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Polysaccharides, oligosaccharides and nitrogenous compounds change during the ageing of Tempranillo and Verdejo sparkling wines

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Abstract

BACKGROUND: Verdejo and Tempranillo are traditional varieties for producing still wines; however, they could provide an alternative for the manufacturing of sparkling wines. Sparkling wines were elaborated by the traditional method, followed by ageing on lees for 9 months. A study on the changes that take place in polysaccharides, oligosaccharides and nitrogenous compounds during the ageing on lees of Tempranillo and Verdejo sparkling wines has been undertaken.

RESULTS: Mannoproteins and the glucose residue of oligosaccharides were the major carbohydrates detected in all vinification stages. Yeast polysaccharides and glucan-like structures of the oligosaccharides increased after 3 months of ageing. The evolution of yeast polysaccharides and the composition of PRAG-like structure were different among grape varieties. A decrease in amino acids and biogenic amines was observed during the ageing. The contents of polysaccharides, oligosaccharides and nitrogenous compound were significantly higher in Tempranillo than in Verdejo sparkling wines at the end of the ageing period.

CONCLUSION: Polysaccharides and oligosaccharides from yeast were more significant autolysis markers of sparkling wines than the nitrogenous compounds. Our data suggest a potential cultivar effect on the evolution of yeast polysaccharides and on the composition of PRAG, which may influence the physico-chemical and sensory properties of sparkling wines.

Keywords: sparkling wine; ageing; polysaccharides; oligosaccharides; nitrogenous compounds

INTRODUCTION

Quality sparkling wines elaborated following the traditional or bottle-fermented method undergo a second fermentation in closed bottles of base wines, followed by ageing of wines with lees for at least 9 months before de-disgorging, since it is the minimum time necessary for sparkling wines with a protected designation of origin (EC Regulation No. 606/2009). In Spain, 'Cavas' are the most well-known sparkling wines, which are elaborated mainly from Macabeo (Viura), Xarel.lo and Parellada white grape varieties. However, other varieties include Malvasía (prime name Alarije, Variety number VIVC n° 213) and Chardonnay (white varieties) and Garnacha Tinta, Monastrell, Pinot Noir and Trepat (red varieties) (BOE No. 50 8487-8491/2007; Order APA/415/2007). Taking into account that the grape varietal landscape in Spain is very rich, other grape varieties could also present good characteristics to obtain quality sparkling wines. In this sense, *Vitis vinifera* L. var. Verdejo (prime name Verdejo Blanco, Variety number VIVC n° 12949) and Tempranillo (prime name Tempranillo Tinto, Variety number VIVC n° 12350) are two of the best Spanish grape varieties to produce high-quality still wines and have significantly increased plantings in recent years,[1] but could also be used for sparkling wine production. Grape variety composition and its ability to age have been described as one of the major factors influencing the character of bottle-fermented sparkling wines,[2,3] In the same way, grape variety has a notable influence on yeast autolysis,[4] the process by which the yeasts release intracellular compounds into the wine that can significantly change its final composition.[5] Among compounds released during autolysis, yeast mannoproteins largely affect the final quality of the wines. They may have utility in bottle fermentation of sparkling wines because they contribute to the flocculation of yeast strains.[6] Mannoproteins can bind volatile compounds and thus retain wine aroma,[7] and they have also shown a positive effect on foam stability,[8–10] On the other

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hand, decreases in the content of polysaccharides from grapes[11,12] and de-arabinosylation of polysaccharides rich in arabinose structures[13,14] have been observed during the ageing process. These facts may have possible consequences for physico-chemical properties of polysaccharides and thus modify the final colloidal equilibrium of wines. With regards to oligosaccharides, the influence of these molecules over astringency in still wines has been recently investigated.[15,16] It was observed that oligosaccharides improve sensory characteristics such as taste and texture, and enhanced the stability of foams, emulsions and mouthfeel in a wide range of food applications.[17] Thus, oligosaccharide composition could have important consequences on foam quality of sparkling wines. However, little is known about the sensory influence, content and evolution of these molecules in white and rosé sparkling wines. Since the quantity, composition and structure of oligosaccharides in wines depend on the grape variety[18] an understanding of their content and kinetic release during the Verdejo and Tempranillo sparkling winemaking is essential.

The amount of nitrogenous compounds in sparkling wines is influenced by the grape variety and ageing time.[19,20] Amino acids are, generally, considered the major compounds released into the wine during the autolysis process which could contribute to the wine's volatile profile[21] and foam properties.[8] Besides, the increase in amino acids could also favour the formation of biogenic amines, although there are very few studies on this subject in sparkling wines.[21,22]

The evolution of complex hyperbranched carbohydrates, defined as polysaccharides and oligosaccharides, during the winemaking process and also during the ageing on yeast lees to elaborate red sparkling wines has been previously performed.[12] However, to the best of our knowledge, there is no literature about the content and evolution of the nitrogenous compounds and complex carbohydrates during the winemaking of Tempranillo and Verdejo sparkling wines. Finally, it is important to point out that autolysis is a very complex event and should be evaluated as many compounds together as possible to show aspects of the release of compounds. Only the knowledge of the ageing ability and the chemical composition of Verdejo and Tempranillo sparkling wines can give opportunities for the adaptation of the characteristics of these grape varieties to the sparkling winemaking procedures.

Considering all the previous comments and studies, the aim of this work was to focus on the study of the polysaccharides, oligosaccharides and nitrogenous compounds of white and rosé base wines elaborated from Verdejo and Tempranillo and the evolution of the sparkling wines during their ageing on lees in bottle.

MATERIALS AND METHODS

Sparkling wine samples

Sparkling wines were manufactured using the traditional or bottle-fermented method in the oenological station of Castilla y León (Valladolid, Spain). White base wines were elaborated with *Vitis vinifera* cv. Verdejo from the Rueda Denomination of Origin (D.O.), and rosé base wines were obtained with *Vitis vinifera* cv. Tempranillo grapes from the Cigales D.O. White grapes were destemmed–crushed in a crusher–destemmer machine (Model ECR-15; CMMC group, Madrid, Spain). The mass obtained was sulfited (50 mg L^{-1}) and pressed (0.2–2 bars, 5–6 h pressing time) in a Europress EHS 103 pneumatic press (Scharfenberger, Germany) to obtain juice. After destemming and crushing, red grapes underwent to prefermentative maceration for 2 days before pressing. Grape juices were settled for 24 h before racking. Juice clarity was determined by measuring the turbidity of must samples, using a turbidimeter (model 2100 N; Hach Instruments, Loveland, CO, USA). No treatment with pectinolytic commercial enzymes was applied. The base wines were produced in duplication following the traditional white or rosé winemaking process in stainless steel tanks of 2000 and 2600 L, respectively. The fermentation processes took place at 16 to 18 °C after the inoculation with 20 g HL^{-1} *S. cerevisiae* var. *bayanus* (IOC 18-2007; Lallemand, Madrid, Spain). After cold stabilisation (−5 °C) and clarification with PVPP (10 g HL^{-1}, Laffort, Bordeaux-Cedex, France) to prevent browning, and bentonite (80 g HL^{-1}, Laffort) to remove unstable proteins of base wines, the *tirage* liquor was added and the wines were bottled. The *tirage* liquor was formed by yeast *S. cerevisiae* var. *bayanus* (30 g HL^{-1}, IOC 18-2007; Lallemand), sucrose (23 g L^{-1}) and bentonite (0.10 g L^{-1}; Laffort). The bottles were finally kept in a cellar at temperature (11–13 °C) and relative humidity (75–85%) controlled for 9 months. Samples for analyses were taken from the base wines (T0) and then after 3 months (T3), 6 months (T6), and 9 months (T9) of ageing on yeast lees. Wines were riddled and disgorged before analysis and 'liqueur "d'expédition"' (dosage solution, consisting of a mixture of sugar and aged wine) was no added. Therefore, each bottle was filled with the same wine to produce Brut Nature wines. Since the second fermentation takes place in individual bottles, three bottles of each varietal sparkling wine at each sampling time were analysed.

Isolation of polysaccharide and oligosaccharide fractions

The polysaccharide and oligosaccharide fractions were isolated as previously described.[23] Polysaccharide fraction was eluted between 40 and 53 min, while oligosaccharide fraction was collected between 54 and 93 min.[16,24] The isolated fractions were freeze-dried, redissolved in water and freeze dried again for four times to remove the ammonium salt.

Polysaccharide analysis

Neutral monosaccharides were released after hydrolysis of the wine polysaccharides by treatment with 2 mol L^{-1} trifluoroacetic acid for 75 min at 120 °C.[25] The different alditol acetates were identified from their retention time by comparison with that of standard monosaccharides. Allose and myo-inositol were used as internal standards. Neutral sugar amounts were calculated relative to the internal standard (myo-inositol).

