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Influence of grape maturity on complex carbohydrate composition of red sparkling wines

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ABSTRACT

This paper studied how grape maturity affected complex carbohydrate composition during red sparkling wine making and wine aging. Grape ripening stage (premature and mature grapes) showed a significant impact on the content, composition and evolution of polysaccharides and oligosaccharides of sparkling wines. Polysaccharides rich in arabinose and galactose, mannoproteins, rhamnogalacturonans II and oligosaccharides in base wines increased with maturity. For both maturity stages, polysaccharides rich in arabinose and galactose, and glucuronic acid glycosyl residue of the oligosaccharides were the major carbohydrates detected in all vinification stages. Total glycosyl content of oligosaccharides decreased during the whole period of aging on yeast lees. The reduction of polysaccharides rich in arabinose and galactose and rhamnogalacturonans type II during the aging was more pronounced in mature samples. To our knowledge, this is the first time to report the polysaccharide and oligosaccharide composition in red sparkling wines.

Keywords: sparkling wine, aging on lees, grape maturity, polysaccharides, oligosaccharides, mannoproteins, RG-II, PRAG**.**

Abbreviations: PRAG, Polysaccharide Rich in Arabinose and Galactose; AG, type II Arabinogalactans; AGPs, type II Arabinogalactan-proteins; RG-I, Rhamnogalacturonans type I; RG-II, rhamnogalacturonans type II; MPs, Mannoproteins; Ara, arabinose; Gal, galactose; TMS, per-O-trimethylsilylated methyl glycosides; GC-EI-MS, Gas Chromatography Electron Impact Mass Spectrometry; SEC-MALLS, Size Exclusion Chromatography-Multi Angle Laser Light Scattering.

INTRODUCTION

Quality sparkling wines elaborated by the traditional method undergo a second fermentation in closed bottles of base wines, and followed by wines aging with lees for at least 9 months since it is the minimum time necessary for sparkling wines with a protected designation of origin (EC Regulation No. 606/2009). The best known sparkling wines produced within this premium category are white or rosé ones from Champagne, Talento-Trento and Cava regions from France, Italy, and Spain, respectively.

The production of sparkling wines by traditional method is lower compared to that of still wines, but the economic impact of this product is very important because of its high added value. For this reason, in recent years a new market strategy in the oenological industry based on the diversification of wine production and on the exploitation of the characteristics and particularities of different varieties of grapes is emerging.[1-5] In this frame, although the most of the sparkling wines elaborated are white and rosé ones, red sparkling wines produced by the traditional method may be considered as a good example of these types of new products.

Grapes destined for producing high quality red sparkling wines must be harvested with a lower grape ripeness than fruit for still wines, with relatively lower pH, higher titratable acidity, and lower soluble sugars. This is because the secondary fermentation will increase the alcohol content and the finished wines should be fresh and light in mouth as well as exhibit the flavors produced by the traditional method.

The lower maturity of grapes for sparkling wine production may influence the carbohydrate composition of its respective wines,[6-9] and thus have implications in the sparkling wine sensory properties. The progressive pectin degradation of the grape skin cell walls,[10] that takes place thorough ripening should favor polysaccharide

solubilization in the juice and thus in wine.[9,11] Additionally, grape maturity could modulate the growth of yeast[12] and in finally the release polysaccharides and oligosaccharides of yeast cell wall during the alcoholic fermentation and the aging on lees of sparkling wines. Environmental factors such as carbon source or level of initial colloid content of the fermenting medium have been shown to influence the amount of cell wall polysaccharides secreted and then liberated into the medium.[13]

Complex carbohydrates are present in wines, and with polyphenols and proteins constitute the macromolecules of wines. They play an important role in stabilizing other molecules in solution and thus are able to modify both the wine processing and organoleptic properties.[14] In sparkling wines, foam and aroma have been correlated with the type, the molecular weight, and the composition of polysaccharides.[15-19] The evolution of polysaccharides during the winemaking process and also during the aging on yeast lees to elaborate white and rosé sparkling wines has been previously performed.[19] However, little is known about the content and evolution of the different polysaccharide families during the winemaking of red sparkling wines. Moreover, although it is largely known the presence of oligosaccharides in still wines,[9,20-25] there is no information on the oligosaccharide composition in sparkling red wines. These natural molecules are related to plants self-defense processes,[26] dietary antioxidants,[27] and several health benefits,[28] they are also known for their physicochemical properties such as chelations of cations,[29] which may be important in enhancing the quality of sparkling wines. Since the structure and amounts of oligosaccharides released into the wines will depend on the wine-making process,[9,24,25] an understanding of their content and kinetic release during the sparkling wine making is essential.

The aim of this paper was to analyze the changes occurring on complex carbohydrate (oligosaccharides and polysaccharides) composition during the red sparkling wine

processing by the traditional method, as well as to study the effect of the grape ripening stage on the carbohydrate composition.

MATERIALS AND METHODS

Sparkling wine samples

Grapes from Tempranillo variety were collected (vintage 2013) at the same vineyard on the Cigales Denomination of Origin (D.O.).

Grapes were harvested in two maturity moments: prematurity grapes, with acidity and sugar level suitable for sparkling wine production, and grapes at their optimum degree of phenolic maturity. Prematurity grapes were harvested 10 days before maturity grapes and there was no significant climate variation during this period. Then, two red sparkling wines were manufactured using the traditional method in the enological station of Castilla y León (Valladolid, Spain). Base wines were elaborated following the traditional red winemaking process in stainless steel tanks of 150 liters in duplicate. The grapes were destemmed, crushed, and slightly sulphited (50 mg/L). Alcoholic fermentation was carried out with commercial *Saccharomyces cerevisiae* yeasts (FERM ES 488, Enartis, Italy). Pectinolytic enzymes were not added. The maceration-fermentation time was 7 days and temperature was maintained at 25 ± 2 °C. Once the alcoholic fermentation was over (reducing sugars < 2 g/L), the wines were gently pressed and racked into new tanks. Wines were inoculated with commercial *Oenococcus oeni* lactic acid bacteria (Viniflora CH16, CHR Hansen, Denmark) to carry out the malolactic fermentation. The base wines were cold-stabilized (-5 ºC) and clarified with Gel-Red porcine gelatin (0.25 mL/L) (Enolviz, Spain). Then the wines were bottled and the tirage liquor, formed by yeast *S. cerevisiae var. bayannus* (0.30 g/L, IOC 18-2007, Institut OEnologique de Champagne, Épernay, France), sucrose (23 g/L) and bentonite sodium activated (100 mg/L) (Laffort, France), was added. After

that, the bottles were kept in a cellar at a temperature (11-13 ºC) and relative humidity (75-85%) controlled for 9 months. The pressure and residual sugars were measured periodically to control the second fermentation. Samples for analyses were taken from the base wines (T0) and then after 3 months (T3), 6 months (T6) and 9 months (T9) of aging on yeast lees. Wines were riddled and disgorged before analysis, and "liqueur d'expédition" was not added. For each stage, three bottles were analyzed, and all the analyses were conducted in triplicate on wines after centrifugation. Standard enological parameters in musts and base wines were determined using official analysis methods (OIV 1990).[30]

