 ± 0.2
apiose	*b*	0.52 ± 0.01	1.13 ± 0.01	3.23 ± 0.08	3.22 ± 0.05	3.36 ± 0.03	2.79 ± 0.09	2.3 ± 0.05	2.0 ± 0.1
arabinose	1.81 ± 0.08	9.9 ± 0.4	30.0 ± 1.2	47.3 ± 3.3	33.8 ± 0.7	23.6 ± 0.4	18.2 ± 0.2	20.1 ± 0.2	19.0 ± 1.3
rhamnose	0.63 ± 0.03	8.16 ± 0.06	18.5 ± 0.1	18.0 ± 0.05	13.6 ± 0.2	11.2 ± 0.8	9.5 ± 0.2	10.7 ± 0.8	9.8 ± 0.8
fucose	*b*	0.30 ± 0.01	0.89 ± 0.02	1.5 ± 0.1	1.40 ± 0.05	1.47 ± 0.02	1.22 ± 0.01	1.45 ± 0.02	0.8 ± 0.02
xylose	*b*	0.31 ± 0.01	0.65 ± 0.05	0.69 ± 0.01	0.82 ± 0.03	5.3 ± 0.3	0.73 ± 0.04	0.69 ± 0.02	0.71 ± 0.05
mannose	1.40 ± 0.09	8.2 ± 0.4	25.0 ± 0.3	31.2 ± 0.5	42.5 ± 0.6	47.0 ± 0.4	54.9 ± 1.4	51.7 ± 1.2	55.2 ± 4.3
Dha[c]	*b*	0.79 ± 0.05	3.1 ± 0.04	3.7 ± 0.1	2.16 ± 0.03	0.99 ± 0.01	3.1 ± 0.1	2.58 ± 0.05	1.5 ± 0.1
galactose	3.29 ± 0.10	17.4 ± 1.5	36.8 ± 2.2	38.1 ± 1.0	40.4 ± 2.3	40.6 ± 4.1	38.6 ± 1.9	40.1 ± 2.8	43.1 ± 2.6
GalA[c]	1.16 ± 0.03	13.6 ± 0.6	32.9 ± 2.0	38.4 ± 0.9	31.9 ± 1.1	30.7 ± 2.7	23.9 ± 0.7	26.6 ± 0.9	24.1 ± 0.8
glucose	1.56 ± 0.05	4.52 ± 0.06	7.2 ± 0.2	6.1 ± 0.6	7.0 ± 0.2	5.5 ± 0.1	5.90 ± 0.08	5.1 ± 0.2	5.0 ± 0.3
GlcA[c]	0.19 ± 0.01	1.16 ± 0.02	2.8 ± 0.2	4.13 ± 0.06	4.0 ± 0.1	4.2 ± 0.2	3.9 ± 0.2	4.1 ± 0.1	4.6 ± 0.1
Kdo[c]	*b*	0.54 ± 0.02	1.47 ± 0.01	3.36 ± 0.09	3.11 ± 0.05	3.36 ± 0.03	3.2 ± 0.1	3.13 ± 0.06	3.0 ± 0.3

[a] Average values of 3 replicates (mean ± SD). [b] <0.1 mg/L. [c] 2-*O*-M Fuc, 2-*O*-methyl fucose; 2-*O*-M Xyl, 2-*O*-methyl xylose; Dha, 3-deoxy-D-*lyxo*-heptulosaric acid; GalA, galacturonic acid; GlcA, glucuronic acid; Kdo, 3-deoxy octulosonic acid.

Table 4. Carbohydrate Composition (mg/L) of Must and Wine Fractions S3 Obtained by HRSEC on a Superdex-75 HR Column and Determined by GC and GC-MS of their TMS Derivatives[a]

