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Changes in Polysaccharide Composition during Sparkling Wine Making and Aging

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ABSTRACT: The evolution in polysaccharide composition and molecular weights during sparkling wine making and aging was studied for the first time in this work. Different autochthonous grape varieties from Spain (Verdejo, Viura, Malvasía, Albarín, Godello, Garnacha and Prieto Picudo) were used to elaborate sparkling wines following the champenoise method. Principal component analysis showed differentiation of wines according to polysaccharide families. This differentiation was due to the process of aging on yeast lees, but not to the variety employed. The content of mannoproteins during aging was positively correlated (r = 0.792) with total polysaccharides from grapes. After six months of aging the highest content of mannoproteins and polysaccharides rich in arabinose and galactose was obtained. Also a shift to lower molecular weights was observed. The combination of these two characteristics could imply a better foam stability and thus sensory quality of sparkling wines.

KEYWORDS: *sparkling wine, grape variety, polysaccharides rich in arabinose and galactose, homogalacturonans, rhamnogalacturonan II, mannoproteins, glucans*

INTRODUCTION

Polysaccharides are one of the main groups of macromolecules in wines. They come from grape berries, yeast, bacteria and fungal grape contamination such as *Botrytis cinerea*. From the enological and quantitative point of view, polysaccharides from grapes and yeast are the most important. Polysaccharides rich in arabinose and galactose (PRAGs) such as type II arabinogalactan-proteins (AGPs) and arabinans, rhamnogalacturonans type I (RG-I) and type II (RG-II), and homogalacturonans (HLs) come from grape berries, while glucans (GLs), mannans and mannoproteins (MPs) are released by yeast either during fermentation or by enzymatic action during aging on yeast lees by autolysis. Exogenous polysaccharides such as arabic gum and carboxymethyl cellulose could also be present in several commercial wines as they are authorized as additives.

Polysaccharides have an important influence on several stages of the winemaking process, including fermentation, filtration and stabilization.[1−3] They are in part responsible for the organoleptic properties of wines.[4−9] However, it has been shown that not all polysaccharides have the same behavior with respect to wines. Their influence on wine processing and sensory properties will depend not only on their quantity but also on the type of polysaccharide. It has been shown that AGPs have greater influence on the filtration procedures than MPs,[10] which are more efficient at reducing protein haze in white wines.[11] RG-II is a stronger accelerator of hydrogen tartrate crystallization than RG-I. RG-II has a concentration-dependent effect on hydrogen tartrate crystallization, accelerating crystallization at low concentrations and inhibition of it at high concentrations.[12] AGPs, on the other hand, have no effect on this phenomenon.[10] Besides, it has been recently shown that RG-II, MPs and AGPs have different influences on aggregation of proanthocyanidins[5] and, therefore, have varied influences on wine characteristics.[6] In the case of sparkling wines, some authors have correlated the foam properties of grape juices, base wines and sparkling wines with the polysaccharide content.[13−17] A connection between the molecular weight and composition of polysaccharides and foaming characteristics has been shown.[18,19] Some authors have even identified the importance of the type of polysaccharide on wine foam properties. Among wine polysaccharides, yeast mannoproteins released during autolysis have been associated with the improvement of foaming properties.[20−23] However it has been shown that not all mannoproteins have the same behavior.[21,22] The positive effect of mannoproteins on foam has been attributed to the presence of a balanced composition of hydrophobic and hydrophilic protein domains. This balance contributes to the creation of points of adsorption to the gas−liquid interface of the bubbles. In this way stability is increased.[21] Moreover, mannoproteins play other roles in sparkling wines since they contribute to the flocculation of yeast strains[24] and improve their elimination from the bottle during disgorging. Finally, these compounds could also serve as markers to follow the autolysis process because they are the major polysaccharides released by yeast.

Given the importance of polysaccharides in the sparkling wine making and sensory properties, an understanding of their content and kinetic release is essential. Different analytical methodologies have been developed to determine grape, must

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and wine polysaccharides. On the one hand, colorimetric methods[25] are frequently used to analyze the global content of neutral and acid polysaccharides. On the other hand, more complex and time-consuming methods based on gas chromatography are used to identify and quantify specific monosaccharides.[26−28] Previous studies have analyzed the evolution of polysaccharide families during the winemaking and aging of still wines.[4,8,29,30] Some research has been carried out on the evolution of neutral or total polysaccharides throughout the sparkling wine making process.[14,18,20] However, none of these studies analyzed the evolution of concrete polysaccharide families.

Therefore, this paper aims to analyze the changes occurring on monosaccharides, polysaccharide families and molecular weights of polysaccharides during the different stages of the sparkling wine processing by the traditional champenoise method. For this purpose different white (Verdejo, Viura, Malvasía, Albarín and Godello) and rosé (Garnacha and Prieto Picudo) sparkling wines were industrially manufactured with maintenance on yeast lees during 30 months. Chemometric techniques were applied to achieve a possible differentiation of the wines according to grape variety along with vinification stage and their monosaccharide and polysaccharide family composition.

MATERIALS AND METHODS

Chemicals. All reagents were analytical grade unless otherwise stated. Standards of different monosaccharides were used to perform the calibration curves. D-(+)-Fucose, L-rhamnose, 2-*O*-methyl-D-xylose, L-(+)-arabinose, D-(+)-galactose, D-(+)-glucose, D-(+)-mannose, Kdo (2-keto-3-deoxyoctonate ammonium salt) and D-apiose solution were supplied by Sigma-Aldrich (Beerse, Belgium), and D-(+)-galacturonic acid, D-glucuronic acid and myo-inositol (internal standard) were obtained from Fluka (Buch, Switzerland). Ethanol 96% (v/v) and acetyl chloride were supplied by Scharlab (Barcelona, Spain), hydrochloric acid 37% was purchased from Carlo Erba (Rodano, Milan, Italy) and hexane, dried methanol, pyridine, hexamethyldisilazane and trimethylclorosilane were obtained from Sigma-Aldrich (Beerse, Belgium). Lithium nitrate of HPLC grade supplied by Sigma (Beerse, Belgium) and Milli-Q deionized water (Millipore, Molsheim, France) were used. A pullulan calibration kit (Shodex P-82) was obtained from Waters (Barcelona, Spain).

Winemaking. All the sparkling wines in this study were manufactured using the traditional method champenoise from grapes from the 2009 harvest in the enological station of Castilla y León (Valladolid, Spain). Five white monovarietal and three rosé monovarietal base wines were prepared using the traditional winemaking process. White base wines were elaborated with *Vitis vinifera* cv. Verdejo and Viura grapes from the Rueda Denomination of Origin (D.O.), *Vitis vinifera* cv. Malvasía grapes from the Toro D.O., *Vitis vinifera* cv. Albarín grapes from the Tierras de León D.O. and *Vitis vinifera* cv. Godello grapes from the Bierzo D.O. Rosé base wines were obtained with *Vitis vinifera* cv. Prieto Picudo grapes from the Tierras de León D.O., and *Vitis vinifera* cv. grapes of Garnacha from the Cigales D.O. Two different viticultural areas of Garnacha were used in this work, and thus two different Garnacha wines were obtained, called Garnacha and Garnacha*, respectively. White grapes were destemmed-crushed and directly pressed to obtain juice. Red grapes were destemmed-crushed and left to prefermentative maceration for 2 days before getting the must. Base wines were made in stainless steel tanks of 150 L by duplicate at 16 to 18 °C after the addition of selected winery yeast strain. The wines were cold-stabilized and clarified, and finally they were bottled and the tirage liquor was added. The bottles were finally kept in the cellar at a temperature (11−13 °C) and relative humidity (75−78%) controlled for 30 months. Stirring was conducted at 29 months of aging in order to remove the lees. Samples for analyses were taken from the base wines (BW) and then after 3 months (T3M), 6 months (T6M), 9 months (T9M), 18 months (T18M) and 30 months (T30M) of aging on yeast lees. These sampling points were selected according to representative aging periods of sparkling wine categories: sparkling wine (≥9 months), Reserve (≥15 months) and Great Reserve (≥30 months). Wines were riddled and disgorged before analysis, and liqueur d'expédition was not added. Three bottles were analyzed at each disgorging time, and all the analyses were conducted in triplicate on wines after centrifugation.