Isolation of polysaccharide and oligosaccharide fractions

The polysaccharide and oligosaccharide fractions were isolated as previously described.[25] The wines (5 mL) were partially depigmented in polyamide CC 6 columns, particle size 0.05–0.16 previously equilibrated with NaCl 1 M. Total wine carbohydrate was not retained on the polyamide column, and was eluted by 2 bed volumes of 1 M NaCl.[31] The eluted fraction was concentrated under vacuum using a rotary evaporator (Buchi, Switzerland). Size exclusion high resolution column chromatography was performed by loading 2 mL of the previously concentrated total wine carbohydrate on a system composed by a 234-Gilson sampling injector (Roissy, France), an LC-10 AS Shimadzu pump (Kyoto, Japan) and a Isco Foxy sampling collector (Lincoln, NE, USA). Elution was performed on a Superdex-30 HR column (60 x 1.6 cm, Pharmacia, Sweden) with a precolumn (0.6 x 4 cm) equilibrated at 1 mL/min with 30 mM ammonium formiate pH 5.6. Elution of polysaccharides and oligosaccharides was followed with an Erma-ERC 7512 (Erma, Japan) refractive index detector combined with Waters Baseline 810 software. Polysaccharide fraction was eluted between 40 and 53 min, while oligosaccharide fraction was collected between 54 and 93 min.[23,24] The

126 isolated fractions were freeze-dried, redissolved in water and freeze dried again for four
127 times to remove the ammonium salt.

128 **Polysaccharide analysis**

129 Neutral monosaccharides were released after hydrolysis of the wine polysaccharides by
130 treatment with 2 mol/L trifluoroacetic acid for 75 min at 120 °C.[32] They were then
131 converted to the corresponding alditol acetate derivatives by reduction and acetylation,
132 and quantified by gas chromatography (GC) analysis on a Shimadzu GC-2010 plus gas
133 chromatograph connected to a flame ionization detector, using a fused silica DB-225
134 (210 °C) capillary column (30 m × 0.32 mm i.d., 0.25 μm film), with hydrogen as the
135 carrier gas, on a Hewlett-Packard Model 5890 gas chromatograph (Hewlett Packard,
136 Palo Alto, CA, USA). The different alditol acetates were identified from their retention
137 time by comparison with that of standard monosaccharides. Allose and myo-inositol
138 were used as internal standards. Neutral sugar amounts were calculated relative to the
139 internal standard (myo inositol).

140 **Oligosaccharide analysis**

141 The neutral and acidic sugar composition was determined after solvolysis with
142 anhydrous MeOH containing 0.5 M HCl (80 ºC, 16 h), by GC of their per-O-
143 trimethylsilylated methyl glycoside derivatives.[33] The TMS derivatives were separated
144 on two DB-1 capillary columns (30 m x 0.25 mm i.d., 0.25 μm film) (temperature
145 programming 120-200 ºC at 1.5 ºC/ min), coupled to a single injector inlet through a
146 two-holed ferrule, with H_2 as the carrier gas on a Shimadzu GCMS-QP2010SE gas
147 chromatograph. The outlet of one column was directly connected to a flame ionization
148 detector at 250 ºC and the second column via a deactivated fused-silica column (0.25 m
149 x 0.11 μm i.d.) was connected to a mass detector. Samples were injected in the pulsed
150 split mode with a split ratio of 20:1. The transfer line to the mass was set at 280 ºC. EI

mass spectra were obtained from m/z 50 to 400 every 0.2 s in the total ion-monitoring mode using an ion source temperature of 200 ºC, a filament emission current of 60 μA, and an ionization voltage of 70 eV.

Determination of molar mass of sparkling wine polysaccharides and oligosaccharides.

Molar-mass distributions, molar weight and number average mass (Mw and Mn in g/mol), and intrinsic viscosity ([η] in mL/g), were determined at 25 ºC by coupling size exclusion chromatography with a multi-angle light scattering device (MALLS), a differential viscometer and a differential refractive index detector. Size exclusion chromatography elution was performed on OH-pack guard column followed by two serial Shodex OH-pack KB-804 and KB-805 columns (0.8 × 30 cm; Shodex Showa Denkko, Japan) at 1 mL/min flow rate in 0.1 M $LiNO_3$ after filtration through 0.1 μm filter unit. The MALLS photometer, a DAWN-HELEOS from Wyatt Technology Inc. (Wyatt Technology Corporation, Santa Barbara, CA, USA), was equipped with a GA-AS laser (λ = 658 nm). The differential viscometer detector (Viscostar II, Wyatt Technology Inc., USA) was equipped with a 4-capillary bridge design. The concentration of each eluted polysaccharide was determined using the differential refractive index detector (Optilab TrEX, Wyatt Technology Inc., USA). All collected data were analyzed using Astra V 6.0.6 software with the zimm plot (order 1) technique for molar-mass estimation and a differential refractive index increment of the polymer in the solvent used. It was employed a dn/dc classical value for polysaccharides (0.146 mL/g)[34].

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 anhydrous, allose, and myo inositol 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).

Statistical analysis

All of the data are expressed as the arithmetic average of three replicates. One-factor ANOVA and two-sample t test were carried out with the package SPSS for Windows (SPSS Statistics v.15.0, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Oenological parameters

Standard enological parameters were determined for the musts, the base wines and sparkling wines at 9 months of aging (Table 1).

For premature sparkling winemaking, grapes were harvested when probable alcohol reached was optimal for classic sparkling wines, with high acidity and low sugar level. These fruit quality parameters for premature must were in agreement to previously reported data for desired maturity for sparkling wine production.[35] For mature sparkling winemaking, grapes were harvested 10 days later, with alcohol and phenolic maturity adequate to elaborate red still wines.

As it was expected, premature base wines had lower alcohol concentrations, higher acidity, lower pH and color intensity than mature ones. It must be emphasized that first alcoholic fermentation in mature base wine finished with high alcohol content to elaborate sparkling wines. Therefore, wine techniques were applied to reduce the alcohol content in sparkling wines made with mature grapes.

Due to the maturity grape stage and the low alcohol content, the extraction of phenolic compounds from grape berries into the wine was low in premature base wines. Color intensity values varied from 8.7 to 11.5 depending on the grape maturity stage. Values obtained after malolactic fermentation in both base wines for volatile acidity confirmed a suitable winemaking with absence of microbial alterations. The resulting sparkling wines completely finished the second fermentation with a residual sugar concentration below 1.70 g/L, and an ethanol content of 12.3 and 14.0 % v/v in premature and mature red sparkling wines, respectively. Internal bottle pressure became similar for both wines. Volatile acidity concentrations were less than 0.32 g/L, which indicated a good preservation state. The second fermentation involved a decrease in color intensity but a slight increase in tonality.