Oligosaccharide analysis

Following the method of Doco *et al.*,[26] the neutral and acidic sugar composition was determined after solvolysis with anhydrous MeOH containing 0.5 mol L^{-1} HCl (80 °C, 16 h), by GC of their per-*O*-trimethylsilylated methyl glycoside derivatives.

Determination of molar mass of sparkling wine polysaccharides and oligosaccharides

Molar-mass distributions (M_w and M_n), polydispersity index (M_w/M_n) and intrinsic viscosity were determined at 25 °C by coupling size exclusion chromatography with a multi-angle light scattering device (MALLS) and a differential refractive index detector, as previously described.[13] All collected data were analysed using Astra V 6.0.6 software (Wyatt Technologies, Santa Barbara, CA, USA) with the zimm plot (order 1) technique for molar-mass

estimation and a differential refractive index increment of the polymer in the solvent used. A d*n*/d*c* classical value was employed for polysaccharides (0.146 mL g^{-1}).[27]

Amino acids and biogenic amines analysis

The amino acids and biogenic amines were analysed by high-performance liquid chromatography with diode array detector (HPLC-DAD) after derivatisation with DEEMM[28]. Calibration curves were obtained using the commercial standards, and L-2-aminoadipic acid was used as the internal standard.

Chemicals

All reagents were analytical grade unless otherwise stated. Ammonium formiate, sodium chloride, phosphorous pentoxide, hydrogen chloride, trifluoroacetic acid, sodium borohydride, ammonia, acetone, glacial acetic acid, ethyl acetate, acetic anhydride, perchloric acid 70%, 1-methylimidazole, chloroform, and *n*-hexane were obtained from Merck (Darmstadt, Germany). Methanol and acetonitrile were provided by Scharlau (Barcelona, Spain) and water Milli-Q was obtained via a Millipore system (Bedford, MA, USA). Methanol anhydrous, allose, myo-inositol, isoamylamine, L-proline, diethyl ethoxymethylenemalonate (DEEMM), putrescine, tyramine, tryptamine, *trans*-4-hydroxy-L-proline, L-2-aminoadipic acid, L-ornithine monohydrochloride, L-tryptophan, L-asparagine, L-threonine, γ-aminobutyric acid (GABA), L-isoleucine, L-glutamine, L-methionine and sodium azide were purchased from Sigma–Aldrich (St Louis, MO, USA). Polyamide SC6 was supplied by Macherey–Nagel (Düren, Germany). Tri-Sil (Reagent Pierce, Interchim) was obtained from Thermo Scientific (Waltham, MA, USA). L-Cysteine, L-leucine, L-phenylalanine, L-lysine, ammonium chloride, L-histidine, agmatine sulfate, cadaverine, L-arginine, histamine, L-alanine, spermidine, glycine, β-alanine, L-aspartic acid, L-glutamic acid, L-tyrosine, L-valine, L-serine and L-phenylethylamine were purchased from Fluka (Buchs, Switzerland).

Statistical analysis

All of the data are expressed as the average of three replicates. One-factor ANOVA considering ageing time as independent variable and two-sample *t* test considering grape variety as independent variable were carried out with the package SPSS for Windows (SPSS Statistics v.15.0; SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Polysaccharide composition

The concentration of the main neutral monosaccharides released after hydrolysis of wine polysaccharides is shown in Table 1. Galactose (Gal), arabinose (Ara), fucose (Fuc), xylose (Xyl), apiose (Api), 2-*O*-methyl-fucose (2OMeFuc), 2-*O*-methyl-xylose (2OMeXyl) and rhamnose (Rha) come from grape cell walls. Gal and Ara are the major constituents of polysaccharides rich in arabinose and galactose (PRAG), while Rha could come from pectic polysaccharides such as type II rhamnogalacturonans (RG-II) or type I rhamnogalacturonans (RG-I), those found in wines as free fragments of grape parietal RG-I[29] or as RG-I fragments bound to the PRAG.[30] The identification of several rare sugars such as apiose, 2-*O*-methyl-fucose, and 2-*O*-methyl-xylose, indicated the presence of the RG-II molecule,[31] while the presence of Xyl residues indicated that traces of hemicelluloses might be solubilised from grape berry cell walls.[32] Although glucose (Glc) is not known as a component of pectic polysaccharides, it could arise from yeast polysaccharides,[11] but also from bacterial polysaccharides.[33] Finally, mannose (Man) comes from yeast mannoproteins (MP).[29]

In general, a significantly higher quantity of sugar residues in the rosé base wine than in the white wine was observed (Table 1). Therefore, prefermentative maceration in rosé wines increased the extraction and solubilisation of grape monosaccharides. Galactose showed the most different content between base wines, indicating that it was easily extracted by endogenous enzymes during prefermentative maceration. However, apiose content was higher in white base wines than in rosé ones. This fact suggested RG-II some resistance to degradation by pectic-degrading enzymes during prefermentative maceration in rosé wines.

Mannose and galactose were the most prevalent sugars in both base wines (72.0% and 14.3% in white base wines respectively; and 58.3% and 28.1% in rosé base wines respectively), followed by glucose, arabinose and rhamnose (6.9%, 3.1% and 2.2% in white base wines respectively; and 4.7%, 3.6% and 3.1% in rosé base wines, respectively). The content of rare sugars, fucose, and xylose was lower than 1% in the base wines. The mannose percentages obtained in Tempranillo and Verdejo base wines were higher than those obtained by different authors in other varietal base wines.[11,12] Since mannoproteins play an important role in sensory properties of sparkling wines, the high percentage found for mannose in base wines indicated that grape varieties studied, yeast strain and fermentation conditions were suitable for sparkling wines production.

In general, in both sparkling wines the content of monosaccharides forming the grape polysaccharides remained constant or decreased during the whole period of ageing. These trend results agree with those obtained by other authors during the ageing of other varietal sparkling wines.[11,12] However, yeast monosaccharides showed different trends among white and rosé sparkling wines. In white wines, the content of mannose increased significantly at 3 and 6 months of ageing, probably due to yeast autolysis, but it was significantly reduced at 9 months of ageing. Decreases in the content of mannose could be attributed to precipitation phenomena as a result of their interaction with other wine components to form unstable colloids. In rosé sparkling wines, mannose content increased throughout all periods studied. Therefore, higher initial content of mannose in white wines could be related to a higher instability and precipitation at the last stage of ageing. Moreover, the lack of a decrease in mannose in rosé sparkling wines could be due to the previous precipitation of colloidal material during the base winemaking, which would reduce the content of this material during ageing. The content of glucose decreased at 6 months of ageing in white sparkling wines; however, an increase during this period of ageing in the rosé wines was observed. From this behaviour, a higher action of endogenous glycosidase activity during ageing of rosé base wines compared to white base wines could be presumed. Thus, white and rosé wines after ageing were mainly composed of mannose (76.8% and 62.4%, respectively), followed by galactose (11.8% and 24.5%, respectively) and glucose (6.1% and 5.9%).

To increase the knowledge of the structure of polysaccharide sugars from sparkling wines, the ratios arabinose to galactose (Ara/Gal) and mannose to glucose (Man/Glc) were calculated. The Ara/Gal ratio in base wines were similar to described in the literature in Champagne AGP.[13] The difference in the molar ratio of arabinose to galactose during winemaking has been described previously. In one study, AGP with a lower arabinose to galactose molar ratio were extracted first and AGP with a higher ratio

Table 1. Glycosyl composition (mg L^{-1}) and characteristic ratios of polysaccharides from sparkling wines during different stages of sparkling wine production: base wines (T0), sparkling wines after 3 months (T3), 6 months (T6), and 9 months (T9) of aging on yeast lees