Precipitation of Total Soluble Wine Polysaccharides. Wine polysaccharides were recovered by precipitation after ethanolic dehydration as previously described.[27] Samples were homogenized and centrifuged using a RC-6 Plus Sorvall refrigerated centrifuge (Du Pont, BH, Germany), and 2 mL of the supernatants were taken and introduced into 15 mL falcon-tubes to be concentrated to dryness in a Joan RC10-10 centrifugal evaporator (Fisher Scientific, Madrid, Spain). Polysaccharides were then precipitated by adding 2 mL of cold ethanol/acid (ethanol 96% containing 0.3 M HCl) and kept for 24 h at 4 °C. Thereafter, samples were centrifuged, the supernatants discarded and the pellets washed several times with 96% ethanol to remove the interference materials. The pellet, which corresponded to total soluble polysaccharides (TSP), was finally freeze-dried using a Virtis freeze-drying apparatus (New York, USA). This polysaccharide extraction was performed in triplicate in each sample.

Identification and Quantification of Monosaccharides by GC−MS. The monosaccharide composition of the TSP precipitates was determined by GC−MS of their trimethylsilyl-ester *O*-methyl glycolsyl-residues obtained after acidic methanolisis and derivatization as previously described.[27] GC was controlled by ChemStation software and equipped with a 7653B automatic injector consisting of an Agilent 7890A gas chromatograph (Agilent Technologies, Waldbronn, Germany) coupled to a 5975C VL quadrupole mass detector (MS). Samples were injected in duplicate. The content of each polysaccharide family in the wine samples was estimated from their concentration of individual glycosyl residues which are characteristic of structurally identified wine polysaccharides.[28,31] PRAGs, representing mainly arabinogalactan-proteins and arabinans in wines, were estimated from the sum of galactosyl, arabinosyl, rhamnosyl and glucuronosyl residues. All the mannose content was attributed to yeast mannoproteins (MPs), and all the glucose content was attributed to yeast glucans (GLs). The RG-II content was calculated from the sum of its diagnostic sugars (apiose, 2-*O*-methyl-l-fucose, 2-*O*-methyl-D-xylose, aceric acid (3-c-carboxy-5-deoxy-l-xylose), Kdo (3-deoxy octulosonic acid), and Dha (3-deoxy-D-lyxo heptusolaric acid)), which represent approximately 25% of the RG-II molecule. For one residue of 2-*O*-methyl fucose, RG-II contains 3.5 rhamnosyl, 2 arabinosyl, 2 galactosyl, 1 glucuronosyl and 9 galacturonosyl residues. Taking into account these molar ratios, it was possible to estimate their respective amounts in the RG-II. The remaining part was attributed to the presence of PRAGs in the case of rhamnose, arabinose and galactose; and the remaining galacturonosyl residues was used to estimate the content of oligomers of homogalacturonans (HLs). The content of total polysaccharides was estimated from the sum of PRAGs, MPs, GLs, RG-II and HLs.

Analysis of Polysaccharides by HRSEC-RID. A high-resolution size-exclusion chromatography (HRSEC) system with a refractive index detector was used to obtain the molecular weight distributions of the wine polysaccharides as previously described.[27] Two serial Shodex OHpack SB-803 and SB-805 columns (0.8 × 30 cm, Showa Denko, Japan) equilibrated at 1 mL/min in 0.1 M $LiNO_3$ were used. Chromatographic separation was carried out on an Agilent modular 1100 liquid chromatograph (Agilent Technologies, Waldbronn, Germany) connected to G1362 refractive index detector. Calibration was performed with narrow pullulan molecular weight standards (Shodex P-82, Waters, Barcelona, Spain): P-5, M_w = 5.9 kDa; P-10, M_w = 11.8 kDa; P-20, M_w = 22.8 kDa; P-50, M_w = 47.3 kDa; P-100, M_w = 112 kDa; P-200, M_w = 212 kDa, P-400, M_w = 404 kDa. The apparent molecular weights were deduced from the calibration equation log M_w = 11.027−0.410 tR (tR = column retention time at peak maximum, and r^2 = 0.999).

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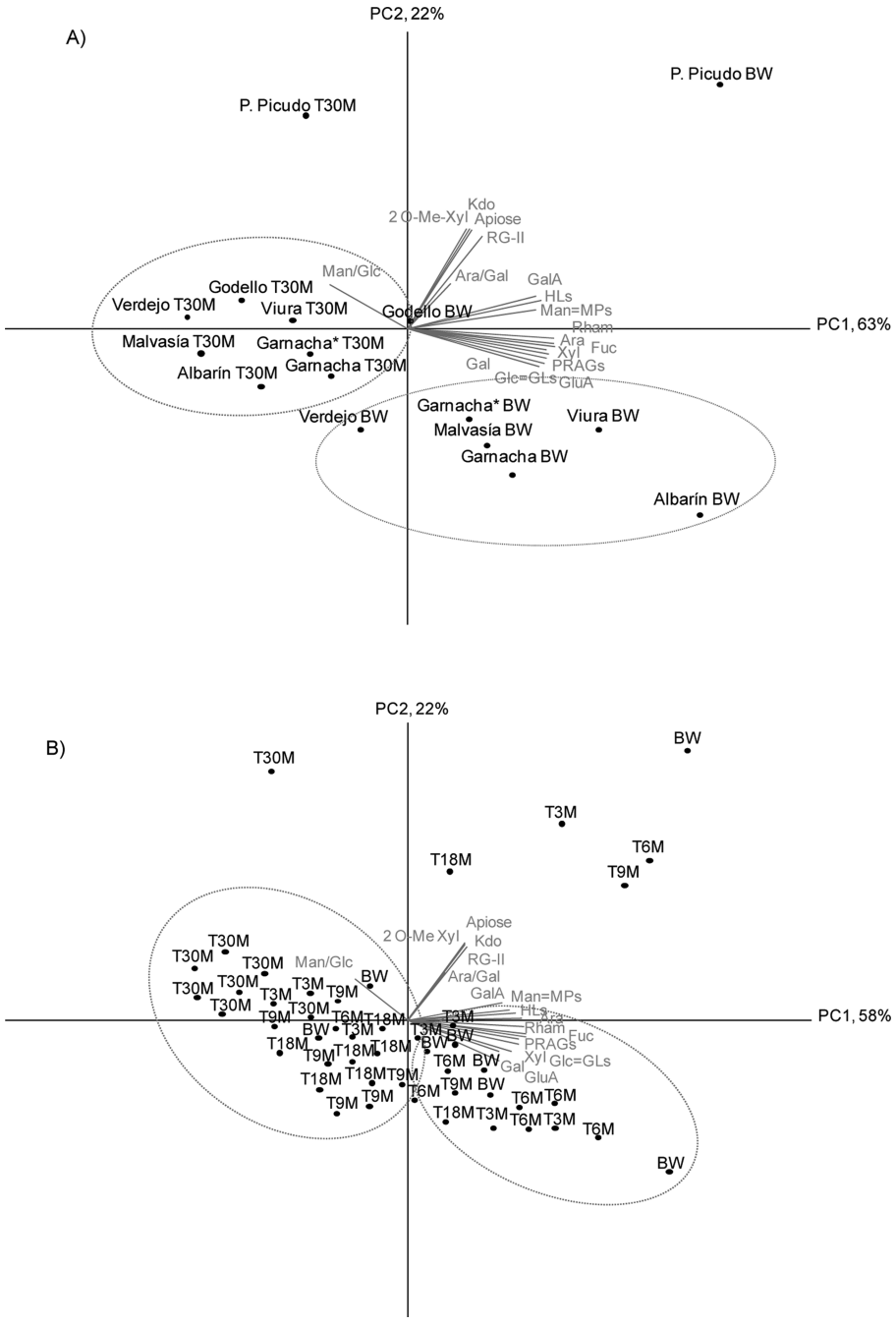

Figure 1. PCA of wines according to the winemaking stage: (A) base wines (BW) and sparkling wines after 30 months of aging on yeast lees (T30M); (B) base wines (BW), and sparkling wines after 3 months (T3M), 6 months (T6M), 9 months (T9M), 18 months (T18M) and 30 months (T30M) of aging on yeast lees. Ara, arabinose; Fuc, fucose; Man, mannose; Gal, galactose; GalA, galacturonic acid; Glc, glucose; Rham, rhamnose; GluA, glucuronic acid; Kdo, 2-keto-3-deoxyoctonate ammonium salt; 2 O-Me-Xyl, 2-*O*-methyl-D-xylose; MP, mannoproteins; PRAG, polysaccharides rich in arabinose and galactose; GL, glucans; HL, homogalacturonans; RG-II, rhamnogalacturonan type II; Ara/Gal ratio; Man/Glc ratio.