Sparkling wine polysaccharide and oligosaccharide fractions

Figure 1 shows the molecular weight distributions of polysaccharides and oligosaccharides of premature and mature red sparkling wines during their aging on yeast lees. The population eluting on the Superdex 30-HR column between 40 and 53 min corresponded to the polysaccharide fraction, while the oligosaccharide population was collected between 54 and 93 min. The first peak obtained in the range 40 to 48 min corresponded to the polysaccharide fraction of highest molecular mass, and it corresponded to polysaccharides rich in arabinose and galactose (PRAG) and mannoproteins (MP).[25,36] The second peak eluted between 49 and 53 min, and corresponded to the fraction containing mainly rhamnogalacturonans type II (RG-II),[25,36] but also PRAG and MP of lower mass. Significant differences between the content and profiles of base wines could be observed. The profiles of polysaccharide and oligosaccharide fractions of red base wines elaborated with mature grapes were higher than in the fractions obtained with the premature grapes. The differences in the

refractive index responses were attributed to differences in maturity stages between the grapes at the time of the harvest. Therefore, the progressive enzymatic degradation of the walls of skin cells during ripening[37] could have increased the presence of soluble polysaccharides[7] in the wine. Additionally, grape ripeness influenced the polysaccharide composition of its respective base wines. The occurrence of a peak tailing at 48 min in premature wines, not observed in mature wines, may indicate that polysaccharides were more easily extracted during the maceration-fermentation of the mature red base wines. The different profiles observed among the samples confirmed the great influence of the grape maturity level on the wine polysaccharide and oligosaccharide fractions, and thus this technique could be used to identify wines according to their grape´s ripening stage. During the aging on yeast lees, significant changes in the areas of the signals were observed (Figure 1), indicating that transformations in the polysaccharide and oligosaccharide quantities were occurring. However, no shifts were observed and chromatograms were almost superimposable, showing no evolution in the molecular weight distributions during this period.

Polysaccharide composition

Table 2 shows the glycosyl residue composition of the polysaccharides. The presence of neutral sugars (mannose, glucose, rhamnose, arabinose, galactose and fucose) confirmed the presence of mannan-, glucan-, arabinan-, arabinogalactan-, homogalacturonan- and rhamnogalacturonan-like structures in the polysaccharides of the red sparkling wines studied. Although glucose is not known as a component of pectic polysaccharides, it could arise from yeast polysaccharides[19]. The presence of xylose residues indicated that traces of hemicelluloses might be solubilized from grape berry cell walls. The identification of several rare sugars, such as apiose, 2-*O*-methyl-fucose and 2-*O*-methyl-xylose, indicated the presence of RG-II molecule.[36]

Grape maturity affected the monosaccharide composition of polysaccharides in base wines. The major differences among the glycosyl composition of polysaccharides in base wines were found in arabinose, galactose and rhamnose content. Premature base wines were composed of mannose (30%), followed by arabinose (27%) and galactose (26%). However, arabinose (32%) and galactose (29%) were found at higher concentrations than mannose (26%) in mature base wines. These percentages were in agreement with the glycosyl composition of other sparkling wines obtained by different authors.[19,38] As previously reported,[11] the amount of galactose, arabinose and rhamnose, which come from grapes, clearly increased with grape maturity. In the same way, base wines elaborated with mature grapes showed higher content in mannose than those elaborated with premature ones. The high concentration of mannose may be due to enhanced yeast metabolism in higher-sugar grape juices.[39] In contrast, base wines elaborated with more mature grapes presented lower quantities of glucose. The total content of glycosyl residues was higher in mature base wines than in premature ones (318 ± 8.3 and 199 ± 5.7 mg/L, respectively). In the same way, during the aging on yeast lees, mature red sparkling wines showed higher quantities of several glycosyl residues than premature ones (approximately 1.7 times higher). Therefore, chemical quantitative analysis corroborated the profiles obtained by size exclusion chromatography (Figure 1).

Glycosyl content and profile of polysaccharides changed as the aging on yeast less process went on. Yeast monosaccharides showed different trends. In both wines, the content of mannose increased significantly at 6 months of aging probably due to yeast autolysis, but it was significantly reduced from 6 to 9 months of aging in mature samples. 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. No clear trend in the glucose concentration was observed. The content of glucose decreased at 3 months of aging in premature red sparkling wines; however, it was observed an increase after 3 months of aging in the mature wines. This lack of a trend in glucose concentration could be related with the different sources of polymeric glucose, with the precipitation of grape hemicelluloses during winemaking, but also with the different yeast autolysis conditions.

The content of monosaccharides forming the grape polysaccharides remained constant or decreased during aging. Grape polysaccharides could react with other wine compounds to form unstable colloids during long periods of aging on yeast lees. These results were in agreement with those of other researchers in white and rosé sparkling wines.[19]

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.

Ara/Gal ratio remained close to 1.3 in both base wines, which is somewhat higher than those described in the literature for still red wine polysaccharides rich in arabinose and galactose (PRAG).[40,41] Analysis of the Ara/Gal ratio indicated that aging on yeast lees slightly modified the total PRAG composition of sparkling wines according their grape maturity stage. Mature red sparkling wines showed a significant increase in Ara/Gal ratio during the aging on yeast lees, suggesting a larger release of arabinose or polysaccharides rich in arabinose arising from the hairy region of the pectic framework. In contrast, the Ara/Gal ratio of premature red sparkling wines remained constant during the aging. The different trends in the Ara/Gal ratio during the aging on yeast lees may influence the PRAG physicochemical properties and thus modify the final colloidal equilibrium[17] and foam properties of the sparkling wines.[18]

The Man/Glc ratio indicated that grape maturity modified the release of polysaccharides from yeast during the autolysis of the cell walls. Mature red sparkling wines showed higher changes in the Man/Glc ratio than premature ones. A significant decrease was observed in mature samples after 3 months of aging. Man/Glc decrease was due to a significant increase in the glucose content, suggesting that glucans (GL) were hydrolyzed by glucanases during this period.

The concentration of mannoproteins (MP), glucans (GL), polysaccharides rich in arabinose and galactose (PRAG) and rhamnogalacturonans type II (RG-II) in red sparkling wines is shown in Figure 2, and it was estimated from the concentration of individual glycosyl residues, as determined by GC after hydrolysis, reduction and acetylation.[41] 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 that of 2-O-methylfucose and 2-O-methyl-xylose.

In all the winemaking stages, the MP concentration was lower in premature red sparkling wines than in mature 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 mature red sparkling wines could be due to the different alcohol content of the wines. Several factors, such as the winemaking conditions[39] or the initial colloid content in must,[42] are related with the MP released by yeasts. In the same way, Doco et al.[43] found higher concentrations of MP in Carignan wines than in Grenache wines, probably due to the different ripening degrees at harvest. Yeast MP were mainly released after 6 months of aging, when autolysis process occurred,[44] which is consistent

with previously published data.[19] All samples had lower GL concentration than those previously reported for white and rosé sparkling wines[19].

The concentration of PRAG and RG-II in red sparkling wines clearly increased with grape maturity. The increase in soluble pectic polysaccharides in grapes throughout maturity could be related with enzymatic activity. As previously reported[45], grape polygalacturonase activity, which is almost unnoticeable during the herbaceous growth of the berry, gradually increases after veraison, and triggers a ripening-associated pectin depolymerization. Therefore, an increase in the soluble polysaccharides in grape berries during ripening[6,8,11] could justify the higher content of PRAG and RG-II in sparkling wines made from riper grapes. In general, aging on yeast lees produced a significant reduction in PRAG and RG-II in wines. This reduction was more pronounced in mature samples, suggesting a higher hydrolytic phenomenon in sparkling wines obtained with mature grapes. Decreases in grape polysaccharides content throughout aging has also been described by other authors[19,46].

Oligosaccharide composition

Table 3 shows glycosyl composition and characteristic ratios of oligosaccharides from red sparkling wines. To the best of our knowledge, there is no literature on this topic relating sparkling wines, and this is the first time that the glycosyl composition of oligosaccharides in these types of wines is described.