Parameter	2-OMeFuc*	2-OMeXyl*	Api*	Ara*	Fuc*	Gal*	Glc*	Man*	Rha*	Xyl*	Total*
White											
T0	0.29 ± 0.02^{cA}	0.22 ± 0.01^{cA}	0.17 ± 0.00^{dB}	2.74 ± 0.26^{bA}	0.17 ± 0.01^{bA}	12.88 ± 1.00^{bA}	6.19 ± 0.24^{bB}	64.67 ± 1.85^{bB}	1.96 ± 0.12^{bA}	0.52 ± 0.02^{cA}	89.81 ± 3.52^{bA}
T3	0.24 ± 0.02^{bA}	0.18 ± 0.02^{bA}	0.15 ± 0.01^{cB}	2.82 ± 0.04^{bA}	0.24 ± 0.01^{cB}	12.27 ± 1.02^{bA}	6.27 ± 0.41^{bB}	72.70 ± 1.51^{cB}	1.78 ± 0.12^{bA}	0.39 ± 0.02^{bA}	97.04 ± 3.16^{bA}
T6	0.20 ± 0.02^{abA}	0.14 ± 0.01^{aA}	0.07 ± 0.00^{aB}	2.51 ± 0.17^{abA}	0.13 ± 0.01^{aA}	11.74 ± 0.99^{abA}	5.35 ± 0.19^{aA}	71.68 ± 1.03^{cB}	1.48 ± 0.02^{aA}	0.37 ± 0.01^{bA}	93.67 ± 2.44^{bA}
T9	0.18 ± 0.01^{aA}	0.14 ± 0.01^{aA}	0.13 ± 0.00^{bA}	2.20 ± 0.14^{aA}	0.15 ± 0.01^{abA}	9.47 ± 0.42^{aA}	4.79 ± 0.11^{aA}	60.06 ± 1.21^{aA}	1.25 ± 0.10^{aA}	0.27 ± 0.02^{aA}	78.64 ± 2.01^{aA}
Rosé											
T0	0.51 ± 0.02^{bB}	0.37 ± 0.00^{bB}	0.15 ± 0.01^{dA}	3.32 ± 0.18^{aB}	0.34 ± 0.01^{cB}	25.62 ± 1.46^{aB}	4.26 ± 0.33^{aA}	53.19 ± 0.99^{aA}	2.83 ± 0.16^{bB}	0.70 ± 0.03^{bB}	91.29 ± 3.18^{aA}
T3	0.40 ± 0.03^{aB}	0.28 ± 0.01^{aB}	0.08 ± 0.00^{bA}	3.01 ± 0.06^{aB}	0.22 ± 0.01^{aA}	23.13 ± 2.11^{aB}	4.66 ± 0.17^{aA}	57.92 ± 2.28^{abA}	2.28 ± 0.13^{aB}	0.65 ± 0.03^{abB}	92.63 ± 4.83^{aA}
T6	0.37 ± 0.02^{aB}	0.29 ± 0.01^{aB}	0.06 ± 0.00^{aA}	3.21 ± 0.17^{aB}	0.31 ± 0.02^{cB}	25.14 ± 0.26^{aB}	5.43 ± 0.30^{bA}	60.83 ± 3.79^{bA}	2.34 ± 0.20^{aB}	0.61 ± 0.02^{aB}	98.59 ± 4.78^{aA}
T9	0.41 ± 0.04^{aB}	0.30 ± 0.03^{aB}	0.12 ± 0.01^{cA}	3.09 ± 0.27^{aB}	0.26 ± 0.01^{bB}	24.62 ± 1.61^{aB}	5.97 ± 0.21^{bB}	62.58 ± 1.10^{bA}	2.43 ± 0.21^{abB}	0.57 ± 0.05^{aB}	100.36 ± 3.52^{aB}

Parameter	Ara/Gal*	Man/Glc*
White		
T0	0.26 ± 0.00^{aB}	10.45 ± 0.55^{aA}
T3	0.28 ± 0.02^{aB}	11.60 ± 0.20^{bA}
T6	0.26 ± 0.00^{aB}	13.41 ± 0.28^{dB}
T9	0.28 ± 0.00^{aB}	12.54 ± 0.03^{cB}
Rosé		
T0	0.16 ± 0.00^{aA}	12.49 ± 0.74^{bB}
T3	0.16 ± 0.01^{aA}	12.42 ± 0.04^{bB}
T6	0.15 ± 0.01^{aA}	11.19 ± 0.09^{aA}
T9	0.15 ± 0.00^{aA}	10.48 ± 0.19^{aA}

Different letters indicate statistical differences ($P < 0.05$). Lower-case letters are used to compare the same wine in each parameter and different ageing times by one-way ANOVA. Upper-case letters are used to compare the different wines in each parameter and each ageing time by the two-sample *t* test.
*Average of the three measurements and standard deviation.
2-OMeFuc, 2-O-CH_3-fucose; 2-OMeXyl, 2-O-CH_3-xylose; Api, apiose; Ara, arabinose; Fuc, fucose; Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose; Xyl, xylose.

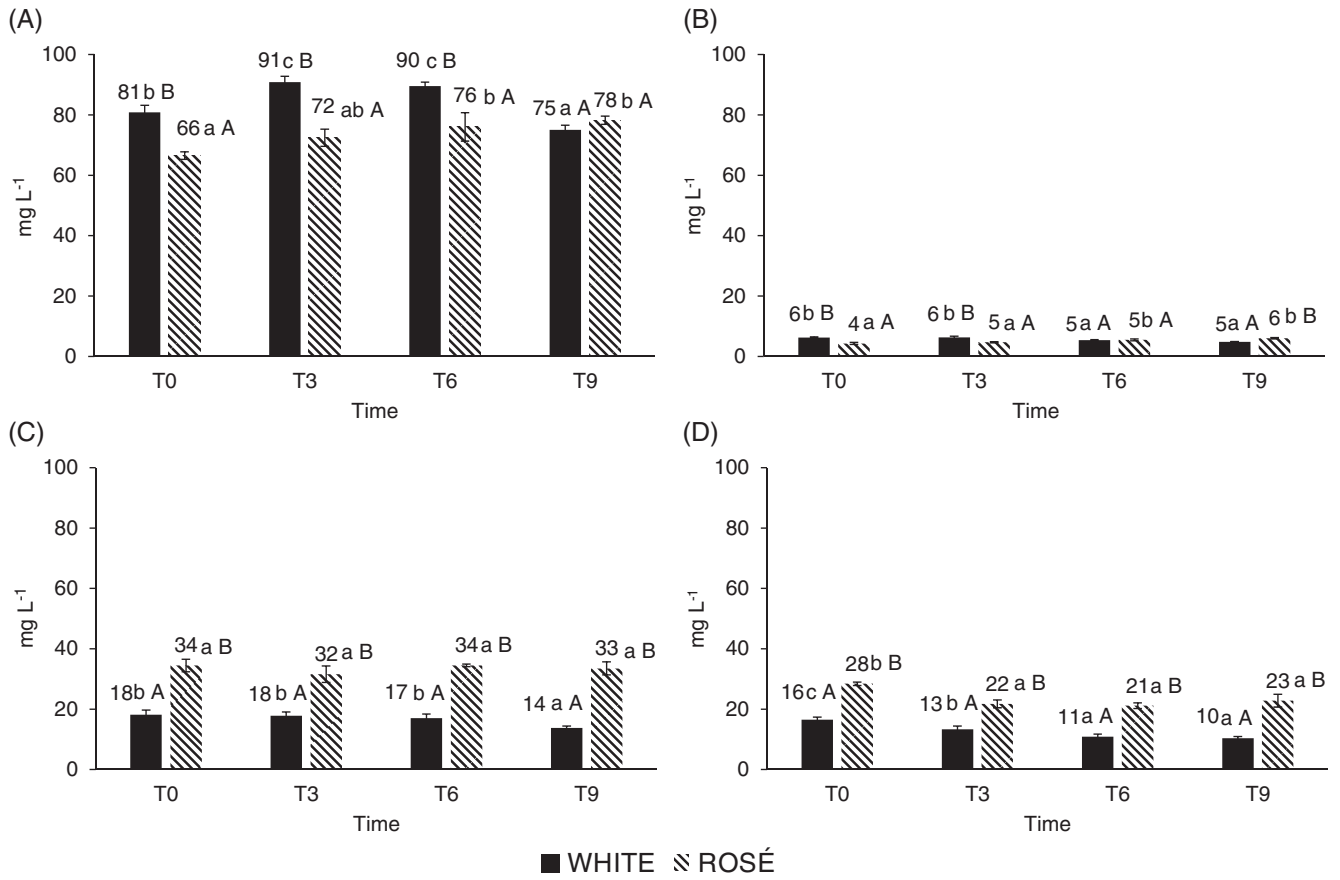

Figure 1. Concentration of mannoproteins (A), glucans (B), polysaccharides rich in arabinose and galactose (C), and rhamnogalacturonan type II (D) in white and rosé sparkling wines during different stages of sparkling wine production: base wines (T0) and sparkling wines after 3 months (T3), 6 months (T6), and 9 months (T9) of ageing on yeast lees. Average of the three measurements and standard deviation. Different letters indicate statistical differences ($P < 0.05$). Lower-case letters are used to compare the same wine in each parameter and different ageing times by one-way ANOVA. Upper-case letters are used to compare the different wines in each parameter and each ageing time by the two-sample *t* test.

were extracted later during the maceration– fermentation step of red winemaking.[34] Thus, lower arabinose to galactose ratios may be expected in white and rosé sparkling wines obtained with lower maceration times. Somewhat surprisingly, white base wines showed a significant higher Ara/Gal ratio than rosé ones, suggesting a larger release of arabinose or polysaccharides rich in arabinose arising from the ramified or hairy region of the pectic framework in the case of white base wines. Higher arabinose to galactose ratio was found by other authors in white base wines than in rosé ones.[11] These results suggested the degradations undergone by PRAG are enzymatic mechanism which could depend on the grape variety used.