Statistical Analysis. Significant differences among samples were analyzed by an analysis of variance (ANOVA) if the data adhered to assumptions of normality. If these assumptions were not adhered to, nonparametric methods were used. Separate principal component analysis (PCA) was carried out on the values of monosaccharide composition, polysaccharide families, arabinose/galactose (Ara/Gal) and mannose/glucose (Man/Glc) ratio grouped according to grape variety and winemaking stage. ANOVA evaluations were performed using the Statistica 8.0 program for Microsoft Windows (Statsoft Inc., Tulsa, Oklahoma) and PCA analysis by using the Senstools Version 3.3.2. Program (Utrecht, The Netherlands).

RESULTS AND DISCUSSION

Differentiation of Sparkling Wines According to Monosaccharide Composition and Polysaccharide Families. Principal component analysis (PCA) was applied to achieve a possible differentiation of the wines according to the variety employed. Figure 1A shows the distribution of base wines and sparkling wines after 30 months of aging on yeast lees, and the monosaccharide composition and polysaccharide families' loads. The two first principal components explained 85% of the accumulative variance. Prieto Picudo wines were

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Table 1. Evolution of Yeast Monosaccharides (mg/L) and Mannose/Glucose Ratio during Different Stages of the Sparkling Wine Production: Base Wines (BW), and Sparkling Wines after 3 Months (T3M), 6 Months (T6M), 9 Months (T9M), 18 Months (T18M), and 30 Months (T30M) of Aging in Bottle on Yeast Lees[a]

		Albarín	Viura	Godello	Malvasía	Verdejo	Garnacha	Garnacha*	Prieto Picudo
BW									
	glucose	146.20 ± 21.45 cd BC	90.61 ± 17.50 ab C	61.23 ± 18.74 a C	87.11 ± 4.97 ab C	49.30 ± 9.27 a B	187.45 ± 21.00 d C	120.64 ± 17.15 bc B	111.69 ± 8.50 bc BC
	mannose	93.62 ± 12.76 d BC	70.69 ± 7.03 c A	37.40 ± 1.25 ab A	55.18 ± 3.63 bc A	28.88 ± 2.63 a A	58.27 ± 6.93 c A	57.13 ± 0.97 c B	106.23 ± 7.68 d B
	mannose/glucose	0.77 ± 0.24 abc ABC	0.94 ± 0.17 bc A	0.73 ± 0.19 ab A	0.76 ± 0.06 abc A	0.70 ± 0.12 ab A	0.37 ± 0.08 a A	0.57 ± 0.07 ab AB	1.14 ± 0.10 c A
T3M									
	glucose	141.76 ± 15.00 cd BC	97.98 ± 12.76 b CD	31.80 ± 9.11 a AB	56.00 ± 12.00 a B	44.89 ± 5.61 a AB	169.89 ± 5.07 d BC	117.17 ± 10.73 bc B	109.95 ± 16.41 bc BC
	mannose	104.18 ± 8.05 cd BC	87.00 ± 0.09 bc B	39.67 ± 0.95 a A	68.00 ± 5.70 b A	44.30 ± 5.18 a B	67.28 ± 14.37 b A	73.55 ± 4.49 b C	120.00 ± 8.89 d BC
	mannose/glucose	0.88 ± 0.15 abc BC	1.07 ± 0.20 bcd A	1.50 ± 0.36 d B	1.46 ± 0.28 cd B	1.18 ± 0.17 bcd A	0.48 ± 0.09 a A	0.75 ± 0.07 ab AB	1.31 ± 0.18 bcd A
T6M									
	glucose	120.00 ± 22.68 ab B	118.00 ± 1.38 ab D	57.10 ± 1.28 a BC	76.09 ± 7.00 ab BC	75.14 ± 18.70 ab BC	210.00 ± 39.90 d C	191.47 ± 42.00 cd C	139.79 ± 17.00 bc C
	mannose	113.05 ± 15.22 de C	98.23 ± 5.61 d BC	42.92 ± 4.60 a A	69.79 ± 10.91 b A	68.00 ± 5.00 b C	94.92 ± 7.00 cd B	73.03 ± 4.24 bc C	134.84 ± 0.03 e C
	mannose/glucose	1.13 ± 0.22 c C	1.00 ± 0.05 c A	0.90 ± 0.08 bc A	1.10 ± 0.17 c AB	1.09 ± 0.23 c A	0.54 ± 0.09 ab AB	0.46 ± 0.09 a A	1.16 ± 0.12 c A
T9M									
	glucose	109.92 ± 14.19 bc AB	52.44 ± 3.47 a B	66.09 ± 7.18 a C	80.54 ± 7.65 ab C	84.76 ± 18.17 ab C	159.11 ± 14.02 d BC	112.66 ± 15.13 bc B	131.32 ± 23.48 cd C
	mannose	40.84 ± 3.02 a A	102.00 ± 2.87 d D	49.00 ± 1.39 ab B	61.00 ± 8.50 bc A	67.80 ± 3.98 c C	65.76 ± 6.23 c A	54.42 ± 1.69 abc B	104.02 ± 8.76 d B
	mannose/glucose	0.45 ± 0.06 a A	2.33 ± 0.14 d C	0.89 ± 0.08 bc A	0.91 ± 0.13 bc AB	0.96 ± 0.18 c A	0.50 ± 0.05 a A	0.58 ± 0.07 ab AB	0.95 ± 0.16 c A
T18M									
	glucose	164.78 ± 6.41 d C	52.69 ± 9.63 a B	57.20 ± 6.22 a BC	55.07 ± 7.00 a B	58.86 ± 10.12 a BC	114.14 ± 20.08 c AB	102.33 ± 15.33 bc B	75.60 ± 5.69 ab B
	mannose	77.87 ± 12.79 c B	68.79 ± 2.54 bc A	41.00 ± 0.56 a A	59.00 ± 4.52 b A	55.30 ± 4.11 ab B	69.39 ± 4.38 bc A	58.25 ± 2.77 b B	83.58 ± 4.28 c B
	mannose/glucose	0.57 ± 0.08 a AB	1.57 ± 0.24 d B	0.86 ± 0.08 ab A	1.29 ± 0.16 cd AB	1.13 ± 0.18 bc A	0.73 ± 0.11 ab B	0.68 ± 0.09 a AB	1.33 ± 0.10 cd A
T30M									
	glucose	70.00 ± 12.00 b A	24.28 ± 0.81 a A	20.64 ± 0.91 a A	26.30 ± 8.99 a A	13.56 ± 3.95 a A	65.84 ± 1.01 b A	24.48 ± 1.56 a A	17.01 ± 1.77 a A
	mannose	23.44 ± 1.30 a A	60.75 ± 3.56 e A	39.00 ± 1.71 cd A	29.27 ± 3.66 ab B	46.19 ± 2.37 d B	68.75 ± 0.86 e A	33.33 ± 2.26 bc A	42.75 ± 6.23 d A
	mannose/glucose	0.40 ± 0.06 a A	3.00 ± 0.17 cd D	2.27 ± 0.12 bc C	1.34 ± 0.41 ab AB	4.09 ± 1.01 c B	1.25 ± 0.02 ab C	1.63 ± 0.13 b C	3.02 ± 0.45 cd B

[a]Values are means ± SD ($n = 3$). Different lowercase letters in the same row indicate that means significantly differ at $p < 0.05$. Different capital letters in the same column indicate that means significantly differ at $p < 0.05$.