No significant differences were found in the total oligosaccharide content between the two base wines (mature and premature base wines: 311 ± 17.6 and 299 ± 20.0 mg/L, respectively). These quantities were in good agreement with those reported for still wines.[22-25] Differences among total glycosyl content of oligosaccharides in both base wines were not as significant as in the total glycosyl content of polysaccharides. These

results suggested that the grape maturity stage had more influence on the wine polysaccharide concentration than on the oligosaccharide concentration.

In all vinification stages, the galacturonic acid residue was the predominant constituent of the oligosaccharides in both maturation stages (52-59%), followed by glucose (13-20%), mannose (7-10%), xylose (6-7%), and arabinose (4-6%). Galactose (2-4%) and 4-methyl glucuronic acid (2%) were also detected, but in smaller quantities. Rhamnose, fucose, glucuronic acid and xylitol were also detected in all the samples with even lower amounts (1%). Our results showed significantly higher quantities of galacturonic acid in red sparkling wines than those reported in literature for still wines.[9,21-23] The high galacturonic acid concentration could be explained by differences in the pectin composition and in the natural pectinase activities present in the grape skins.

The content of glucose and mannose glycosyl residues didn't increase in the oligosaccharide fraction during aging on yeast lees, probably due to a reduction of the hydrolytic enzymes activities involved in the autolytic process, and/or the higher precipitation or combination rate of oligosaccharides than their solubilization into the wine could explain this phenomena. Total glycosyl content of oligosaccharides decreased in both sparkling wines during the whole period of aging. Reductions in premature red sparkling wines were higher than in mature ones (40% vs 9%) during the wine aging. These data suggested that oligosaccharides from riper grapes showed more solubility and stability, which could have implications on sparkling wine sensory properties.

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 (Table 3).

373 The Ara/Gal ratio is characteristic of the PRAG-like structures.[47,48] The ratio was two
374 fold higher than that of red still wine polysaccharides, usually close to 1.[43] Mature red
375 sparkling wines showed higher Ara/Gal ratios than premature ones during the aging on
376 yeast lees. The increase of this ratio in the oligosaccharide fraction suggested a higher
377 release of arabinose or oligosaccharides rich in arabinose arising from the pectic
378 framework in mature wines than in premature ones. Moreover, two trends were
379 observed during the wine aging. Ara/Gal increased in mature red sparkling wines but
380 the opposite was observed in premature ones, suggesting a significant degradation of
381 PRAG structures in wines made with the less mature grapes.
382 The relative richness of the wine oligosaccharides in homogalacturonans versus
383 rhamnogalacturonans could be deduced from the Rha/GalA ratio.[49] The low value
384 observed for this ratio in oligosaccharides from red sparkling wine (0.02-0.03) indicated
385 that homogalacturonans were the major compounds. The values observed for this ratio
386 were lower than those obtained for red still wines.[21-25]
387 The ratio of (Ara + Gal) to Rhamnose was calculated to estimate the relative importance
388 of the neutral side-chains to the rhamnogalacturonan backbone. During all vinification
389 stages, the (Ara + Gal)/Rha ratio was considerably lower in premature red sparkling
390 wine oligosaccharides in comparison with mature ones. It could indicate that the
391 rhamnogalacturonan oligomers present in mature red sparkling wines carry more neutral
392 lateral chains. The modifications of (Ara + Gal)/Rha ratio obtained for oligosaccharide
393 fraction indicated that arabinan and arabinogalactan side chains carried by the rhamnose
394 residues of the pectin hairy zone were changed during the aging.
395 The values obtained for Rha/GalA and (Ara + Gal)/Rha ratios indicated that red
396 sparkling wine oligosaccharides contained more structures from the hairy regions of

pectins (rhamnogalacturonan-like structures carrying neutral lateral chains) as a result of degradation of grape cell wall berries by pectinases.

Regarding Man/Glc ratio of oligosaccharides, glucose was the prevalent sugar, and mannose represented only 7 to 17%. The greater proportion of glucose suggested that the oligosaccharides released into the medium were essentially gluco-oligosaccharides. Throughout ageing, the Man/Glc ratio in oligosaccharides decreased in both maturity stages.

MP and PRAG are known to be involved in the foaming properties of sparkling wines.[18] However, no information is available on the properties of arabino- and manno oligosaccharides. Only the influence of oligosaccharides over astringency has been recently investigated.[23,50] Therefore, it would be interesting to study their impacts on sparkling wines, especially their implications in the foaming properties. The knowledge of the oligosaccharide composition and content of sparkling wines should allow to know their physicochemical properties and their interactions with other components present in these types of wines.

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

Figure 3 shows the elution profiles of the polysaccharidic fractions from premature and mature red sparkling wines using HPSEC coupled to on-line differential refractometer, viscosimeter, and multi-angle light scattering (MALLS). Concentration signal derived from the differential refractometer, whereas molar mass derived from light scattering were given. The polysaccharides refractive index elution profiles from premature grapes displayed three principal populations whose concentration signal peaks are in the ranges 29-32 min (first population), 32-35 min (second population) and 35-39 min (third population), no matter what time of aging on yeast lees (Figure 3, DRI signal). In the

422 case of wine elaborated from mature grapes (Figure 3, DRI signal), similar populations
423 were found, although the range of second populations extended until 36 min.
424 Comparing maturity degrees, the first population appeared clearly higher in the wine
425 elaborated with mature grapes no matter the aging on yeast lees. Besides, it can be
426 observed that the second and third populations were not well separated in wines from
427 premature grapes, whereas these two peaks appeared clearly differentiated for the wines
428 elaborated with mature ones.

429 Regarding the time of aging on yeast lees, wines after 9 months of aging on yeast lees
430 showed lower values in the case of second population without regards the maturity
431 degree of grapes. Moreover, in the case of wine coming from mature grapes, first and
432 third populations also appeared lower after 9 months of aging in comparison with
433 initial, 3 months and 6 months wines. Concerning wines from prematurity grapes, the
434 highest third peak corresponded to wine with no month of aging.

435 The molar mass of the eluting molecules for all samples decreased with increasing
436 elution time in agreement the normal size exclusion separation mechanism (Figure 3,
437 Mw signal). In general, molar mass was higher in wines coming from premature grapes
438 between 29 and 34 min, whereas wines from mature grapes showed higher molar mass
439 between 34 and 37 min.

440 The molar mass, the polydispersity index (Mw/Mn) and the intrinsic viscosity ([η])
441 values from studied wines are shown in Table 4. The molar mass appeared notably
442 higher for premature wines in all three populations (P1 (29-32 min): between 368300
443 and 420000 g/mol; P2 (32-35 min): between 94900 and 106500 g/mol; P3 (35-39 min):
444 between 14790 and 15650) in comparison with mature wines (P1 (29-32 min): between
445 264400 and 313900 g/mol; P2 (32-35 min): between 91170 and 95910 g/mol; P3 (35-39
446 min): between 13230 and 15450). The polydispersity index (Mw/Mn) was in general

lower in third population in comparison with first and second population for all the studied wines. Peak P3 (35-39 min) mainly corresponds to RG-II which have a perfectly defined structure (10000 g/mol for the dimer and 5000 g/mol for the monomer),[48] and which therefore has a similar polydispersity index (Mw/Mn) of 1. The intrinsic viscosity was notably higher for premature wines in third population (between 12.9 and 14.6 mL/g) compared with mature wines (between 5.2 and 7.5 mL/g). In contrast, this parameter appeared lower in the case of first population from premature wines (between 41.2 and 51.6 mL/g) compared with same population from mature wines (between 51.1 and 56.3 mL/g).