The difference in the Ara/Gal ratio in white and rosé wines may influence the PRAG physico-chemical properties and thus modify the final colloidal equilibrium[35] and foam properties of the sparkling wines.[8] Contrary to results reported by other authors[14] ageing on yeast lees did not modify the total PRAG composition of sparkling wines. This discrepancy could be explained by different grape maturities in these works. In this line, Martínez-Lapuente *et al.*[12] found that the maturity of grape affected the Ara/Gal characteristic ratio during the ageing of sparkling wines.

White sparkling wines showed a significant increase in Man/Glc ratio during the ageing on yeast lees, due mainly to significant decrease in the glucose content, suggesting that glucans (GL) were hydrolysed during this period. However, the opposite trend was observed in the Man/Glc ratio of rosé sparkling wines. This contradictory behaviour could be linked to the kinetic of glucanase activity. However, this aspect is still unknown in sparkling wine.[5,10]

The concentrations of mannoproteins (MP), glucans (GL), polysaccharides rich in arabinose and galactose (PRAG), and rhamnogalacturonans type II (RG-II) in white and rosé sparkling wines are shown in Fig. 1, and they were estimated from the concentrations of individual glycosyl residues, as determined by GC after hydrolysis, reduction and acetylation.[36] All the mannose content was attributed to yeast MP, and all the glucose content was attributed to yeast GL. The sum of galactose and arabinose residues was used to estimate PRAG, representing mainly AGP, arabinogalactans, and arabinans in wines. The concentration of RG-II was calculated from those of 2-*O*-methylfucose and 2-*O*-methyl-xylose.

From T0 to 6 months of ageing the MP content was lower in rosé sparkling wines than in white ones. Considering that the yeast strain used in all wines was the same, and that all the mannose can be attributed to yeast MP, the higher MP amounts observed in white sparkling wines could be due to the different chemical characteristics of the wines. Certain factors, such as the winemaking conditions,[37] the initial colloid content in must,[38] or ripening degrees at harvest[39] could influence the MP released by yeasts. Several works have highlighted the initial colloidal

content of grape must as a factor affecting yeast metabolism and alcoholic fermentation.[38,40,41] One of the effects of the initial colloidal content described is related to MP production and their release to the media by yeasts: the lower the initial colloidal content, the higher the MP release.[38,41] Since rosé base wines contain a higher amount of polysaccharides from grapes (Fig. 1), it is quite likely that the initial colloidal content of musts also increases, which accounts for the lower content of yeast MP. Rosé sparkling wines had higher concentration of PRAG and RG-II than white ones. Grape polysaccharides are released from the pectic network of berry cell walls under the action of several endogenous and exogenous enzymes during the earlier stages of winemaking. Therefore, prefermentative maceration in rosé base wines increased the solubilisation of grape polysaccharides from grape cell-walls to must. Higher reduction in PRAG and RG-II was observed in white sparkling during the ageing. This fact suggested a higher hydrolytic phenomenon in white sparkling wines than in rosé ones. Decreases in grape polysaccharides content throughout ageing have also been described by other authors,[11,12,14] and may be a consequence of the formation of unstable complexes between polysaccharides and other wine compounds.[11] The main polysaccharide families in final sparkling wines were MP with average percentages of 72% and 56% of the total polysaccharide content in Verdejo and Tempranillo sparkling wines, respectively. The sum of MP and GL (60–77% of total polysaccharide families) was in the range obtained in other white and rosé varietal sparkling wines.[11] However, the percentage of polysaccharides from yeast in Tempranillo and Verdejo sparkling wines were two-fold higher than obtained in red ones.[12] Since yeast MP improves foaming stability of sparkling wines,[8–10] the differences observed in the composition of the polysaccharide families could suggested a better foam quality in white and rosé sparkling wines than red ones.

Oligosaccharide composition

Table 2 shows the glycosyl composition and characteristic ratios of oligosaccharides from white and rosé sparkling wines. Significant differences were found in the total oligosaccharide contents between the two base wines (white and rosé base wines: 83.4 ± 3.8 and 112.0 ± 4.0 mg L^{-1}, respectively). The differences in oligosaccharide concentration between the two base wines could be related to the different wine making techniques used for white and rosé wine. The highest quantity of oligosaccharides detected in the fractions derived from rosé wine could be partly related to longer contact time between skins and must during the production of rosé base wine than production of white wine. It is known that the integrity of cell walls and their possible weakening modulates the extraction of various components, and in particular polysaccharides and oligosaccharides[42] during winemaking. Important differences were observed among glycosyl residue patterns of oligosaccharides and polysaccharides. In all vinification stages, the glucose residue was the predominant constituent of the oligosaccharides in both sparkling wines (34–44%), followed by mannose (11–15%), xylose (9–14%), galactose (9–13%), and galacturonic acid (6–8%). Arabinose (4–5%), 4-methyl glucuronic acid (3–6%) and rhamnose (2–6%) were also detected, but in smaller quantities. Fucose, glucuronic acid, and xylitol were also detected in all the samples with even lower amounts (3%). The total glycosyl content of oligosaccharides remained stable in both Verdejo and Tempranillo sparkling wines during the whole period of ageing. However, a higher release in glucose than in mannose glycosyl residues of the oligosaccharide fraction was observed during the ageing in both wines. A reduction of the hydrolytic enzyme activity involved in the autolytic process, and/or a higher precipitation or combination rate of oligomannans than their solubilisation into the wine could explain it.

The proportion of cell wall oligosaccharides from yeasts (the sum of oligoglucans and oligomannans) increased during the ageing in both sparkling wines (white and rosé base wines: 51–56% and 49–54%, respectively). As observed in polysaccharide composition, the percentage of oligosaccharides from yeast in white and rosé sparkling wines were higher than in red ones.[12] The oligosaccharide concentrations from the isolated fractions in the final sparkling wines were similar to those reported in still wines from Chardonnay.[43] We have observed significant differences of oligosaccharides total content between Tempranillo (ranged between 109 and 112 mg L^{-1}, depending on the ageing time) and Verdejo (ranged between 79 and 84 mg L^{-1}, depending on the ageing time) sparkling wines. As previously demonstrated,[18] the varietal influence on the content of these compounds in the finished wine can be obviously suggested. Furthermore, it seems essential for us to underline the close relationship between higher oligosaccharide extraction and prefermentative maceration in the case of rosé wines, which might undoubtedly contribute to explaining these found differences.

Several characteristic ratios were calculated from oligosaccharide sugar composition: Ara/Gal, rhamnose to galacturonic acid (Rha/GalA), arabinose + galactose to rhamnose (Ara + Gal)/Rha, and Man/Glc. The Ara/Gal ratio is characteristic of the PRAG-like structures.[14,29] White sparkling wines showed higher Ara/Gal ratios than rosé ones during all the ageing time on yeast lees. The increase of this ratio in the oligosaccharide fraction suggested a higher release of arabinose or oligosaccharides rich in arabinose arising from the pectic framework in white wines compared to that in rosé ones. Moreover, two trends were observed during the wine ageing. Ara/Gal increased in rosé sparkling wines, but the opposite was in general observed in white ones, suggesting a significant degradation of PRAG structures in wines made with the white grapes. The relative richness of the wine oligosaccharides in homogalacturonans versus rhamnogalacturonans could be deduced from the Rha/GalA ratio.[44] A lower value observed for this ratio was observed in oligosaccharides from white sparkling wine than rosé ones, suggesting that homogalacturonans were major compounds in white samples. The ratio of (Ara + Gal) to rhamnose was calculated to estimate the relative importance of the neutral side chains to the rhamnogalacturonan backbone. In general, the (Ara + Gal)/Rha ratio was considerably lower in rosé sparkling wine oligosaccharides in comparison with white ones. This could indicate that the rhamnogalacturonan oligomers present in white sparkling wines carry more neutral lateral chains. Regarding the Man/Glc ratio of oligosaccharides, the proportion of glucose was notably higher than mannose in both wines during the entire ageing time. This ratio was considerably higher in rosé sparkling wine oligosaccharides in comparison with white ones. The higher content of oligomannans in rosé sparkling wines would explain the higher ratio of mannose to glucose in rosé sparkling wines. Throughout ageing, the Man/Glc ratio in oligosaccharides decreased in both sparkling wines.