	vinification stages								
sugars	0AF	30AF	60AF	99AF	BMF	EMF	BOA	EOA	BA
aceric acid	*b*	0.12 ± 0.01	0.25 ± 0.02	0.43 ± 0.01	3.15 ± 0.06	0.36 ± 0.01	0.38 ± 0.02	0.31 ± 0.01	0.28 ± 0.02
2-*O*-M Fuc[c]	*b*	0.09 ± 0.01	0.21 ± 0.01	0.41 ± 0.02	2.57 ± 0.09	0.38 ± 0.01	0.26 ± 0.01	0.31 ± 0.01	0.42 ± 0.02
2-*O*-M Xyl[c]	*b*	0.08 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	2.89 ± 0.05	0.10 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01
apiose	*b*	0.09 ± 0.01	0.28 ± 0.01	0.21 ± 0.01	3.22 ± 0.05	0.10 ± 0.01	0.20 ± 0.01	0.11 ± 0.01	0.95 ± 0.03
arabinose	4.05 ± 0.08	7.2 ± 0.2	7.8 ± 0.4	15.4 ± 0.3	33.8 ± 0.7	10.5 ± 0.4	7.9 ± 0.3	7.3 ± 0.2	7.0 ± 0.2
rhamnose	0.45 ± 0.03	0.77 ± 0.02	1.9 ± 0.1	4.09 ± 0.04	13.6 ± 0.2	2.4 ± 0.1	2.9 ± 0.3	2.5 ± 0.1	2.80 ± 0.03
fucose	*b*	0.12 ± 0.01	0.25 ± 0.02	0.94 ± 0.08	1.40 ± 0.05	0.36 ± 0.02	0.18 ± 0.01	0.37 ± 0.01	0.45 ± 0.04
xylose	0.44 ± 0.03	0.33 ± 0.02	0.68 ± 0.05	1.77 ± 0.01	0.82 ± 0.03	0.8 ± 0.1	0.80 ± 0.04	0.77 ± 0.04	0.63 ± 0.15
mannose	3.03 ± 0.09	3.58 ± 0.04	5.8 ± 0.3	25.7 ± 0.5	42.5 ± 0.6	14.8 ± 0.4	17.1 ± 1.4	18.9 ± 1.4	19.60 ± 0.02
Dha[c]	*b*	0.57 ± 0.05	0.27 ± 0.01	1.03 ± 0.08	2.16 ± 0.03	0.47 ± 0.01	0.47 ± 0.02	0.68 ± 0.02	0.42 ± 0.12
galactose	3.9 ± 0.1	5.8 ± 0.2	6.0 ± 0.1	20.4 ± 1.0	40.4 ± 2.3	15.3 ± 0.4	20.9 ± 0.2	19.2 ± 0.5	18.2 ± 0.6
GalA[c]	2.50 ± 0.03	3.0 ± 0.2	10.0 ± 0.4	14.4 ± 0.6	31.9 ± 1.1	9.3 ± 0.2	8.38 ± 0.01	6.0 ± 0.2	6.8 ± 0.13
glucose	18.0 ± 0.5	12.40 ± 0.06	10.7 ± 0.2	66.1 ± 0.8	7.0 ± 0.2	34.3 ± 0.1	24.8 ± 0.1	29.2 ± 1.5	30.20 ± 0.08
GlcA[c]	0.71 ± 0.01	0.37 ± 0.02	0.56 ± 0.02	1.74 ± 0.05	4.0 ± 0.1	0.77 ± 0.05	1.14 ± 0.06	0.97 ± 0.01	1.02 ± 0.09
Kdo[c]	*b*	0.12 ± 0.01	0.25 ± 0.01	0.43 ± 0.02	3.11 ± 0.05	0.36 ± 0.03	0.38 ± 0.03	0.31 ± 0.02	0.51 ± 0.02

[a] Average values of 3 replicates (mean ± SD). [b] <0.1 mg/L. [c] 2-*O*-M Fuc, 2-*O*-methyl fucose; 2-*O*-M Xyl, 2-*O*-methyl xylose; Dha, 3-deoxy-D-*lyxo*-heptulosaric acid; GalA, galacturonic acid; GlcA, glucuronic acid; Kdo, 3-deoxy octulosonic acid.

generated under the conditions used for precipitation and separation procedures even though the shift from dimeric to monomeric state requires very low pH values (*14*).

Evolution of Must and Wine-Soluble Polysaccharide Families during Vinification and Aging. The evolution of major must and wine polysaccharides was monitored during winemaking and aging (**Figure 6**), and the results obtained showed good agreement with the observations described in the previous sections. Wine and must AGP and MP were calculated from the sum of high-molecular-weight AGP and MP from fractions S1 and smaller AGP and MP from fractions S2.

The rate of extraction and solubilization of grape and yeast polysaccharides differed depending on the polysaccharide family in question. AGP, localized in soluble form within grape cell walls (*26*), were easily extracted by endogenous enzymes during grape maturation, crushing, and the early maceration-fermenta-