Table 5 shows the molar mass distribution analysis of complex carbohydrate fraction from red sparkling wines as determined by Size Exclusion Chromatography coupled on-line to Multi Angle Laser Light Scattering (SEC-MALLS) and differential refractometer. Regarding these data, six delimited ranges (Molar mass range: range 1 = 2500-20000 g/mol; range 2 = 20000-100000 g/mol; range 3 = 100000-250000 g/mol; range 4 = 250000-500000 g/mol; range 5 = 500000-1000000 g/mol and range 6 = 1000000-10000000) can be observed among different wines. These ranges limits have been selected from their correspondence with values obtained from different polysaccharide families by SEC-MALLS analysis: RG-II monomer: Mw: 5000 g/mol; RG-II dimer: 10000 g/mol; MP_{0c}: Mw=58000 g/mol; AGP_2: Mw = 165000 g/mol; MP_{0a}: 350000 g/mol; MP_3: Mw = 1000000 g/mol (data not reported). In this way, it has been found a molar mass around 145000 g/mol in AGP_0 from red wines.[48]

Regarding the maturity degree, the wine polysaccharide fractions from premature grapes showed higher values in range 1, no matter the time of aging on yeast lees (between 37% and 42% of cumulative percentage) compared with wine polysaccharide fractions from mature grapes (around 25% of cumulative percentage of its molar mass

in this range). In contrast, wine polysaccharide fractions from mature grapes presented around 35% of mass in range 3, whereas wine polysaccharide fractions from premature grapes present lower percentages of masse in this range (between 24% and 29%). Obvious differences can also be detected regarding range 5: premature sparkling wines showed between 1.5 and 1.9% whereas the value was 0% in all the mature ones. Concerning the aging of wines with lees, the difference in range 2 between premature sparkling wines with 3 months of aging (28%) and premature sparkling wines with 9 months of aging (18%) was remarkable. In contrast, the percentages in each range appeared notably similar between mature sparkling wines regardless of the aging time.

Taking into account that all the winemaking conditions were the same, except grape maturity stage, results obtained by several analytical techniques suggest a grape ripening influence on sparkling wine carbohydrate concentration, composition and structure. Nevertheless, more works should be carried out to further investigate the possible effect of other factors, such as grape variety, terroir effect or different winemaking procedures, on the oligosaccharides fraction from sparkling wine.

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FIGURE CAPTIONS

Figure. 1. Purification by high-resolution size-exclusion chromatography on Superdex 30-HR column of total complex carbohydrate fractions isolated from premature and mature red 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. (Relative Refractive Index versus Retention Time (Minutes))

Figure 2. Concentration of Mannoproteins (MP), Glucans (GL), Polysaccharides Rich in Arabinose and Galactose (PRAG), and Rhamnogalacturonan type II (RG-II) in premature (in red line) and mature (in black line) red sparkling wines during different stages of wine production: base wines (T0), sparkling wines after 3 months (T3), 6 months (T6), and 9 months (T9) of aging on yeast lees. Average of the three measurements and standard deviation. Different letters indicate statistical differences ($p < 0.05$). Small letters are used to compare the wines of the same maturity level in each parameter and different aging time by one-way ANOVA. Capital letters are used to compare the wines of the different maturity level in each parameter and each aging time by two-sample t test.

Figure 3. SEC-MALLS chromatograms and weight average molar mass distributions of the polysaccharide fraction in premature (in red line) and mature (in black line) red 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. Molar weight distribution (Mw; g/mol; thick line) and Refractive Index (DRI; relative scale; dashed line).

TABLES

Table 1. Standard enological parameters in must, base wines and sparkling wines at 9 months of aging.

Parameter[a]	Premature	Mature
Musts		
Brix	19.7	22.4
pH	3.25	3.46
TA	6.3	6.1
Malic acid (g/L)	3.30	1.89
Tartaric acid (g/L)	4.73	5.19
Potassium (mg/L)	1207	2000
Base wines		
pH	3.47	3.71
TA	5.1	4.8
Alcohol	11.1	13.0
Malic acid (g/L)	0.09	0.09
Tartaric acid (g/L)	1.90	2.30
VA	0.32	0.20
Potassium (mg/L)	1100	730
CI	8.7	11.5
Hue	0.63	0.53
Sparkling wines		
pH	3.49	3.70
TA	5.2	4.9
Alcohol	12.3	14.0
Reducing sugar (g/L)	1.50	1.70
Malic acid (g/L)	0.09	0.09
Tartaric acid (g/L)	1.80	2.20
VA	0.32	0.30
CI	6.7	8.8
Hue	0.64	0.57
Pressure (bars)	5.3	5.1

[a] TA: titratable acidity as g tartaric acid/L. Alcohol: % ethanol by volume at 20ºC. VA: volatile acidity as g acetic acid/L. CI: color intensity as sum of absorbances at 420, 520, and 620 nm. Hue: A420/A520.

Table 2. Glycosyl content (mg/L) and characteristic ratios of polysaccharides from red sparkling wines during different stages of sparkling wine elaboration: base wines (T0), sparkling wines after 3 months (T3), 6 months (T6), and 9 months (T9) of aging on yeast lees [a]

	2-OMeFuc[b]	2-OMeXyl[b]	Api[b]	Ara[b]	Fuc[b]	Gal[b]	Glc[b]	Man[b]	Rha[b]	Xyl[b]	Total	Ara/Gal[b]	Man/Glc[b]
Premature													
T0	1.4 ± 0.1 b A	0.8 ± 0.1 b A	0.7 ± 0.1 a A	54.8 ± 2.9 b A	1.0 ± 0.1 a A	51.3 ± 1.1 a A	12.3 ± 0.8 c A	59.7 ± 0.1 ab A	16.1 ± 0.3 b A	1.3 ± 0.2 b A	199 ± 5.7 b A	1.28 ± 0.0 b A	4.85 ± 0.3 a A
T3	1.3 ± 0.2 ab A	0.6 ± 0.0 a A	0.6 ± 0.1 a A	47.0 ± 2.2 a A	0.8 ± 0.1 a A	48.8 ± 1.2 a A	8.6 ± 0.3 a A	56.5 ± 1.8 a A	13.4 ± 1.1 a A	0.9 ± 0.1 a A	178 ± 7.1 a A	1.16 ± 0.0 a A	6.54 ± 0.0 b A
T6	1.1 ± 0.0 b A	0.6 ± 0.1 a A	0.6 ± 0.0 a A	51.9 ± 2.3 ab A	0.8 ± 0.0 a A	55.0 ± 3.3 a A	10.7 ± 0.2 b A	65.3 ± 3.2 c A	12.3 ± 0.9 a A	1.0 ± 0.0 a A	199 ± 10.0 b A	1.13 ± 0.1 a A	6.10 ± 0.2 b A
T9	1.2 ± 0.1 ab A	0.6 ± 0.0 a A	0.7 ± 0.0 a A	51.6 ± 1.2 ab A	1.0 ± 0.1 a A	52.1 ± 4.1 a A	12.2 ± 0.7 c A	63.4 ± 0.4 bc A	13.5 ± 0.5 a A	1.1 ± 0.1 ab A	197 ± 7.2 ab A	1.19 ± 0.1 ab A	5.21 ± 0.3 a A
Mature													
T0	1.9 ± 0.0 c B	1.1 ± 0.0 a B	0.7 ± 0.1 a A	101.8 ± 1.4 b B	1.6 ± 0.2 a B	93.5 ± 4.1 b B	6.3 ± 0.2 a B	81.8 ± 2.1 a B	28.0 ± 0.2 b B	1.7 ± 0.1 ab B	318 ± 8.3 ab B	1.31 ± 0.0 a B	12.97 ± 0.1 b B
T3	1.9 ± 0.1 c B	1.2 ± 0.1 a B	0.9 ± 0.2 a A	103.6 ± 2.2 b B	1.6 ± 0.0 a B	86.4 ± 4.0 ab B	12.4 ± 3.2 b B	81.1 ± 1.2 a B	28.6 ± 0.3 b B	1.6 ± 0.0 a B	319 ± 8.8 ab B	1.44 ± 0.0 b B	6.53 ± 1.7 a A
T6	1.8 ± 0.0 b B	1.2 ± 0.0 a B	0.9 ± 0.1 a B	106.2 ± 2.7 b B	1.6 ± 0.2 a B	93.1 ± 2.3 b B	10.6 ± 0.5 ab A	91.8 ± 6.4 b B	29.1 ± 2.0 b B	1.7 ± 0.1 ab B	338 ± 14.8 b B	1.37 ± 0.0 ab B	8.68 ± 0.1 a B
T9	1.7 ± 0.0 a B	1.2 ± 0.1 a B	0.8 ± 0.2 a A	96.7 ± 1.5 a B	1.4 ± 0.1 a B	80.5 ± 2.8 a B	9.3 ± 0.4 ab B	79.1 ± 1.2 a B	25.2 ± 0.1 a B	2.0 ± 0.2 b B	298 ± 6.6 a B	1.44 ± 0.0 b B	8.50 ± 0.3 a B