Determination of molar mass: the structural features by SEC-MALLS of polysaccharide fractions from white and rosé sparkling wines

The elution profiles and M_w distributions (MWD) of the polysaccharidic fractions from white and rosé wines in function of the elution time are shown in Fig. 2. A concentration signal was derived

Table 2. Glycosyl composition (mg L^{-1}) and characteristic ratios of oligosaccharides from sparkling wines during different stages of sparkling wine production: base wines (T0), sparkling wines after 3 months (T3), 6 months (T6), and 9 months (T9) of ageing on yeast lees

Parameter	Rha*	Fuc*	Ara*	Xyl*	Man*	Gal*	Glc*	Gal A*	Glc A*	Xylitol*	4-OMeGlc A*	Total
White												
T0	2.04 ± 0.12^{aA}	0.74 ± 0.02^{bA}	4.18 ± 0.28^{abA}	11.35 ± 0.84^{bA}	9.48 ± 0.06^{bA}	7.08 ± 0.51^{aA}	32.72 ± 2.57^{aA}	6.64 ± 0.41^{bA}	2.44 ± 0.22^{cA}	1.80 ± 0.04^{bB}	4.90 ± 0.41^{bB}	83.37 ± 3.81^{aA}
T3	1.89 ± 0.13^{aA}	0.63 ± 0.02^{aA}	3.67 ± 0.33^{abA}	9.83 ± 0.77^{abA}	9.07 ± 0.02^{aA}	6.84 ± 0.17^{aA}	34.99 ± 0.09^{abA}	5.42 ± 0.13^{aA}	1.88 ± 0.09^{abB}	1.42 ± 0.04^{aA}	4.21 ± 0.08^{aB}	79.85 ± 2.23^{aA}
T6	2.05 ± 0.19^{aA}	0.63 ± 0.04^{aA}	4.36 ± 0.25^{bA}	9.91 ± 0.39^{abA}	9.38 ± 0.04^{bA}	7.81 ± 0.31^{aA}	36.31 ± 0.15^{bA}	5.55 ± 0.45^{aA}	2.24 ± 0.10^{bcA}	1.50 ± 0.09^{aA}	4.11 ± 0.19^{aA}	83.86 ± 2.51^{aA}
T9	1.80 ± 0.10^{aA}	0.69 ± 0.03^{abA}	3.58 ± 0.22^{aA}	9.56 ± 0.25^{aA}	8.97 ± 0.01^{aA}	7.13 ± 0.53^{aA}	34.95 ± 0.08^{abA}	5.39 ± 0.35^{aA}	1.82 ± 0.12^{aA}	1.35 ± 0.04^{aA}	3.92 ± 0.10^{aB}	79.18 ± 2.31^{aA}
Rosé												
T0	6.46 ± 0.52^{aB}	1.76 ± 0.12^{aB}	4.85 ± 0.27^{aB}	11.12 ± 0.66^{aA}	17.14 ± 0.04^{aB}	14.69 ± 0.61^{bB}	38.12 ± 0.10^{aB}	9.31 ± 0.85^{bB}	2.13 ± 0.05^{cA}	1.51 ± 0.12^{aA}	3.90 ± 0.28^{cA}	111.98 ± 4.04^{aB}
T3	6.07 ± 0.35^{aB}	1.87 ± 0.18^{aB}	4.74 ± 0.41^{aB}	10.46 ± 0.13^{aA}	17.12 ± 1.41^{aB}	14.52 ± 1.36^{bB}	44.62 ± 1.41^{bB}	7.09 ± 0.52^{aB}	1.60 ± 0.05^{aA}	1.47 ± 0.02^{aA}	2.90 ± 0.24^{aA}	112.46 ± 4.12^{aB}
T6	5.93 ± 0.21^{aB}	1.72 ± 0.07^{aB}	4.86 ± 0.18^{aB}	10.57 ± 0.43^{aA}	16.78 ± 0.09^{aB}	12.73 ± 0.70^{abB}	41.94 ± 2.46^{abB}	8.23 ± 0.46^{abB}	2.05 ± 0.08^{bcA}	1.48 ± 0.07^{aA}	3.76 ± 0.16^{bcA}	110.06 ± 3.88^{aB}
T9	5.84 ± 0.11^{aB}	1.60 ± 0.06^{aB}	4.71 ± 0.12^{aB}	10.20 ± 0.80^{aA}	16.49 ± 0.02^{aB}	12.24 ± 0.20^{aB}	42.49 ± 2.78^{abB}	8.25 ± 0.81^{abB}	1.90 ± 0.10^{bA}	1.41 ± 0.14^{aA}	3.33 ± 0.10^{abA}	108.46 ± 5.28^{aB}

Parameter	Ara/Gal*	Rha/Gal A*	(Ara + Gal)/Rha*	Man/Glc*
White				
T0	0.71 ± 0.00^{cB}	0.36 ± 0.00^{aA}	5.41 ± 0.06^{aB}	0.29 ± 0.01^{bA}
T3	0.64 ± 0.04^{abB}	0.41 ± 0.02^{bcA}	5.41 ± 0.10^{aB}	0.26 ± 0.00^{aA}
T6	0.67 ± 0.01^{bcB}	0.44 ± 0.01^{cA}	5.80 ± 0.27^{bB}	0.26 ± 0.00^{aA}
T9	0.60 ± 0.01^{aB}	0.40 ± 0.00^{bA}	5.78 ± 0.08^{abB}	0.26 ± 0.00^{aA}
Rosé				
T0	0.40 ± 0.01^{aA}	0.82 ± 0.01^{aB}	2.89 ± 0.10^{abA}	0.45 ± 0.04^{bB}
T3	0.39 ± 0.00^{aA}	1.01 ± 0.02^{bB}	3.03 ± 0.11^{bA}	0.38 ± 0.01^{aB}
T6	0.46 ± 0.01^{bA}	0.85 ± 0.02^{aB}	2.85 ± 0.04^{abA}	0.40 ± 0.01^{abB}
T9	0.46 ± 0.00^{bA}	0.84 ± 0.07^{aB}	2.79 ± 0.00^{aA}	0.39 ± 0.02^{aB}

Different letters indicate statistical differences ($P < 0.05$). Lower-case letters are used to compare the same wine in each parameter and different ageing times by one-way ANOVA. Upper-case letters are used to compare the different wines in each parameter and each ageing time by two-sample *t* test.
*Average of the three measurements and standard deviation.
Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; Gal A, galacturonic acid; Glc A, glucuronic acid; 4-OMeGlc A, 4-*O*-methyl glucuronic acid.

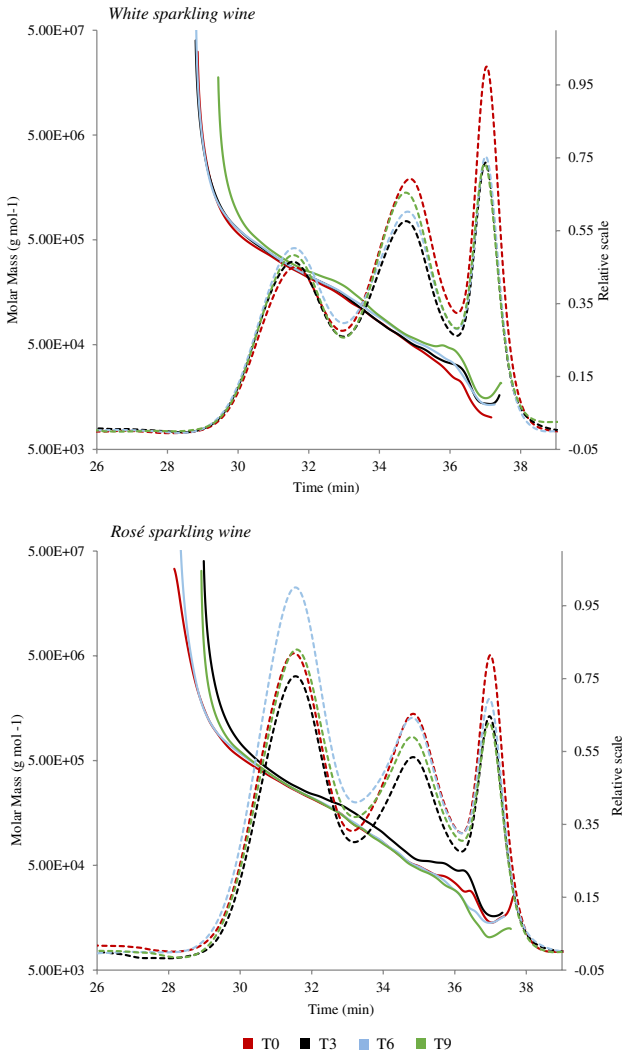

Figure 2. SEC-MALLS chromatograms and weight-average molar mass distributions of the polysaccharide fraction in white and rosé sparkling wines during different stages of sparkling wine production: base wines (T0) and sparkling wines after 3 months (T3), 6 months (T6), and 9 months (T9) of ageing on yeast lees. Molar weight distribution (M_w, g mol^{-1}, thick line) and refractive index (DRI, relative scale, dashed line).

from the differential refractometer, whereas the molecular mass derived from light scattering were given. Three principal peaks from the polysaccharides refractive index elution profiles from white and rosé wines can be observed. These three populations range between 28 and 33 min (first population), 33–36 min (second population) and 36–39 min (third population), with no regard for the time of ageing on yeast lees (Fig. 2, DRI signal).