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widely separated from the rest of base and sparkling wines because they were highly related to the RG-II polysaccharide and their constituent monosaccharides. However, the rest of the varietal wines could not be separated in the PCA space according to the polysaccharide composition. On the contrary, the process of aging on lees affected the monosaccharide profile differentiation between varieties. Base wines were clearly separated from sparkling wines with 30 months of aging. Except for Man/Glc ratio, base wines were highly related to all studied loads, and the process of aging on yeast lees increased this ratio.

In order to check which stages of aging most influenced the polysaccharide composition of sparkling wines, a new PCA including all the stages was conducted (Figure 1B). Wines were properly located in the vectorial dimension defined by the first two factors, which accounted for 80% of the total variance in the PCA space. Wines were clearly differentiated according to their winemaking stage. There were no differences in the composition of the base wines and the wines obtained after 3 and 6 months of aging on yeast lees. These wines were highly related to all monosaccharide and polysaccharide families. On the contrary, wines after 9, 18, and 30 months of aging showed a weak relation with these compounds only being correlated with the Man/Glc ratio. Therefore, the final months of aging on yeast lees produced a movement of the wines in the PCA space, clearly marked by a decrease in all polysaccharide families but an increase in the Man/Glc ratio.

Evolution of Yeast Monosaccharides and Polysaccharide Families during Sparkling Wine Making and Aging. Table 1 shows the mannose and glucose content (mg/L) and the mannose/glucose ratio in base wines and sparkling wines over aging time. Between both sugars present in the wine glucose was usually found at a higher concentration. It represented more than 60% of the total content of mannose and glucose. Glucose is the prevalent sugar in grape berries[32] being that it is the main component of cellulose and hemicellulosic xyloglucans. However these structural polysaccharides are minor compounds in musts.[33] On the other hand, the presence of glucose in wines may also be related to microbial polysaccharides (*Botrytis cinerea*, *Oenococcus oeni*) or condensed anthocyanins. In this research, grapes were harvested in good sanitary conditions, malolactic fermentation was not conducted, and all wines showed very low anthocyanin content.[34] Therefore, it is reasonable to presume that all the glucose content in the wines was due to yeast glucans released during the fermentation. Thus, we used the content of glucose to estimate the quantity of glucans (GLs) in the same way that the quantity of mannose is used to estimate the quantity of mannoproteins (MPs).[28]

Release of mannoproteins and glucans during aging on yeast lees was attributed to the autolytic process from the yeast. Mannose content increased from 0 to 6 months of aging while glucose content increased only during the 3 to 6 month period of aging. This difference in the release time could be due to the fact that MPs in the cell wall of *Saccharomyces cerevisiae* are trapped or covalently linked to the GLs.[35] Thus MPs are released first by endo- and exo- β-(1,3)-glucanases, after which GLs are released. Therefore, the amount of MPs or GLs released could be regulated to the time in which a sparkling wine is in the bottle.

The content of MPs and GLs remained constant or decreased gradually over periods longer than 6 months. Thus, mannose and glucose concentration was lower in all final sparkling wines than in their corresponding base wines. In fact, the concentration of mannose and glucose were approximately 3 times higher in wines at 6 months of aging than in wines at 30 months of aging. These results contrasted with those obtained by other authors,[14,18] who observed an increase in neutral monosaccharides during 12 months of aging with yeast. This lack of increase of MPs and GLs may be attributed to different aspects. First, the autolytic conditions employed (low pH and low aging temperature, presence of ethanol, and high pressure of CO_2) and the lack of stirring of lees in sparkling wines during the aging time could have caused a reduction of the hydrolytic enzymes activities involved in the autolytic process and a lower release of yeast polysaccharides. Second, the precipitation rate of the released polysaccharides during this period was probably higher than their solubilization into the wine. Thus, decreases in the content of MPs and GLs were attributed to precipitation phenomena as a result of their interaction with other wine components to form unstable colloids. Although these interactions have not been studied regarding wine aging on lees, some authors have described the establishment of unstable colloids between MPs and other wine constituents in still wines at the end of maceration-fermentation.[9] The distribution of the molecular weights of polysaccharides (Figure 3) indicated decreases mainly affected compounds of low molecular weight. These results suggested that small MPs and GLs were more reactive with other wine components.

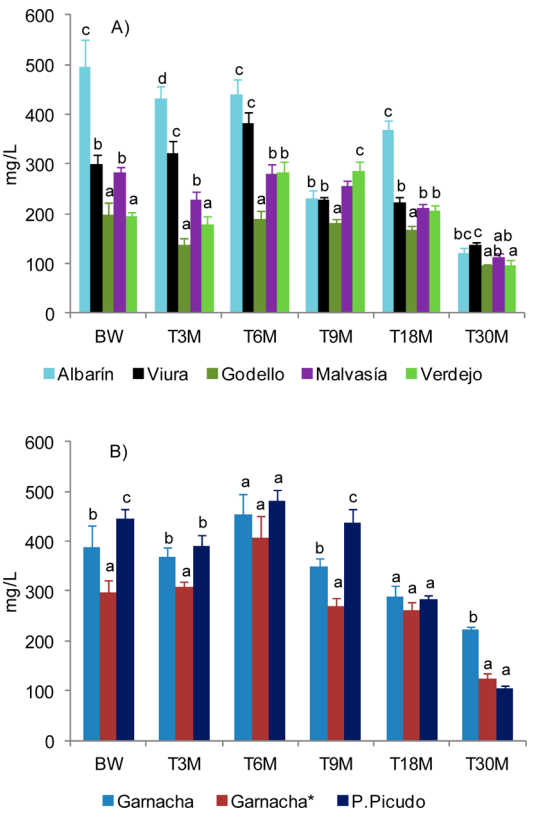

Figure 2. Evolution of total polysaccharide families in (A) white and (B) rosé sparkling wines over the aging time. Base wines (BW), and sparkling wines after 3 months (T3M), 6 months (T6M), 9 months (T9M), 18 months (T18M) and 30 months (T30M) of aging on yeast lees. Values are means ± SD (n = 3). Different letters in the same vinification stage represent means significantly different at $p < 0.05$.

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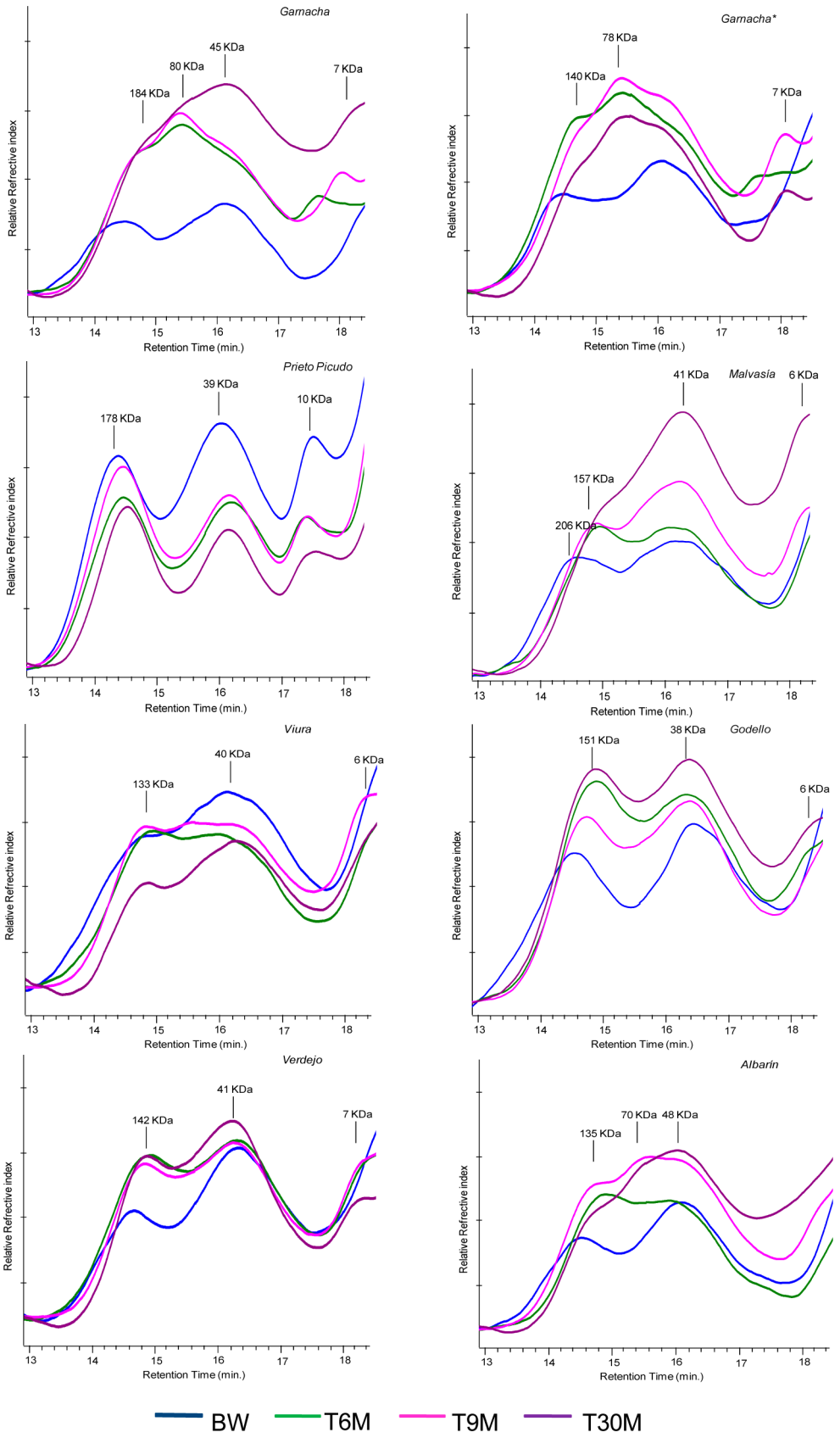