[a] Different letters indicate statistical differences ($p < 0.05$). Small letters are used to compare the wines of the same maturity level in each parameter and different aging time by one-way ANOVA. Capital letters are used to compare the wines of the different maturity level in each parameter and each aging time by two-sample t test.

[b] 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.

Table 3. Glycosyl content (mg/L) and characteristic ratios of oligosaccharides from red sparkling wines during different stages of sparkling wine elaboration: base wines (T0), sparkling wines after 3 months (T3), 6 months (T6), and 9 months (T9) of aging on yeast lees [a]

	Rha[b]	Fuc[b]	Ara[b]	Xyl[b]	Man[b]	Gal[b]	Glc[b]	Gal A[b]	Glc A[b]	Xylitol[b]	4-OMeGlc A[b]	Total
Premature												
T0	4.3 ± 0.3 a A	3.3 ± 0.2 c A	11.6 ± 0.7 bc A	17.9 ± 1.9 b A	26.0 ± 1.1 c A	7.5 ± 0.6 ab A	41.1 ± 4.1 ab A	176.8 ± 10.1 c A	3.1 ± 0.3 b A	1.8 ± 0.1 ab A	5.6 ± 0.6 b A	299 ± 20.0 c A
T3	3.4 ± 0.2 a A	2.2 ± 0.2 ab A	10.3 ± 0.5 b A	15.1 ± 1.3 b A	21.6 ± 2.1 ab A	7.3 ± 0.2 ab A	34.5 ± 3.8 a A	144.0 ± 8.3 b A	2.6 ± 0.2 b A	2.0 ± 0.0 b A	4.9 ± 0.1 b A	248 ± 16.9 b A
T6	4.1 ± 0.2 a A	2.6 ± 0.2 b A	12.5 ± 0.7 c A	17.0 ± 1.1 b A	24.4 ± 1.3 bc A	7.9 ± 0.8 b A	50.0 ± 2.2 b A	172.9 ± 15.0 c A	3.1 ± 0.2 b A	2.1 ± 0.2 b A	5.3 ± 0.4 b A	302 ± 22.1 c A
T9	2.6 ± 0.1 a A	2.1 ± 0.2 a A	6.7 ± 0.4 a A	10.8 ± 0.7 a A	18.1 ± 1.8 a A	6.2 ± 0.2 a A	36.2 ± 3.2 a A	91.8 ± 7.2 a A	1.7 ± 0.0 a A	1.3 ± 0.3 a A	3.0 ± 0.3 a A	180 ± 15.1 a A
Mature												
T0	3.2 ± 0.5 a B	2.7 ± 0.1 ab B	18.0 ± 0.7 c B	20.9 ± 1.1 bc A	24.2 ± 1.0 b A	11.0 ± 1.0 c B	42.2 ± 7.8 a A	174.2 ± 3.0 c A	3.9 ± 0.8 a A	3.3 ± 1.0 a B	7.6 ± 0.7 a B	311 ± 17.6 b A
T3	3.0 ± 0.5 a A	3.0 ± 0.2 b B	16.8 ± 1.0 bc B	21.1 ± 1.0 c B	26.0 ± 0.3 b B	9.3 ± 0.5 b B	54.7 ± 5.7 a B	172.8 ± 1.9 c B	3.6 ± 0.3 a B	3.2 ± 0.2 a B	7.2 ± 0.4 a B	321 ± 12.1 b B
T6	2.5 ± 0.2 a B	2.5 ± 0.1 a A	13.7 ± 0.8 a A	16.4 ± 0.5 a A	20.6 ± 1.4 a B	6.9 ± 1.0 a A	44.4 ± 4.3 a A	160.2 ± 4.2 b A	3.7 ± 0.2 a B	2.0 ± 0.1 a A	6.0 ± 0.5 a A	279 ± 12.4 a A
T9	2.5 ± 0.1 a A	2.5 ± 0.1 a B	15.0 ± 0.2 ab B	18.8 ± 0.2 b B	21.6 ± 0.0 a B	7.0 ± 0.1 a B	56.8 ± 6.8 a B	147.0 ± 1.1 a B	3.3 ± 0.2 a B	2.8 ± 0.3 a B	6.0 ± 0.9 a B	283 ± 10.1 a B

[a] Different letters indicate statistical differences ($p < 0.05$). Small letters are used to compare the wines of the same maturity level in each parameter and different aging time by one-way ANOVA. Capital letters are used to compare the wines of the different maturity level in each parameter and each aging time by two-sample t test.

[b] 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.

Table 3. Continued.

	Ara/Gal[b]	Rha/Gal A[b]	(Ara+Gal)/Rha[b]	Man/Glc[b]
Premature				
T0	1.87 ± 0.0 c A	0.03 ± 0.0 a A	4.49 ± 0.0 a A	0.63 ± 0.0 b A
T3	1.68 ± 0.0 b A	0.03 ± 0.0 a A	5.27 ± 0.1 a A	0.63 ± 0.0 b A
T6	1.89 ± 0.1 c A	0.03 ± 0.0 a A	5.03 ± 0.1 a A	0.49 ± 0.0 a A
T9	1.30 ± 0.0 a A	0.03 ± 0.0 a A	4.98 ± 0.1 a A	0.50 ± 0.0 a A
Mature				
T0	1.96 ± 0.1 a A	0.02 ± 0.0 a B	9.24 ± 0.8 a B	0.57 ± 0.1 c A
T3	2.17 ± 0.0 a B	0.02 ± 0.0 a B	8.89 ± 0.9 a B	0.48 ± 0.0 b B
T6	2.37 ± 0.1 b B	0.02 ± 0.0 a B	8.51 ± 0.3 a B	0.46 ± 0.0 b B
T9	2.56 ± 0.0 b B	0.02 ± 0.0 a B	9.27 ± 0.3 a B	0.38 ± 0.0 a B

[a] Different letters indicate statistical differences ($p < 0.05$). Small letters are used to compare the wines of the same maturity level in each parameter and different aging time by one-way ANOVA. Capital letters are used to compare the wines of the different maturity level in each parameter and each aging time by two-sample t test.