Comparing profiles from rosé and white wines, the first peak is notably higher in the wine elaborated with white grapes. However, the second and third populations are marginally higher in the case of rosé wines in all but one case (second peak at after 6 months of ageing). With respect to the time of ageing on yeast lees, white wine after 6 months of ageing shows clearly the highest profile in the case first peak. In the case of rosé wine, the third peak appears as the highest at the initial time. When the MWD is observed, a decrease with increasing elution time can be check out, which agrees the normal size exclusion separation mechanism (Fig. 2, M_w signal). On the one hand, white wine shows higher MWD at initial time for the end of second peak and for the third peak, as well as after 3 months of ageing for the end of first peak and for the second and third peak. On the other hand, MWD appears slightly higher in rosé wines after 6 months of ageing for second population and the start of third population, together with all three populations after 9 months of ageing. These finding must be corroborated by quantitative data, however (Table 3).

Table 3 shows the molar mass, the polydispersity index and the intrinsic viscosity values from studied wines. The molar mass appears in general similar for first population when rosé (ranged between 3.1×10^5 and 3.6×10^5 g mol^{-1}) and white wines (ranged between 3.1×10^5 and 3.5×10^5 g mol^{-1}) are compared. Concerning the second population, the molar mass is similar at the initial time for both types of wines (rosé wine: 6.4×10^4 g mol^{-1}; white wine: 6.3×10^4 g mol^{-1}). However, the second population from rosé wines increases gradually to achieve a maximum value after 9 months of ageing (7.8×10^4 g mol^{-1}), whereas the same population from white wines achieve the maximum value after 3 months of ageing (7.5×10^4 g mol^{-1}) and subsequently declined. Finally, the third population is higher in white wines at initial time (1.8×10^4 g mol^{-1}) and after 3 months of ageing (2.3×10^4 g mol^{-1}) comparing with rosé wines (1.2×10^4 g mol^{-1} and 1.7×10^4 g mol^{-1}, respectively). This value decreases for white wines from this point, achieving the minimum value after 9 months of ageing (1.3×10^4 g mol^{-1}). In contrast, the molar mass of third population increased gradually until it reaches 2.1×10^4 g mol^{-1} after 9 months of ageing in the case of rosé wines. The polydispersity index is, in general, lower in third population in comparison with first and second population for all the studied wines. Concerning the intrinsic viscosity, for first population this parameter is so similar after 3 and 6 months from white (32 and 37 mL g^{-1}, respectively) and rosé (30 and 34 mL g^{-1}, respectively) wines. However, the first population from white wine shows a decreasing trend between the intrinsic viscosity at initial time (42 mL g^{-1}) and intrinsic viscosity 9 months of ageing (27 mL g^{-1}), whereas this parameter keeps constant in the case of rosé wines. More or less similar behaviour can be observed for the second population, although in this case values are lower. The third population shows the same trend, emphasising that in this case intrinsic viscosity is clearly lower compared with the first and second peaks.

Figure 3 shows the distribution analysis of polysaccharide fraction from rosé and white wines as determined by size exclusion chromatography coupled on-line to multi angle laser light scattering (SEC-MALLS) and differential refractometer. We can observe largely differences between different wines along five delimited ranges (molar mass ranges: range 1 = 2500–20 000 g mol^{-1}; range 2 = 20 000–100 000 g mol^{-1}; range 3 = 100 000–250 000 g mol^{-1}; range 4 = 250 000–500 000 g mol^{-1}; and range 5 = 500 000–1 000 000 g mol^{-1}). These range limits have been selected from their correspondence with values obtained from different polysaccharide families by SEC-MALLS analysis: RGII monomer: M_w 5000 g mol^{-1}; RGII dimer: 10 000 g mol^{-1}; MP_{0c}: M_w = 58 000 g mol^{-1}; AGP_2: M_w = 165 000 g mol^{-1}; MP_{0a}: 350 000 g mol^{-1}; MP_3: M_w = 1 000 000 g mol^{-1} (data not reported).

Rosé wines show evident higher cumulative percentages in ranges 1 and 2 at initial time (30% and 38%, respectively), after 3 months (21% and 44%, respectively) and after 6 months (13% and 32%) of ageing compared with white wines (initial time: 17% and 29%; after 3 months of ageing, respectively; and after 6 months of ageing: 16% and 26%, respectively). In contrast, the cumulative percentages in ranges 3, 4 and 5 appear higher in white wines at initial time (19%, 30% and 4%, respectively), after 3 months (16%, 31% and 7%, respectively) and after 6 months

Table 3. Parameters* obtained for the polysaccharides isolated from sparkling wines during different stages of sparkling wine production: base wines (T0), sparkling wines after 3 months (T3), 6 months (T6), and 9 months (T9) of aging on yeast lees

Parameter	Peak†	M_w (g mol^{-1})	M_n (g mol^{-1})	Polydispersity index (M_w/M_n)	Intrinsic viscosity (mL g^{-1})
White					
T0	1	317 000	264 300	1.20	41.8
	2	63 060	55 500	1.14	23.0
	3	18 170	17 270	1.05	13.1
T3	1	353 300	285 600	1.24	29.6
	2	74 650	67 370	1.11	12.6
	3	22 920	20 570	1.11	4.4
T6	1	322 700	263 700	1.22	33.8
	2	62 740	541 00	1.16	13.0
	3	16 860	163 80	1.03	5.6
T9	1	313 300	256 400	1.22	26.7
	2	61 120	52 550	1.16	10.2
	3	13 130	12 430	1.06	1.8
Rosé					
T0	1	305 300	258 500	1.18	32.0
	2	64 240	52 930	1.21	13.5
	3	12 060	11 430	1.06	6.5
T3	1	323 100	268 900	1.20	32.2
	2	65 070	56 730	1.15	12.7
	3	16 910	15 820	1.07	4.8
T6	1	331 500	275 300	1.20	36.5
	2	73 270	62 620	1.17	16.0
	3	15 930	15 240	1.05	5.9
T9	1	359 700	303 600	1.19	35.9
	2	77 960	68 080	1.15	14.6
	3	20 520	19 070	1.08	6.0

*Molar-mass distributions, M_w, M_n, determined by coupling size exclusion chromatography performed on two serial Shodex OH-pack columns with a multi-angle light scattering device (MALLS), in 0.1 mol L^{-1} $LiNO_3$ ($dn/dc = 0.146$ mL g^{-1}).
M_w/M_n corresponding to the polydispersity index.
Intrinsic viscosity ([η]) determined by a differential viscometry detector equipped with a four-capillary bridge design.
†Peak 1: ranges 29–32 min (first population); peak 2: ranges 32–35 min (second population); peak 3: ranges 35–39 min (third population).

(22%, 31% and 5%, respectively) of ageing compared with rosé wines (initial time: 11%, 18% and 2%, respectively; after 3 months of ageing: 10%, 22% and 4%, respectively; and after 6 months of ageing: 16%, 21% and 4%, respectively). It is important to highlight that during rosé wines elaboration a prefermentative maceration took place, which implies a certain time of contact between grape skin and juice. For that reason, a further degradation of compounds from Tempranillo grape skin can be inferred. It seems therefore coherent to deduce that range 1, which correspond with lower molar mass molecules, shows higher values in the case of rosé wine during first 6 months of ageing. Concerning wines after 9 months of ageing, ranges 2, 3 and 4 keep the same behaviour that samples from previous time of ageing, while cumulative percentages in ranges 1 and 5 appear similar (19% and 3%, respectively) for rosé and white wines.

Amino acids and biogenic amines composition

Table 4 shows the content of each free amino acid and biogenic amines from white and rosé sparkling wines. The most abundant amino acids in the base wines were proline (79% and 61% of the total amino acids in white and rosé base wines, respectively). Of the other amino acids, those present in the greatest proportions in the base wines are α-alanine, β-aminobutyric acid, glutamic acid, and arginine (Table 4). These results are similar to those obtained in base wines of the Macabeo, Xarel.lo, Chardonnay and Parellada varieties.[20] At 3 months of ageing, a release of threonine was observed in both sparkling wines and could reflect the degradation of mannoproteins of the yeast cell wall.[45] These results corroborated the mannose release at 3 months of ageing in both sparkling wines (Table 1). Most of the amino acids exhibited a gradual decrease of content with the ageing time. In fact, 9 months later, a strong decrease of the free amino acid sum was detected in sparkling wines, which became about 19–29% of the initial value for white and rosé sparkling wines, respectively. Peptides and amino acids are, generally, considered the major compounds of those released into the wine during autolysis, and the protease A is the main enzyme involved in the yeast proteolytic activity.[46] Therefore, two possible explanations could be suggested for the decrease in amino acid content during the ageing: first, that protease A produced peptides rather than amino acids; and, second, that amino acids released were later transformed by decarboxylation, deamination and/or synthesis reactions which led to the formation of a number of traditional sparkling wine aroma compounds.[5,46] Similar results were obtained by other authors, who observed a decrease in amino acid content during the ageing of sparkling wines.[20,47,48] The amino acid content of the final sparkling wines were in the range reported in white sparkling wines made from other grape varieties.[20,49]