Figure 3. HRSEC-RID chromatograms of total soluble polysaccharides during the sparkling wine winemaking. Base wines (BW), and sparkling wines after 6 months (T6M), 9 months (T9M) and 30 months (T30M) of aging on yeast lees. Chromatograms obtained using two serial Shodex OHpack KB-803 and KB-805 columns.

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The mannose/glucose ratio (Man/Glc) remained constant until 18 months of aging, yet significantly increased from 18 to 30 months of aging (Table 1). Therefore, sparkling wines with 30 months of aging showed a Man/Glc ratio approximately 2.6 times higher than in the rest of the wines. Man/Glc increase from 18 to 30 months of aging was due to a significant reduction in the glucose content, indicating that GLs would form more unstable compounds susceptible to precipitation than MPs.

Evolution of Grape Monosaccharides and Polysaccharide Families during Sparkling Wine Making and Aging. The content of monosaccharides forming the grape polysaccharides and the arabinose/galactose ratio and polysaccharide families from grapes are shown in Table 2. These monosaccharides resulted from the breakdown and solubilization of native grape polysaccharides which were released by enzymatic degradation during the early steps of their processing to base wine.

Among grape monosaccharides, galactose and arabinose were the two most prevalently detected in all base wines samples (41 ± 19% and 26 ± 9%, respectively), indicating a high content of polysaccharides rich in arabinose and galactose (PRAGs). Galacturonic acid, which represented from 10 ± 1% to 37 ± 11%, was used as an indicator of homogalacturonans (HLs). Rhamnose and glucuronic acid were also detected in smaller amounts in wine samples as they also form PRAGs and rhamnogalacturonan type II (RG-II) polysaccharides. Rare sugars such as 2-*O*-methyl-xylose, apiose and Kdo were only detected in Prieto Picudo wines, indicating that the RG-II polysaccharide was only present in this wine. The absence of the RG-II molecule in all white wines was attributed to the winemaking process. RG-II is a molecule tightly bound to the cell wall matrix of grape cell walls, and it is resistant to pectinolytic enzymes. Therefore RG-II needs a longer maceration time to solubilize.[4,33] White base wines were elaborated without prefermentative maceration, and alcoholic fermentation was conducted in total absence of skin contact, which would prevent the extraction of RG-II into the wine. On the contrary, Prieto Picudo and both Garnacha base wines were given two days of prefermentative maceration before obtaining the musts. These rosé wines were elaborated with equal conditions of prefermentative maceration, alcoholic fermentation and grape maturity at time of harvest.[34] The differences observed with respect to RG-II molecule may be due to differences in the weakness of the grape skins that could modulate the extraction of wine components, which suggest a certain varietal characteristic.

Grape monosaccharides decreased similarly in all sparkling wines during the whole period of aging. Therefore, final sparkling wines had lower concentrations of all glycosyl residues than their corresponding base wines. All base wines were composed of grape PRAGs and HLs, which represented 75 ± 26% and 23 ± 18% of total polysaccharide families from grapes, respectively, except for Prieto Picudo base wines, which also contained the RG-II polysaccharide family. PRAGs were the most prevalent polysaccharide family, indicating that they were easily released into the wine by the action of endogenous enzymes as they are localized in soluble form within grape cell walls.[32] The proportion of HLs was higher than that observed by our group in still wines.[4,9] This fact was attributed to the concentration to dryness used to precipitate polysaccharides, which could have resulted in a higher concentration of oligosaccharides and HLs of low molecular weight.[27]

Similar concentrations of PRAGs and HLs were found in rosé base wines and in white base wines, thus indicating a lack of solubilization of these compounds during the prefermentative maceration in rosé base wines. As previously explained, RG-II extraction only occurred in Prieto Picudo base wines, in which it represented 5.5 ± 0.5% of total polysaccharides from grapes.

The evolution of various types of polysaccharide families was different during the stages of the sparkling wine processing. HLs and RG-II decreased during the first 6 months of aging, and PRAGs remained constant. Aging periods of more than 6 months prompted a considerable reduction in all polysaccharide families. As observed with MPs and GLs, grape polysaccharides also reacted with other wine compounds to form unstable colloids during long periods of aging on yeast lees. During this period of more than 6 months of aging, reductions in HLs were higher than in PRAGs and RG-II (86% vs 41%) in all sparkling wines, therefore, indicating a higher reactivity of HLs toward other wine constituents.

The arabinose/galactose ratio (Ara/Gal) is characteristic of the wine arabinogalactan-protein composition. Other authors have described aging on yeast lees produces a decrease in the Ara/Gal ratio because the terminal arabinose residues were removed. This reduction of arabinose residues indicates a dearabinosylation of arabinogalactan-proteins.[29] Although we also observed a significant decrease in this ratio for Viura and Verdejo sparkling wines, the ratio remained constant in the rest of the wines. Therefore, decisive conclusions could not be obtained.

Evolution of Total Polysaccharide Families during Sparkling Wine Making and Aging. Total monosaccharides were calculated as the sum of arabinose, fucose, mannose, galactose, galacturonic acid, glucose, rhamnose, glucuronic acid, 2-keto-3-deoxyoctonate ammonium salt and 2-*O*-methyl-D-xylose. Prieto Picudo had the highest value of total monosaccharides among rosé base wines (439.71 ± 18.21 mg/L) while Albarín base wines showed the highest value among white wines (488.24 ± 34.28 mg/L). Monosaccharide composition was similar in all base wines: it was composed of glucose, followed by galactose, mannose and arabinose. In the same way, monosaccharide composition was similar in all final wines, which were composed of mannose (35 ± 11%), followed by glucose (25 ± 15%), galactose (21 ± 13%) and arabinose (11 ± 5%). These percentages are in agreement with the composition of other sparkling wines obtained by different authors.[20,36]

Total polysaccharide families were calculated as the sum of MPs, GLs, PRAGs, HLs and RG-II (Figure 2). Among rosé base wines, Prieto Picudo showed the highest amount of total polysaccharides (446.36 ± 18.21 mg/L), whereas Albarín base wine showed the highest quantity among the white wines (494.29 ± 37.72 mg/L). However, base wines with the highest concentrations of polysaccharides had a greater drop in their polysaccharide content during aging, compared to base wines with low concentrations. Thus, total polysaccharides decreased 78 ± 6% in Prieto Picudo and 73 ± 9% in Albarín from 6 months of aging on, reaching similar final values as the rest of the sparkling wines. This fact suggests an important quantity of the extra polysaccharides precipitated during aging. Therefore, techniques employed to increase the extraction and release of polysaccharides during winemaking would not be as interesting as expected because the higher initial content of polysaccharides could be related to a higher precipitation. With regard to