[b] 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.

Table 4. Parameters[a] obtained for the polysaccharides isolated from red sparkling wines during different stages of sparkling wine elaboration: base wines (T0), sparkling wines after 3 months (T3), 6 months (T6), and 9 months (T9) of aging on yeast lees.

	Peak[b]	Mw (g/mol)	Mn (g/mol)	Mw/Mn	Intrinsic viscosity (mL/g)
Premature					
T0	1	376 500	334 700	1.13	45.8
	2	97 560	78 920	1.24	22.2
	3	15 560	14 340	1.09	14.6
T3	1	394 600	331 400	1.19	51.6
	2	94 900	76 590	1.24	25.3
	3	14 790	13 360	1.11	14.5
T6	1	368 300	318 800	1.16	42.9
	2	99 560	81 640	1.22	21.2
	3	15 620	14 100	1.11	14.2
T9	1	420 000	351 400	1.2	41.2
	2	106 500	87 970	1.21	20.6
	3	15 650	13 980	1.12	12.9
Mature					
T0	1	264 400	234 300	1.13	52.8
	2	91 170	79 440	1.15	20.7
	3	13 230	12 640	1.05	6.8
T3	1	297 600	246 500	1.21	53.2
	2	92 200	82 240	1.12	21.6
	3	15 450	14 320	1.08	7.5
T6	1	313 900	273 500	1.15	56.3
	2	95 910	82 120	1.17	21.4
	3	13 720	13 000	1.06	6.4
T9	1	295 000	254 000	1.16	51.1
	2	92 220	80 310	1.15	20.3
	3	14 150	13 380	1.06	5.2

[a]Molar-mass distributions, *Mw*, *Mn*, determined by coupling size exclusion chromatography performed on two serial Shodex OH-pack columns with a multi-angle light scattering device (MALLS), -MALLS in 0.1 M LiNO3 (*dn/dc* = 0.146 mL/g). Intrinsic viscosity ([η]) determined by a differential viscometry detector equipped with a four-capillary bridge design.

[b]peak 1: ranges 29-32 min (first population); peak 2: ranges 32-35 min (second population); Peak 3: ranges 35-39 min (third population)

Table 5. Distribution analysis determined by light scattering (dn/dc = 0.146 mL/g) obtained of polysaccharides fractions isolated from red sparkling wines during different stages of sparkling wine elaboration: base wines (T0), sparkling wines after 3 months (T3), 6 months (T6), and 9 months (T9) of aging on yeast lees.

(%)				
Premature	T0	T3	T6	T9
Molar mass (g/mol) 2.5-20·10^3	37.0	38.8	36.9	42.1
Molar mass (g/mol) 20-100·10^3	25.5	28.2	22.4	18.4
Molar mass (g/mol) 100-250·10^3	26.2	23.5	28.8	26.6
Molar mass (g/mol) 250-500·10^3	9.3	7.7	10.0	11.0
Molar mass (g/mol) 500-1000·10^3	1.5	1.7	1.9	1.6
Molar mass (g/mol) 1000-10000·10^3	0.5	-	-	0.3
(%)				
Mature	T0	T3	T6	T9
Molar mass (g/mol) 2.5-20·10^3	24.6	25.0	25.0	25.1
Molar mass (g/mol) 20-100·10^3	28.4	28.5	29.0	28.5
Molar mass (g/mol) 100-250·10^3	35.5	34.3	34.7	34.0
Molar mass (g/mol) 250-500·10^3	11.3	11.9	11.4	12.4
Molar mass (g/mol) 500-1000·10^3	-	-	-	-
Molar mass (g/mol) 1000-10000·10^3	0.3	0.3	-	-

Figure 1.

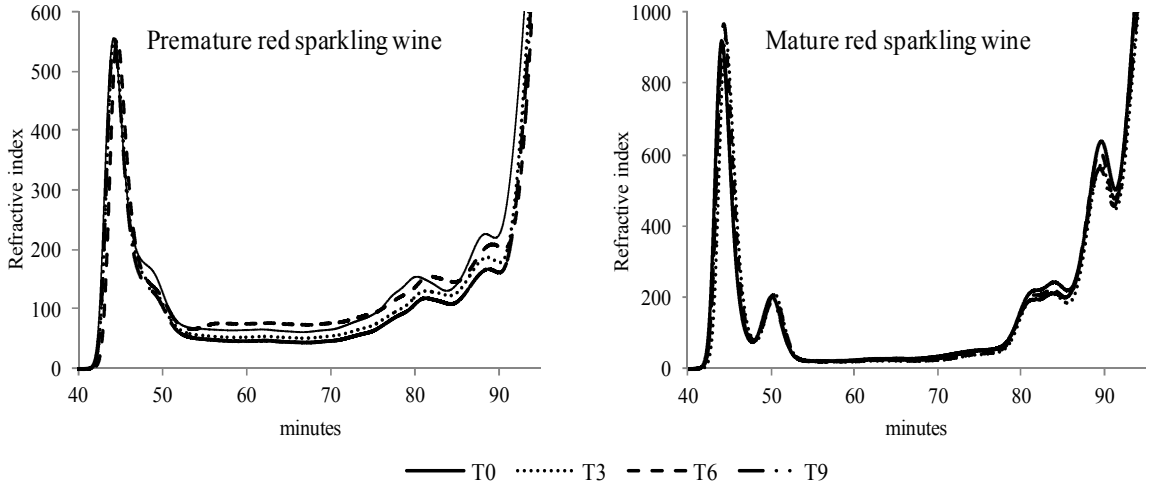

Premature red sparkling wine
Refractive index
minutes
Mature red sparkling wine
Refractive index
minutes
T0
T3
T6
T9

Figure 2.