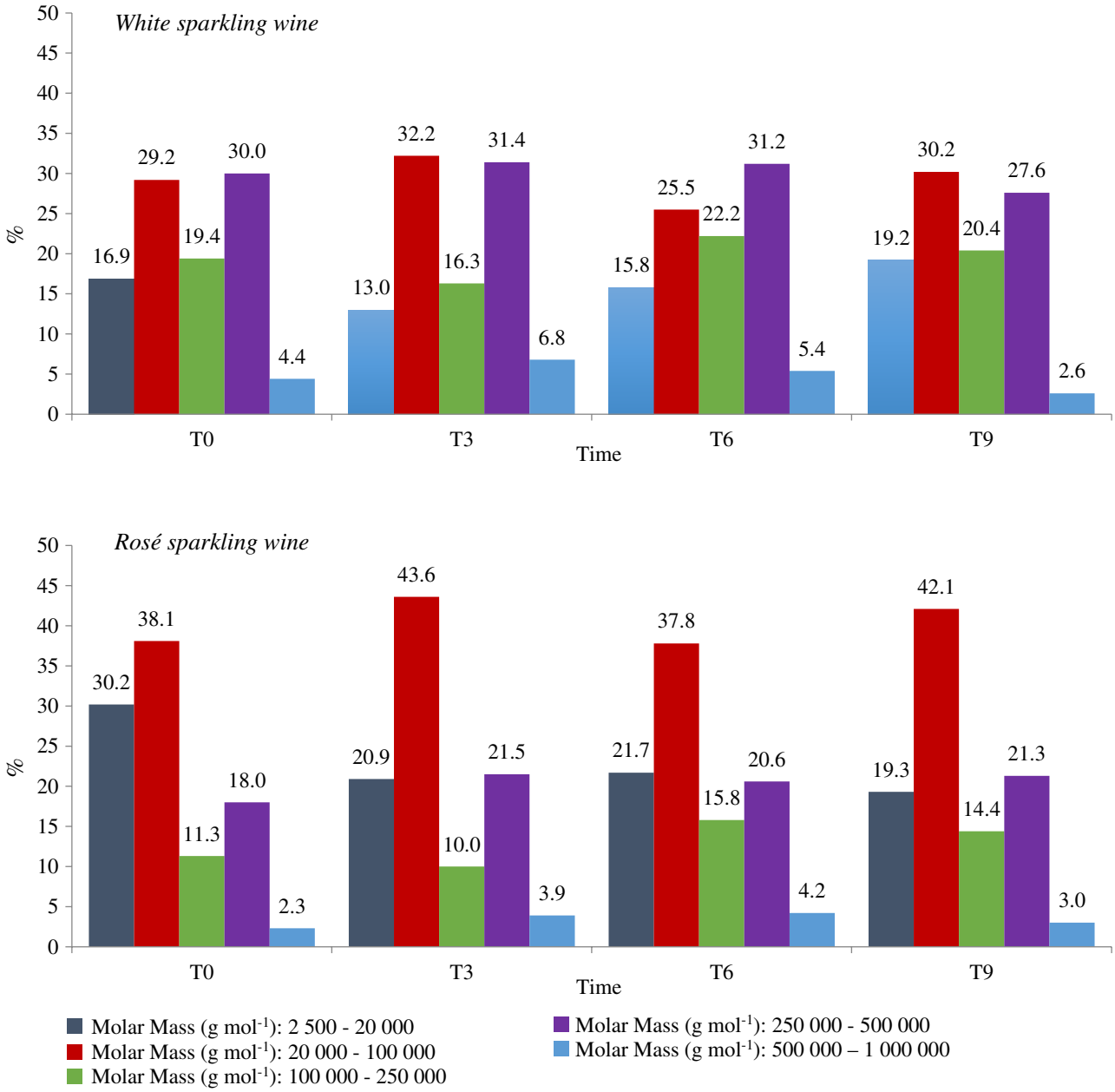

Figure 3. Distribution analysis determined by light scattering ($dn/dc = 0.145$ mL g^{-1}) obtained of polysaccharides fractions isolated from white and rosé sparkling wines during different stages of sparkling wine production: base wines (T0), sparkling wines after 3 months (T3), 6 months (T6), and 9 months (T9) of ageing on yeast lees.

Statistically significant differences in the biogenic amine concentrations were found between white and rosé base wines, and these differences were maintained over the ageing time. The rosé wines showed the highest content of total biogenic amines, which coincides with the highest amino acid concentration found in that wine. This relationship has also been found by other authors in still and sparkling wines.[21,50] As observed in amino acids, the levels of some biogenic amines decreased during the ageing on lees, which could be explained by a potential consumption by the alcoholic fermentative yeast, by a spontaneous chemical degradation, or by adsorption on bentonite added to the *tirage* liquor.[51] It was reported that no remarkable increase in the concentration of biogenic amines could be observed during secondary alcoholic fermentation, with the conclusion that yeasts do not appear to be responsible for the production of most amines found in wines.[52] Moreover, other studies even reported a decrease in biogenic amines during alcoholic fermentation,[53] wine ageing and storage.[54]

In general, very low levels of biogenic amines were found in the wines studied, and these concentrations are far below the limits that can cause toxic effects.[55,56]

CONCLUSIONS

Our work suggests that there may be a potential cultivar effect on the yeast polysaccharide concentration of sparkling wines, demonstrated in particular with evolution of mannoproteins and glucans in Tempranillo and Verdejo sparkling wines. Similarly, the

Table 4. Amino acid and biogenic amine composition (mg L^{-1}) of sparkling wines during different stages of sparkling wine production: base wines (T0), sparkling wines after 3 months (T3), 6 months (T6), and 9 months (T9) of ageing on yeast lees