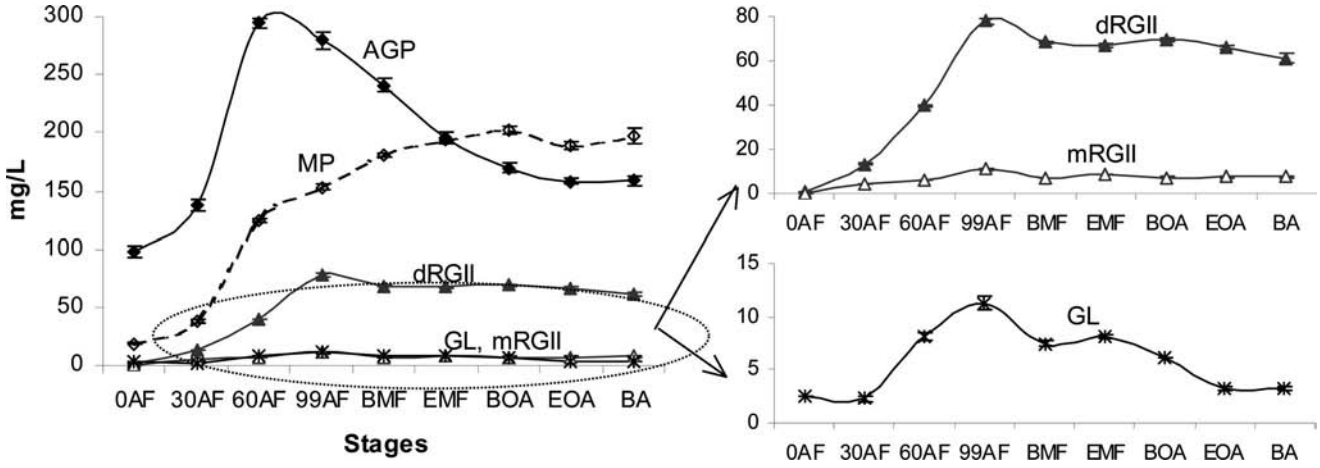

Figure 6. Evolution of major polysaccharide families in must and wine samples during vinification and aging. AGP, arabinogalactan–proteins; MP, mannans and mannoproteins; dRG-II, rhamnogalacturonan-II dimers; mRG-II, rhamnogalacturonan-II monomers; GL, oligomers of homo and rhamnogalacturonans. See text for conditions and calculations.

tion, increasing around 200% between 0AF and 60AF. Unlike AGP, RG-II were resistant to the endogenous pectolytic enzymes of grape berries and seemed not to be affected by crushing of berries. The extraction of RG-II dimers was as well almost negligible between 0AF and 30AF but increased 500% between 30AF and 99AF, indicating that dRG-II were more tightly bound to the cell wall matrix of grape cell walls, needing maceration time to solubilize. As regards the other grape polysaccharides, RG-II monomers and GL displayed similar behavior to RG-II dimers although they were present in very low quantities, mRG-II content being more than 6-fold lower than dRG-II content. The liberation of yeast mannoproteins was progressive during maceration-fermentation, with a higher extraction rate between 30 and 60AF, coinciding with the yeast exponential phase of growth. The reduction in sugar content previously observed at the end of maceration-fermentation only affected AGP molecules; the other polysaccharides were highly extracted during this period.

As a result, 0AF and 30AF must samples were largely dominated by AGP, although, as mentioned previously, low-molecular-weight glucosyl polysaccharides were the most prevalent molecules in these samples. The content of RG-II was almost negligible in musts and only traces of GL could be quantified, which was somewhat unexpected because it is the main pectic polysaccharide occurring in grapes (*27*) and it has also been detected in high amounts in blanc musts (*30*). AGP were also the majority polysaccharides in young wines after maceration-fermentation, followed by MP, dRG-II, and GL. These compounds represented about 50%, 30%, 15% and 2%, respectively, of total quantified soluble polysaccharides, in quite similar proportions to those described for other red varieties (*11*).

Postmaceration performed to enhance wine color did not yield any additional polysaccharide increase; on the contrary, it prompted a considerable reduction in AGP, dRG-II, mRG-II, and GL. Mannoproteins were the only molecules liberated during this period, resulting in an overall decrease of 8% in total polysaccharide content. MP increased 20%, possibly sufficient to improve the organoleptic qualities of wines after postmaceration. As described previously, malolactic fermentation also induced a reduction in total sugar content. However, contrary to what was thought, it only affected AGP molecules and the content of the other macromolecules remained stable, and MP even rose slightly during this period. Probably due to yeast cell wall fragmentation, the liberation of yeast mannoproteins was still high after alcoholic fermentation, thus compensating for their partial precipitation (**Table 1**).

As expected, the content of major polysaccharide families remained stable during oak and bottle wine aging, coinciding with what was observed in previous studies for other red wine varieties (*22*). In contrast to young wines, and due to the changes described previously, AGP were no longer the majority polysaccharides after malolactic fermentation and MP were the most prevalent polysaccharides in aged wines, where AGP, MP, and RG-II represented 37, 45, and 15%, respectively, of total quantified polysaccharides.