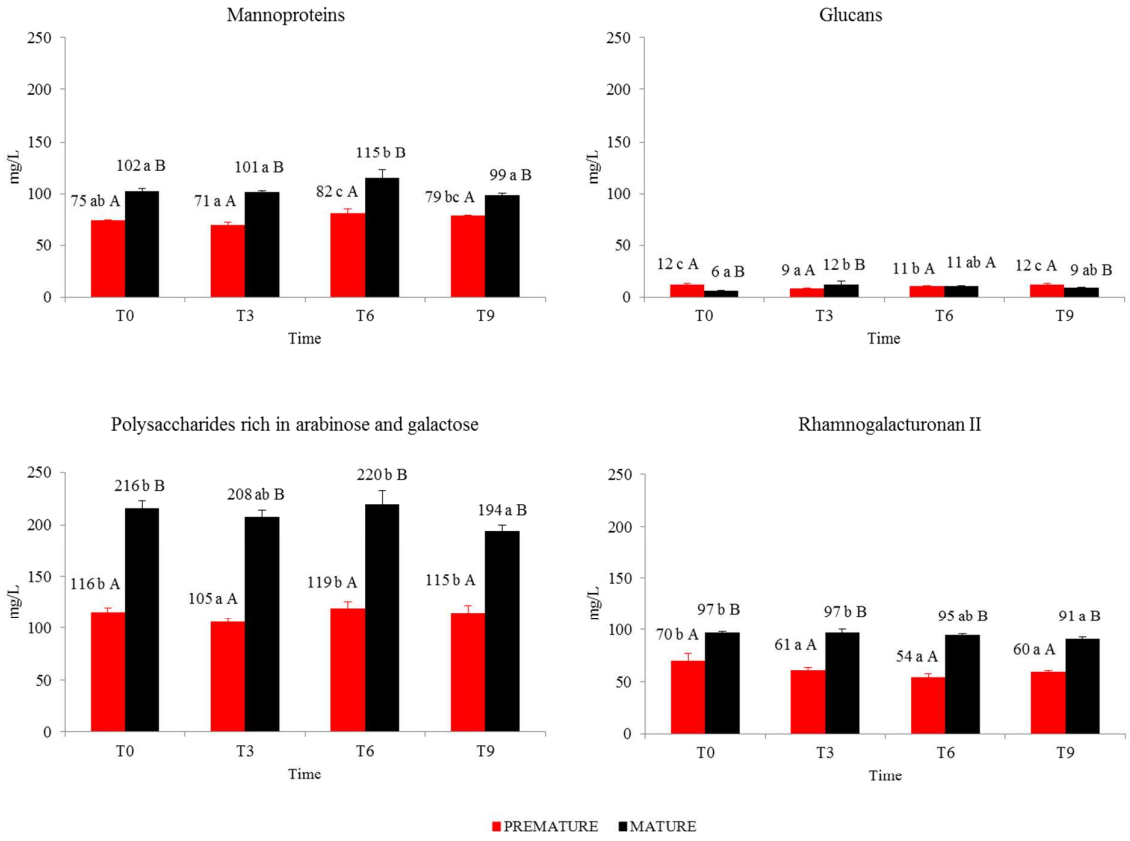
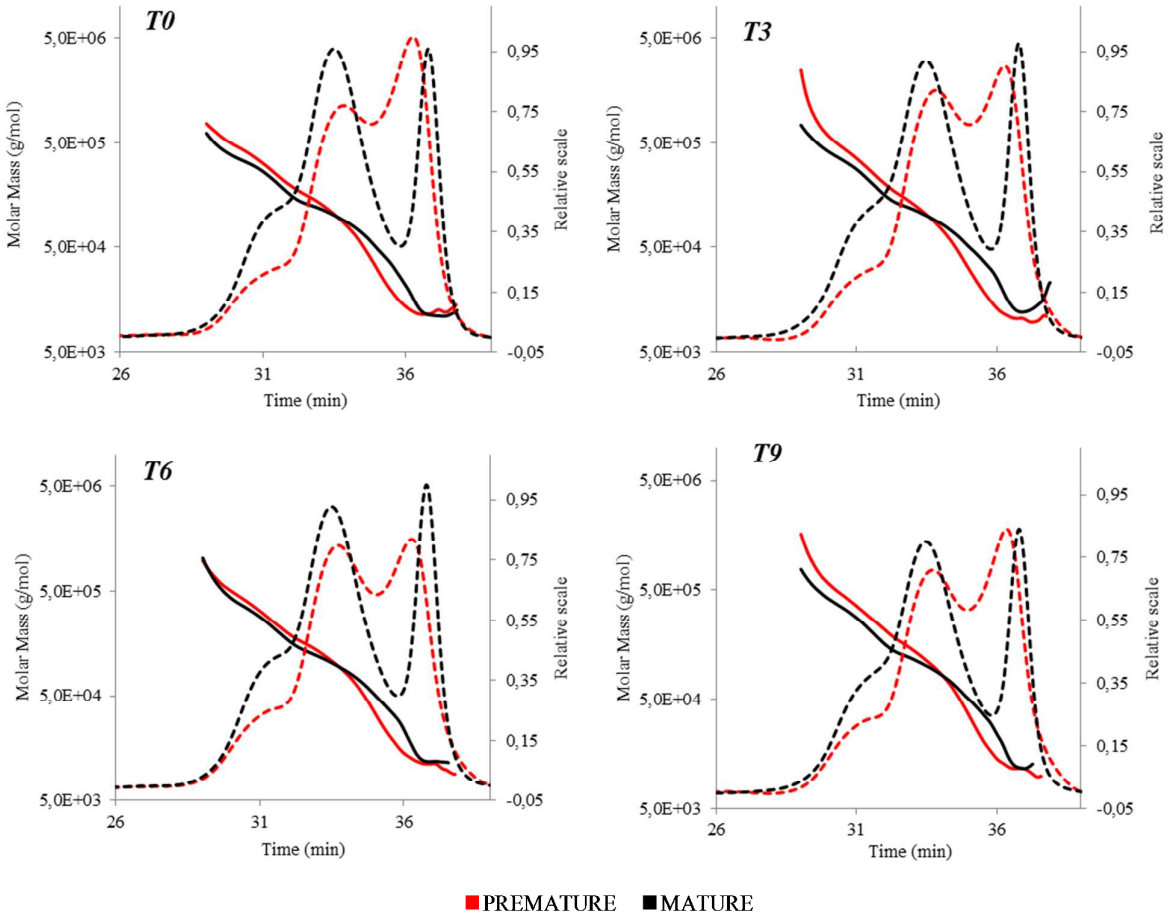
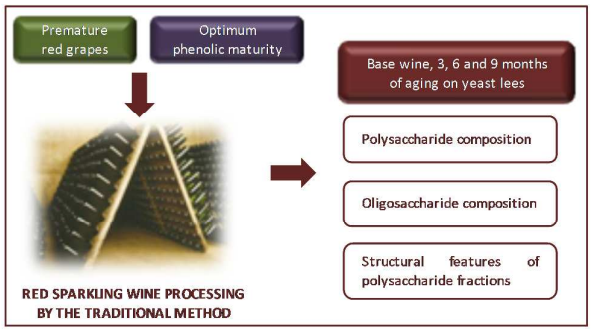

Mannoproteins
mg/L
250
200
150
100
50
0
75 ab A
102 a B
71 a A
101 a B
82 c A
115 b B
79 bc A
99 a B
T0
T3
T6
T9
Time
Glucans
12 c A
6 a B
9 a A
12 b B
11 b A
11 ab A
12 c A
9 ab B
Polysaccharides rich in arabinose and galactose
116 b A
216 b B
105 a A
208 ab B
119 b A
220 b B
115 b A
194 a B
Rhamnogalacturonan II
70 b A
97 b B
61 a A
97 b B
54 a A
95 ab B
60 a A
91 a B
PREMATURE
MATURE

Figure 3.


T0
T3
T6
T9
Molar Mass (g/mol)
5,0E+06
5,0E+05
5,0E+04
5,0E+03
Relative scale
0,95
0,75
0,55
0,35
0,15
-0,05
26
31
36
Time (min)
PREMATURE
MATURE

TOC graphic


Premature red grapes
Optimum phenolic maturity
Base wine, 3, 6 and 9 months of aging on yeast lees
Polysaccharide composition
Oligosaccharide composition
Structural features of polysaccharide fractions
RED SPARKLING WINE PROCESSING BY THE TRADITIONAL METHOD