	White				Rosé			
Amino acid	T0	T3	T6	T9	T0	T3	T6	T9
L-Aspartic acid	4.60 ± 0.37^{bA}	0.53 ± 0.07^{aA}	0.27 ± 0.02^{aA}	0.48 ± 0.23^{aA}	12.90 ± 0.31^{cB}	2.04 ± 0.35^{aB}	2.39 ± 0.08^{abB}	2.75 ± 0.15^{bB}
L-Glutamic acid	7.18 ± 0.39^{bA}	0.64 ± 0.00^{aA}	0.57 ± 0.05^{aA}	0.78 ± 0.17^{aA}	19.02 ± 0.28^{bB}	7.32 ± 1.92^{aB}	6.02 ± 0.03^{aB}	6.25 ± 0.01^{aB}
L-Asparagine	3.00 ± 0.18^{bA}	2.04 ± 0.08^{aA}	2.92 ± 0.14^{bA}	1.74 ± 0.26^{aA}	27.78 ± 1.07^{cB}	15.26 ± 2.82^{abB}	18.60 ± 0.11^{bB}	12.98 ± 0.12^{aB}
L-Serine	0.43 ± 0.02^{abA}	0.33 ± 0.02^{aA}	0.35 ± 0.04^{abA}	0.44 ± 0.06^{bA}	1.20 ± 0.07^{bB}	0.84 ± 0.12^{aB}	0.95 ± 0.11^{aB}	0.86 ± 0.00^{aB}
Hydroxyproline	3.30 ± 0.02^{cA}	2.90 ± 0.01^{bcB}	2.55 ± 0.12^{abB}	2.13 ± 0.48^{aA}	3.87 ± 0.22^{cB}	1.83 ± 0.21^{bA}	1.14 ± 0.32^{aA}	1.56 ± 0.03^{abA}
L-Glutamine	1.07 ± 0.03^{cA}	0.31 ± 0.01^{bA}	0.18 ± 0.01^{aA}	0.18 ± 0.03^{aA}	10.37 ± 0.13^{cB}	2.26 ± 0.33^{bB}	0.77 ± 0.01^{aB}	0.40 ± 0.00^{aB}
L-Histidine	1.44 ± 0.12^{aA}	1.38 ± 0.01^{aA}	1.33 ± 0.09^{aA}	1.57 ± 0.25^{aA}	1.48 ± 0.08^{aA}	2.26 ± 0.30^{bB}	2.38 ± 0.01^{bB}	2.32 ± 0.03^{bB}
Glycine	1.01 ± 0.08^{aA}	1.18 ± 0.04^{aA}	1.06 ± 0.02^{aA}	1.78 ± 0.31^{bA}	5.30 ± 0.17^{aB}	6.45 ± 1.11^{aB}	6.27 ± 0.10^{aB}	6.46 ± 0.26^{aB}
L-Threonine	1.25 ± 0.10^{cA}	1.51 ± 0.04^{dA}	0.65 ± 0.07^{bA}	0.28 ± 0.05^{aA}	1.76 ± 0.05^{aB}	6.29 ± 1.26^{bB}	6.31 ± 0.18^{bB}	5.29 ± 0.09^{bB}
β-Alanine	0.84 ± 0.05^{aA}	0.84 ± 0.03^{aA}	1.02 ± 0.06^{aA}	1.13 ± 0.21^{aA}	1.77 ± 0.02^{aB}	2.50 ± 0.49^{bB}	1.57 ± 0.17^{aB}	1.92 ± 0.09^{abB}
L-Arginine	3.62 ± 0.11^{cA}	1.18 ± 0.04^{aA}	1.31 ± 0.10^{abA}	1.64 ± 0.20^{bA}	20.81 ± 0.33^{bB}	10.47 ± 1.82^{aB}	10.25 ± 0.63^{aB}	9.37 ± 0.41^{aB}
α-Alanine	5.04 ± 0.24^{bA}	2.23 ± 0.03^{aA}	2.30 ± 0.18^{aA}	2.36 ± 0.17^{aA}	14.32 ± 0.21^{bB}	9.99 ± 1.79^{aB}	11.75 ± 0.19^{aB}	9.55 ± 0.04^{aB}
γ-Aminobutyric acid	1.95 ± 0.06^{cA}	0.64 ± 0.03^{aA}	0.60 ± 0.04^{aA}	0.82 ± 0.07^{bA}	55.57 ± 0.69^{bB}	48.67 ± 8.50^{abB}	53.24 ± 0.20^{abB}	43.46 ± 0.21^{aB}
L-Proline	231.60 ± 6.63^{abA}	273.46 ± 0.71^{bA}	229.41 ± 2.58^{abA}	210.73 ± 36.77^{aA}	397.82 ± 6.45^{cB}	343.14 ± 22.24^{abB}	355.03 ± 0.87^{bB}	320.85 ± 0.81^{aB}
L-Tyrosine	0.63 ± 0.01^{cA}	0.52 ± 0.03^{bA}	0.51 ± 0.02^{bA}	0.17 ± 0.05^{aA}	10.31 ± 0.51^{bB}	5.13 ± 0.61^{aB}	5.07 ± 0.07^{aB}	4.51 ± 0.01^{aB}
L-Valine	9.10 ± 0.60^{cA}	8.01 ± 0.00^{bA}	7.21 ± 0.00^{bA}	5.32 ± 0.43^{aA}	12.26 ± 0.85^{cB}	9.52 ± 0.00^{bB}	8.58 ± 0.00^{bB}	7.02 ± 0.09^{aB}
L-Methionine	1.23 ± 0.20^{aA}	1.40 ± 0.01^{aA}	1.48 ± 0.08^{aA}	1.29 ± 0.21^{aA}	3.79 ± 0.31^{bB}	2.99 ± 0.58^{abB}	2.41 ± 0.11^{aB}	3.14 ± 0.11^{abB}
L-Cysteine	0.47 ± 0.01^{aA}	0.64 ± 0.05^{bA}	0.34 ± 0.02^{aA}	0.37 ± 0.09^{aA}	2.22 ± 0.19^{cB}	1.05 ± 0.11^{aB}	0.84 ± 0.01^{aB}	1.51 ± 0.06^{bB}
L-Isoleucine	1.02 ± 0.06^{cA}	0.37 ± 0.06^{bA}	0.35 ± 0.03^{bA}	0.22 ± 0.00^{aA}	2.86 ± 0.02^{cB}	1.10 ± 0.22^{bB}	1.27 ± 0.01^{bB}	0.75 ± 0.05^{aB}
L-Tryptophan	0.22 ± 0.02^{bA}	0.08 ± 0.02^{aA}	0.09 ± 0.01^{aA}	0.14 ± 0.03^{aA}	0.95 ± 0.04^{bcB}	1.04 ± 0.28^{cB}	0.51 ± 0.09^{aB}	0.65 ± 0.02^{abB}
L-Leucine	4.46 ± 0.25^{bA}	0.44 ± 0.01^{aA}	0.50 ± 0.03^{aA}	0.77 ± 0.16^{aA}	12.58 ± 0.21^{cB}	2.90 ± 0.48^{abB}	3.22 ± 0.03^{bB}	2.36 ± 0.02^{aB}
L-Phenylalanine	4.00 ± 0.23^{bA}	0.67 ± 0.01^{aA}	0.73 ± 0.03^{aA}	0.97 ± 0.21^{aA}	10.43 ± 0.19^{bB}	3.84 ± 0.67^{aB}	4.57 ± 0.04^{aB}	3.79 ± 0.01^{aB}
L-Ornithine	0.37 ± 0.02^{aA}	1.15 ± 0.04^{bA}	1.18 ± 0.09^{bA}	1.18 ± 0.19^{bA}	15.23 ± 0.23^{bB}	14.62 ± 0.46^{bB}	16.24 ± 0.05^{cB}	13.06 ± 0.08^{aB}
L-Lysine	4.64 ± 0.39^{bA}	0.50 ± 0.06^{aA}	0.44 ± 0.04^{aA}	0.87 ± 0.14^{aA}	12.14 ± 0.21^{bB}	4.11 ± 0.64^{aB}	4.55 ± 0.24^{aB}	4.65 ± 0.08^{aB}
Total amino acids	292.48 ± 6.75^{bA}	302.96 ± 0.98^{bA}	257.35 ± 2.68^{abA}	237.35 ± 36.78^{aA}	656.76 ± 6.62^{cB}	505.63 ± 24.88^{bB}	523.92 ± 1.41^{bB}	465.47 ± 1.15^{aB}
Histamine	0.25 ± 0.01^{cA}	0.28 ± 0.04^{cA}	0.18 ± 0.03^{bA}	0.09 ± 0.02^{aA}	0.46 ± 0.01^{bB}	0.27 ± 0.06^{aA}	0.21 ± 0.00^{aA}	0.23 ± 0.02^{aB}
Agmatine	0.86 ± 0.01^{bB}	0.91 ± 0.12^{bB}	0.33 ± 0.01^{aA}	0.23 ± 0.11^{aA}	0.38 ± 0.03^{abA}	0.34 ± 0.01^{abA}	0.44 ± 0.14^{bA}	0.22 ± 0.00^{aA}
Spermidine	0.53 ± 0.14^{bA}	0.28 ± 0.01^{aA}	0.22 ± 0.00^{aA}	0.34 ± 0.09^{abA}	2.00 ± 0.02^{cB}	1.68 ± 0.32^{bcB}	0.50 ± 0.01^{aB}	1.47 ± 0.11^{bB}
Tyramine	0.08 ± 0.01^{aA}	0.08 ± 0.01^{aA}	NQ	NQ	0.24 ± 0.02^{cB}	0.17 ± 0.01^{bB}	0.10 ± 0.00^{a}	0.09 ± 0.01^{aB}
Putrescine	0.98 ± 0.06^{aA}	0.96 ± 0.02^{aA}	0.96 ± 0.08^{aA}	1.02 ± 0.18^{aA}	2.91 ± 0.05^{abB}	2.84 ± 0.49^{abB}	3.31 ± 0.04^{bB}	2.54 ± 0.00^{aB}
Tryptamine	NQ	0.10 ± 0.01^{aA}	0.09 ± 0.01^{aA}	NQ	NQ	0.20 ± 0.04^{aB}	0.16 ± 0.01^{aB}	NQ
Cadaverine	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Phenyelethylamine	NQ	NQ	NQ	NQ	NQ	NQ	NQ	0.12 ± 0.02
Isoamylamine	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Total amines	2.70 ± 0.16^{bA}	2.61 ± 0.13^{bA}	1.77 ± 0.09^{aA}	1.69 ± 0.23^{aA}	6.00 ± 0.07^{cB}	5.50 ± 0.59^{bcB}	4.73 ± 0.14^{abB}	4.67 ± 0.11^{bB}

Different letters indicate statistical differences ($P < 0.05$). Lower-case letters are used to compare the same wine in each parameter and different ageing times by one-way ANOVA. Upper-case letters are used to compare the different wines in each parameter and each ageing time by two-sample *t* test.
NQ, below the quantification limit of the analytical method employed (< 0.08 mg L^{-1}).

polysaccharide composition of PRAG was different in both wines, as their arabinose/galactose ratio demonstrated. It is imperative, however, for us to remark that prefermentative maceration in the case of rosé wines might also contribute, together with the cultivar influence, to the observed variations. Mannoproteins and the glucose glycosyl residue of the oligosaccharides were the major carbohydrates detected in all vinification stages. The total glycosyl content of polysaccharides and oligosaccharides remained practically constant during the whole period of ageing on yeast lees. However, an increase of mannoproteins and glucose glycosyl residue of the oligosaccharides was observed after 3 months of ageing. Most of the amino acids exhibited a gradual decrease of content with the ageing time. In general, the levels of biogenic amines in the sparkling wines studied were very low, which does not represent a negative effect on their quality and safety. Results obtained showed that the behaviour pattern among glucan- and mannose-containing polysaccharides and oligosaccharides and nitrogenous compounds was not the same during the ageing of sparkling wines. Polysaccharides and oligosaccharides from yeast are more significant autolysis markers of sparkling wines than are the nitrogenous compounds.

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