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Table 2. Evolution of Grape Monosaccharides and Polysaccharide Families (mg/L) and Arabinose/Galactose Ratio during Different Stages of the Sparkling Wine Production: Base Wines (BW), and Sparkling Wines after 3 Months (T3M), 6 Months (T6M), 9 Months (T9M), 18 Months (T18M), and 30 Months (T30M) of Aging in Bottle on Yeast Lees[a]

		Albarín	Viura	Godello	Malvasía	Verdejo	Garnacha	Garnacha*	Prieto Picudo
BW									
	arabinose	61.41 ± 9.60 e D	42.51 ± 1.95 bc C	24.71 ± 4.10 a B	31.83 ± 1.45 abc C	33.69 ± 1.65 abc B	43.32 ± 0.67 cd C	30.50 ± 0.04 ab B	54.73 ± 5.34 de C
	galactose	125.03 ± 20.2 c C	55.01 ± 0.71 b C	26.98 ± 9.12 a AB	59.84 ± 3.24 b B	61.93 ± 1.36 b CD	66.09 ± 3.36 b BC	50.21 ± 11.89 ab A	73.30 ± 6.83 b C
	rhamnose	18.07 ± 1.95 d D	11.95 ± 0.50 bc BC	8.96 ± 1.00 ab B	9.64 ± 0.76 ab C	4.09 ± 0.41 a AB	12.37 ± 4.21 bc BC	7.57 ± 2.19 ab AB	16.28 ± 1.89 cd C
	fucose	3.95 ± 0.48 e C	1.91 ± 0.05 bc CD	1.58 ± 0.24 b B	2.32 ± 0.11 cd C	0.79 ± 0.11 a A	1.66 ± 0.06 bc A	1.71 ± 0.21 bc AB	2.69 ± 0.26 d D
	galacturonic acid	30.51 ± 4.13 abc C	24.06 ± 0.70 abc BC	37.36 ± 9.35 c C	34.69 ± 8.09 bc B	11.96 ± 1.64 a AB	15.80 ± 2.56 ab B	27.28 ± 11.50 abc B	66.02 ± 11.00 d C
	glucuronic acid	9.46 ± 0.92 c C	5.85 ± 1.48 b BC	2.30 ± 1.46 a AB	5.17 ± 0.31 b B	3.84 ± 0.04 ab AB	5.30 ± 1.08 b A	4.07 ± 1.17 ab B	5.64 ± 0.64 b B
	2-*O*-methyl xylose	nd	nd	nd	nd	nd	nd	nd	0.21 ± 0.06 B
	apiose	nd	nd	nd	nd	nd	nd	nd	0.75 ± 0.15 C
	Kdo	nd	nd	nd	nd	nd	nd	nd	2.16 ± 0.08 C
	arabinose/galactose	0.59 ± 0.11 a A	0.93 ± 0.04 ab CD	1.10 ± 0.37 c B	0.64 ± 0.04 a A	0.65 ± 0.03 a B	0.79 ± 0.03 ab C	0.73 ± 0.14 ab A	0.90 ± 0.10 ab A
	PRAGs	204.22 ± 24.94 d D	109.13 ± 7.03 bc CD	57.35 ± 10.74 a B	101.17 ± 3.63 b B	104.03 ± 2.63 b CD	120.58 ± 3.59 bc D	88.91 ± 11.94 ab B	141.09 ± 8.72 c C
	HLs	40.25 ± 13.34 b C	30.25 ± 1.78 ab AB	42.97 ± 9.86 b B	40.02 ± 8.16 b B	11.48 ± 1.65 a BC	22.30 ± 2.63 ab B	30.71 ± 11.50 ab B	74.88 ± 12.74 c C
	RG-II	nd	nd	nd	nd	nd	nd	nd	12.46 ± 0.71 B
T3M									
	arabinose	43.11 ± 2.44 de C	43.25 ± 3.44 de C	15.63 ± 2.54 a A	28.00 ± 4.69 bc BC	23.97 ± 6.72 ab B	36.52 ± 5.00 cd BC	33.72 ± 2.16 bcd B	50.70 ± 5.41 e C
	galactose	97.01 ± 6.13 d C	50.06 ± 4.50 b C	28.33 ± 9.50 a AB	53.00 ± 9.87 bc B	35.71 ± 3.79 ab AB	69.26 ± 4.74 c C	50.25 ± 1.73 b A	69.00 ± 4.85 c BC
	rhamnose	12.28 ± 0.89 c C	9.94 ± 0.66 b AB	4.27 ± 0.05 a A	5.58 ± 0.80 a B	5.57 ± 0.16 a BC	10.07 ± 0.01 b ABC	8.53 ± 0.26 b AB	13.09 ± 1.24 c BC
	fucose	2.50 ± 0.47 b B	1.74 ± 0.30 ab BC	0.85 ± 0.12 a A	1.23 ± 0.80 a AB	1.12 ± 0.09 a AB	1.62 ± 0.39 ab A	1.81 ± 0.05 ab AB	1.88 ± 0.01 ab C
	galacturonic acid	24.42 ± 1.38 ab BC	27.00 ± 10.85 b BC	13.58 ± 5.38 a AB	11.55 ± 1.19 a A	21.05 ± 4.69 ab C	11.26 ± 0.35 a AB	20.25 ± 1.60 ab AB	15.57 ± 2.39 ab A
	glucuronic acid	7.89 ± 0.70 d C	5.20 ± 1.06 bc BC	3.08 ± 0.78 a ABC	4.74 ± 0.28 bc B	2.40 ± 0.21 a A	5.43 ± 0.03 c A	3.70 ± 0.30 ab B	4.98 ± 0.41 bc B
	2-*O*-methyl xylose	nd	nd	nd	nd	nd	nd	nd	0.13 ± 0.00 AB
	apiose	nd	nd	nd	nd	nd	nd	nd	0.64 ± 0.02 BC
	Kdo	nd	nd	nd	nd	nd	nd	nd	1.42 ± 0.17 B
	arabinose/galactose	0.53 ± 0.04 a A	1.04 ± 0.10 b D	0.66 ± 0.21 a AB	0.63 ± 0.13 a A	0.81 ± 0.20 ab B	0.63 ± 0.08 a ABC	0.81 ± 0.05 ab A	0.88 ± 0.09 ab A
	PRAGs	153.86 ± 6.64 f C	104.38 ± 5.78 cd C	49.17 ± 9.87 a AB	89.54 ± 10.95 bc B	65.33 ± 15.00 ab B	116.16 ± 6.92 de CD	92.24 ± 2.80 cd B	131.55 ± 7.31 ef C
	HLs	30.86 ± 2.54 b C	31.08 ± 11.02 b AB	15.73 ± 5.43 a A	13.34 ± 1.65 a A	23.37 ± 5.14 ab D	16.38 ± 3.49 ab AB	24.20 ± 1.97 ab AB	21.78 ± 5.15 ab A
	RG-II	nd	nd	nd	nd	nd	nd	nd	7.92 ± 0.68 AB
T6M									
	arabinose	49.55 ± 2.92 de CD	43.79 ± 9.89 cde C	16.95 ± 3.59 a AB	33.39 ± 0.22 cd C	33.00 ± 8.00 ab B	27.93 ± 2.37 ab A	35.80 ± 5.39 cd B	59.23 ± 6.88 e C
	galactose	107.22 ± 7.73 c C	69.08 ± 6.13 b D	32.94 ± 8.77 a AB	61.45 ± 12.00 b B	72.00 ± 10.00 b D	69.00 ± 4.00 b C	58.10 ± 12.00 b A	69.41 ± 2.25 b BC
	rhamnose	14.54 ± 1.15 bcd C	15.42 ± 4.00 cd C	7.96 ± 1.00 ab B	8.71 ± 1.99 abc C	6.53 ± 2.40 a BC	14.89 ± 3.20 bcd C	10.56 ± 1.92 abcd B	17.22 ± 2.51 d C
	fucose	3.16 ± 0.42 b BC	2.41 ± 0.30 ab E	1.49 ± 0.21 a B	2.06 ± 0.35 ab BC	1.51 ± 0.54 a BC	1.47 ± 0.69 a A	2.06 ± 0.48 ab B	2.58 ± 0.01 ab D
	galacturonic acid	26.89 ± 4.46 a BC	31.67 ± 10.41 ab C	27.52 ± 9.45 ab BC	25.09 ± 3.05 a B	20.55 ± 5.00 a C	26.97 ± 6.00 a C	32.05 ± 5.00 ab B	47.12 ± 9.62 b B
	glucuronic acid	8.99 ± 0.64 b C	6.63 ± 0.77 ab C	5.00 ± 0.80 a C	5.10 ± 1.15 a B	6.86 ± 1.80 ab C	8.87 ± 1.57 b B	4.92 ± 1.20 a B	3.91 ± 1.20 a AB
	2-*O*-methyl xylose	nd	nd	nd	nd	nd	nd	nd	0.14 ± 0.05 AB
	apiose	nd	nd	nd	nd	nd	nd	nd	0.43 ± 0.05 A
	Kdo	nd	nd	nd	nd	nd	nd	nd	0.82 ± 0.02 A
	arabinose/galactose	0.55 ± 0.04 a A	0.76 ± 0.15 ab ABC	0.62 ± 0.18 a AB	0.65 ± 0.11 a A	0.55 ± 0.13 a AB	0.49 ± 0.04 a A	0.74 ± 0.16 ab A	1.02 ± 0.10 b A