The evolution of arabinogalactan–proteins and mannoproteins was analyzed in detail and they were classified according to their molecular size (**Figure 7**). We noted that these were the highest molecular weight AGP and MP, the most prevalent both in wine and must samples, while smaller molecules, collected in fractions S2, represented less than 30% in wine samples and even less in musts (<10%). Therefore, AGP and MP from grape and yeast cell walls were basically high-molecular-weight molecules and their solubilization rate was similar to the smaller compounds except for two aspects. On the one hand, the smaller AGP and MP were first liberated while the increase in larger molecules began as from 30AF. On the other hand, the precipitation of AGP and MP observed during late maceration-fermentation, postmaceration, and malolactic fermentation (see previous sections) had a greater extent on the bigger AGP and MP. A substantial increase in AGP and MP fragments between 60 and 99AF indicated as well an enzymatic degradation of both AGP and MP during this period. However, during postmaceration and malolactic fermentation, precipitation was probably the major phenomenon influencing the polysaccharidic balance. Precipitation during these stages may be a consequence of the formation of unstable complexes between polysaccharides and other wine polyphenolic compounds (*25*), although in the case of MP, it was fully compensated by their continuous liberation.

As regards the sugar composition of the different polysaccharide families, the molar ratio of arabinose to galactose for predominant high-molecular-weight AGP was between 0.4 and 1, and this ratio changed with the vinification process (**Figure 8**). AGP with lower arabinose/galactose molar ratios were extracted first, while AGP with higher ratios were extracted in the later stages of maceration-fermentation. After this period, high-molecular-weight AGP showed similar arabinose/galactose ratios to those described in the bibliography for AGP isolated

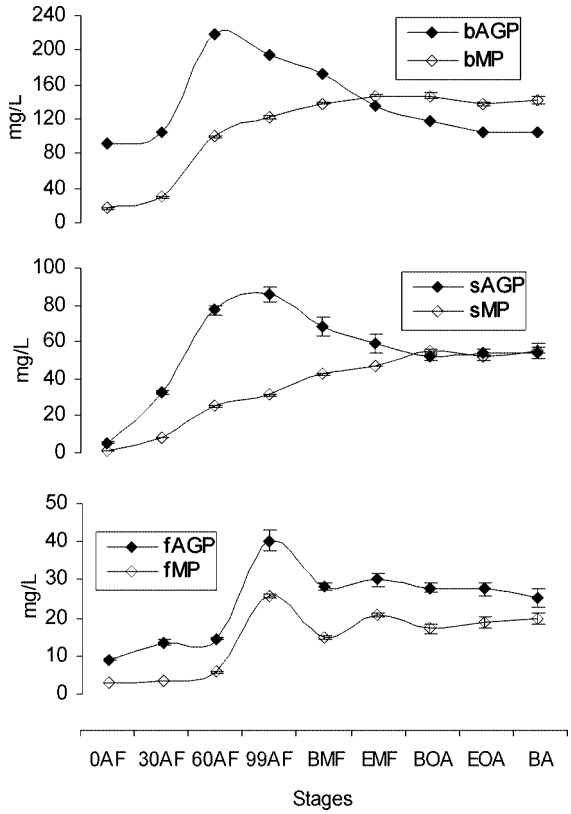

Figure 7. Concentration of arabinogalactan–proteins (AGP) and mannoproteins (MP) during vinification and aging. bAGP, bMP, high-molecular-weight AGP and MP collected in fractions S1, sAGP, sMP, low-molecular-weight AGP and MP collected in fractions S2, and fAGP, fMP, polysaccharide fragments of AGP and MP collected in fractions S3. See text for conditions and calculations.

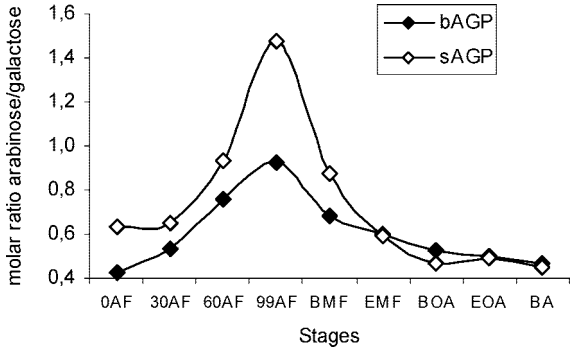

Figure 8. Molar ratio of arabinose to galactose from high-molecular-weight AGP (bAGP) and low-molecular-weight AGP (sAGP) during vinification and aging. See text for conditions and calculations.

from red wines obtained after fermentation of other red grape varieties (*10, 11*). The AGP with the highest arabinose/galactose ratios seemed to be the most affected by precipitation, as this ratio was substantially reduced during postmaceration and malolactic fermentation. The arabinose/galactose molar ratio of smaller AGP showed a similar evolution, although it was substantially higher for smaller AGP extracted after maceration-fermentation. The molar ratio of both RG-II dimers and monomers found coincided with results published previously for the purification of RG-II fractions (*11, 12*).

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NOTE ADDED AFTER ASAP PUBLICATION

The original posting of November 15, 2007, contained minor errors in the second paragraph of the Introduction and in ref *30*. This has been corrected with the posting of November 27, 2007.

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