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Table 2. continued

		Albarín	Viura	Godello	Malvasía	Verdejo	Garnacha	Garnacha*	Prieto Picudo
	PRAGs	172.48 ± 8.30 d CD	125.44 ± 11.74 bc D	57.19 ± 9.52 a B	104.47 ± 16.00 b B	116.34 ± 12.98 bc D	109.59 ± 4.92 bc CD	103.67 ± 13.23 b B	140.58 ± 7.39 c C
	HLs	34.71 ± 5.43 ab C	41.15 ± 16.44 ab B	33.18 ± 9.84 ab B	29.27 ± 3.05 a B	22.61 ± 5.48 a D	38.07 ± 6.98 ab C	37.76 ± 6.51 ab B	56.31 ± 12.88 b BC
	RG-II	nd	nd	nd	nd	nd	nd	nd	8.29 ± 0.04 AB
T9M									
	arabinose	22.52 ± 2.55 ab B	17.58 ± 1.51 a AB	17.51 ± 2.12 a AB	32.92 ± 1.93 c C	34.89 ± 1.53 c B	35.37 ± 0.39 c B	29.68 ± 3.21 bc B	57.75 ± 5.52 d C
	galactose	43.61 ± 4.41 ab B	33.34 ± 1.79 a AB	35.43 ± 2.57 a AB	56.81 ± 0.35 bcd B	67.52 ± 7.11 de CD	58.89 ± 2.76 cde ABC	50.89 ± 3.95 bc A	71.26 ± 9.18 e BC
	rhamnose	5.10 ± 0.57 a B	6.59 ± 0.32 ab A	5.10 ± 0.32 a A	7.82 ± 0.09 ab BC	8.65 ± 1.58 b C	9.39 ± 0.61 b AB	7.57 ± 0.89 ab AB	17.31 ± 2.43 c C
	fucose	1.10 ± 0.10 bc A	0.94 ± 0.04 ab A	0.72 ± 0.02 a A	1.66 ± 0.00 de BC	1.79 ± 0.07 e C	1.71 ± 0.19 e A	1.36 ± 0.15 cd A	2.52 ± 0.13 f D
	galacturonic acid	5.28 ± 0.28 a A	12.81 ± 1.30 bc AB	5.80 ± 0.30 a A	9.96 ± 1.96 ab A	16.18 ± 2.15 c BC C	15.57 ± 2.68 bc B	9.57 ± 1.51 ab A	42.11 ± 4.06 d B
	glucuronic acid	4.03 ± 0.54 a B	3.73 ± 0.65 a AB	3.19 ± 0.44 a ABC	5.50 ± 1.07 a B	5.54 ± 1.34 a BC	5.78 ± 0.59 a A	5.24 ± 0.32 a B	5.73 ± 2.00 a B
	2-*O*-methyl xylose	nd	nd	nd	nd	nd	nd	nd	0.13 ± 0.03 AB
	apiose	nd	nd	nd	nd	nd	nd	nd	0.47 ± 0.03 AB
	Kdo	nd	nd	nd	nd	nd	nd	nd	0.78 ± 0.02 A
	arabinose/ galactose	0.62 ± 0.08 a A	0.63 ± 0.05 a AB	0.59 ± 0.07 a AB	0.70 ± 0.03 a A	0.62 ± 0.06 a AB	0.72 ± 0.03 a BC	0.70 ± 0.08 a A	0.97 ± 0.13 b A
	PRAGs	73.22 ± 5.13 b B	57.04 ± 2.44 a A	58.50 ± 3.37 ab B	99.70 ± 2.25 cd B	112.69 ± 7.40 d D	104.85 ± 2.85 cd BC	89.83 ± 5.12 c B	142.57 ± 10.92 e C
	HLs	7.33 ± 0.76 a AB	17.02 ± 1.56 bc A	8.53 ± 0.84 ab A	13.32 ± 2.15 abc A	20.10 ± 2.30 c CD	20.17 ± 2.69 c B	13.11 ± 2.16 abc A	51.59 ± 8.17 d B
	RG-II	nd	nd	nd	nd	nd	nd	nd	6.96 ± 1.17 AB
T18M									
	arabinose	25.84 ± 3.00 b B	27.37 ± 1.93 bc B	16.27 ± 2.30 a A	23.94 ± 1.85 b B	24.29 ± 2.66 b B	27.71 ± 1.79 bc A	28.36 ± 1.86 bc B	33.76 ± 2.47 c B
	galactose	67.53 ± 9.54 c B	40.04 ± 2.19 a B	40.09 ± 3.30 a B	53.98 ± 2.77 b B	50.96 ± 1.75 ab BC	55.97 ± 6.12 bc AB	51.29 ± 3.23 ab A	56.75 ± 2.36 bc B
	rhamnose	8.15 ± 1.09 cd B	7.93 ± 0.54 bcd AB	4.64 ± 0.32 a A	5.48 ± 0.54 ab B	5.78 ± 0.89 abc BC	7.41 ± 1.06 bcde AB	7.11 ± 0.77 bcd AB	9.52 ± 0.63 e B
	fucose	2.32 ± 0.30 b B	1.46 ± 0.20 a ABC	0.94 ± 0.09 a A	1.39 ± 0.23 a ABC	0.96 ± 0.04 a AB	1.28 ± 0.22 a A	1.23 ± 0.22 a A	1.48 ± 0.10 a B
	galacturonic acid	18.78 ± 7.95 b B	18.28 ± 5.00 b ABC	5.04 ± 0.64 a A	7.73 ± 0.41 a A	7.68 ± 0.59 a A	9.39 ± 1.51 ab AB	8.60 ± 1.40 a A	12.65 ± 0.65 ab A
	glucuronic acid	4.94 ± 0.38 ab B	6.85 ± 0.35 c C	4.07 ± 0.47 ab BC	5.43 ± 0.80 b B	3.69 ± 0.55 a AB	4.39 ± 0.45 ab A	4.80 ± 0.09 ab B	4.40 ± 0.58 ab AB
	2-*O*-methyl xylose	nd	nd	nd	nd	nd	nd	nd	0.10 ± 0.01 A
	apiose	nd	nd	nd	nd	nd	nd	nd	0.42 ± 0.04 A
	Kdo	nd	nd	nd	nd	nd	nd	nd	0.82 ± 0.02 A
	arabinose/ galactose	0.46 ± 0.07 a A	0.82 ± 0.06 e BCD	0.49 ± 0.07 a A	0.53 ± 0.04 ab A	0.57 ± 0.05 abc AB	0.59 ± 0.06 abc AB	0.66 ± 0.05 bcd A	0.71 ± 0.05 cd A
	PRAGs	101.80 ± 10.02 d B	77.98 ± 2.95 b B	62.64 ± 4.06 a B	86.60 ± 3.43 bc B	82.24 ± 3.25 b BC	91.83 ± 6.39 bcd AB	88.30 ± 3.73 bcd B	99.49 ± 3.48 cd B
	HLs	23.43 ± 8.17 c BC	22.51 ± 5.12 bc AB	7.47 ± 1.00 a A	9.96 ± 0.69 a A	10.16 ± 1.07 a B	13.05 ± 1.75 ab AB	11.86 ± 1.62 a A	17.59 ± 1.78 abc A
	RG-II	nd	nd	nd	nd	nd	nd	nd	7.81 ± 0.05 AB
T30M									
	arabinose	7.03 ± 0.30 a A	12.86 ± 2.70 ab A	13.24 ± 1.83 ab A	15.80 ± 2.87 b A	7.16 ± 0.76 a A	25.79 ± 2.79 c A	15.44 ± 3.88 b A	11.99 ± 1.61 ab A
	galactose	14.12 ± 0.93 a A	27.50 ± 1.06 abc A	18.58 ± 0.82 ab A	31.22 ± 0.89 bc A	26.25 ± 8.89 abc A	47.70 ± 3.51 d A	36.65 ± 10.29 cd A	14.89 ± 3.10 a A
	rhamnose	1.67 ± 0.11 a A	5.11 ± 1.28 cd A	3.81 ± 0.57 bc A	2.06 ± 0.41 ab A	1.84 ± 0.21 a A	4.79 ± 0.66 cd A	5.64 ± 0.56 d A	2.36 ± 0.33 ab A
	fucose	0.52 ± 0.03 a A	1.23 ± 0.24 b AB	0.65 ± 0.20 a A	0.62 ± 0.10 a A	0.54 ± 0.04 a A	0.87 ± 0.16 ab A	1.23 ± 0.20 b A	0.50 ± 0.09 a A
	galacturonic acid	2.66 ± 0.21 a A	4.84 ± 0.87 bc A	nd	4.24 ± 1.04 ab A	nd	6.07 ± 0.60 bc A	6.38 ± 0.64 c A	6.57 ± 1.00 c A
	glucuronic acid	1.01 ± 0.15 a A	2.41 ± 0.12 b A	1.41 ± 0.33 a A	2.41 ± 0.65 b A	1.34 ± 0.36 a A	4.17 ± 0.18 c A	1.52 ± 0.07 a A	1.79 ± 0.19 ab A
	2-*O*-methyl xylose	nd	nd	nd	nd	nd	nd	nd	0.12 ± 0.02 A
	apiose	nd	nd	nd	nd	nd	nd	nd	0.39 ± 0.05 A
	Kdo	nd	nd	nd	nd	nd	nd	nd	0.62 ± 0.01 A

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Table 2. continued

	Albarín	Viura	Godello	Malvasía	Verdejo	Garnacha	Garnacha*	Prieto Picudo
arabinose/galactose	0.60 ± 0.04 ab A	0.56 ± 0.10 ab A	0.85 ± 0.10 bc AB	0.61 ± 0.09 ab A	0.33 ± 0.10 a A	0.65 ± 0.07 abc BC	0.51 ± 0.16 a A	0.97 ± 0.20 c A
PRAGs	23.12 ± 0.99 a A	44.51 ± 2.92 bcd A	35.02 ± 2.05 ab A	51.57 ± 3.10 cd A	35.72 ± 8.93 abc A	81.16 ± 4.50 e A	55.71 ± 11.01 d A	30.29 ± 3.50 ab A
HLs	3.37 ± 0.21 ab A	8.20 ± 1.51 c A	2.01 ± 0.50 ab A	4.16 ± 1.04 b A	0.87 ± 0.09 a A	7.37 ± 0.77 c A	9.92 ± 1.97 c A	7.30 ± 1.01 c A
RG-II	nd	nd	nd	nd	nd	nd	nd	6.32 ± 0.02 A

[a]Values are means ± SD (n = 3). Different lowercase letters in the same row indicate that means significantly differ at $p < 0.05$. Different capital letters in the same column indicate that means significantly differ at $p < 0.05$. nd: below detection limit.

final sparkling wines, Garnacha reached the highest content of total polysaccharides (223.11 ± 4.76 mg/L), followed distantly by Viura (137.74 ± 4.71 mg/L) and last by the rest of sparkling wines (<130 mg/L). These results indicated that the content of polysaccharides was independent of the color of the grapes and the type of winemaking (with or without prefermentative maceration). The values found were in the range described in other studies for sparkling wines.[14,17,18,20] Final sparkling wines were essentially composed of PRAGs, MPs, GLs and HLs, with average percentages of 35 ± 16%, 35 ± 11%, 25 ± 15% and 4 ± 2%, respectively. The sum of MPs and GLs (47−78% of total polysaccharide families) was higher than those found in still wines, obviously due to the lysis process during the aging period. To the best of our knowledge, there is no literature on this aspect relating sparkling wines, and this is the first time concrete polysaccharide families in these types of wines are described.

Despite the foam properties of sparkling wines being controlled by a large number of molecules that act in a synergistic way,[37] MPs released by yeast during autolysis are particularly important because their hydrophobic nature causes them to preferentially adsorb to the gas/liquid interface of foam bubbles.[38] On the other hand, PRAGs could also play an important role in the foam quality and stability due to its protein fraction. The results of our investigation indicated how the highest content of mannoproteins was obtained at 6 months of aging. We also observed how the content of polysaccharides coming from grapes was positively correlated with the content of MPs (r = 0.792; $p < 0.01$) during the entire winemaking and aging process. Therefore, the content of PRAGs and HLs also reached its highest concentrations after 6 months of aging. In this sense, these results suggest that longer aging time is not necessary to obtain greater amount of polysaccharides.

Distribution of the Molecular Weights of Polysaccharides during Sparkling Wine Making and Aging. HRSEC-RID on Shodex column allowed us to follow the qualitative changes in the molecular weight distribution of polysaccharides during sparkling wine making (Figure 3). Chromatograms of base wines were analyzed in order to establish differences due to variety. In this sense, Prieto Picudo base wines showed a different profile than the rest of the base wines. Prieto Picudo base wines were characterized by three populations that eluted at 14.2, 16.0, and 17.2 min and corresponded to fractions of 178, 39, and 10 kDa, respectively. According to the literature,[9,27,28,31,39] the first two populations corresponded to complex mixture of high and medium molecular weight PRAGs from grape berries and high and medium molecular weight MPs and GLs released by the yeast. The third population corresponded mainly to grape RG-II dimers, and also to low molecular weight PRAGs and MPs. The rest of base wines showed two major peaks eluting at 14.2 and 16.1 min. However, they did not show the presence of a third population. These results were in agreement with those obtained by GC−MS, illustrating how Prieto Picudo base wines had the RG-II polysaccharide family. Except for Prieto Picudo, all base wines showed a similar molecular weight distribution as that previously described in white musts.[33]

All samples showed a slight shift from higher to lower molecular weight polysaccharides from base wine to 6 months of aging on yeast lees. This could be attributed to the release of MPs and GLs of lower molecular weights due to the random breaking of the cell wall into a succession of different size

fragments. However, this could also be contributed to the hydrolysis of the macromolecules by exo-β-(1,3)-glucanases, α-manosidases and proteases[40] released into the wine. These results were in agreement with those of other researchers, who also observed a change to lower molecular weights in the polysaccharide size distribution during aging.[30,31,41−43] Moreover, the occurrence of peak tailing at ∼16 kDa was observed, thus, suggesting a partial degradation of the polysaccharides during aging over lees, and modification of their properties and solubilization.

Several authors have observed that small MPs inhibit tannin aggregation[5] and their efficiency as particle stabilizers decreases as their molecular weight increases.[44] Moreover, small MPs have also been shown to be responsible for tartaric stability.[45] The fraction responsible for the foaming properties in sparkling wines is constituted by MPs with a relative molecular weight between 10 and 30 kDa.[21]Therefore, the shift to lower molecular weight polysaccharides could result in an improvement of the wine colloidal stability and foam properties. As the tirage phase went on, no more shifts were observed.

In conclusion, it is important to point out that the highest amount of polysaccharides was obtained at 6 months of aging along with a change to lower molecular weights. These changes could imply a better foam stability and thus better sensory quality.

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Notes

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