Polysaccharide characterization of commercial dry yeast preparations and their effect on white and red wine composition

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Running title: Commercial dry yeast preparations and their effect on wines

Abstract

The aim was to characterize several commercial dry yeast derivative preparations and to study their effect on different quality parameters of white and red wines. The monosaccharide and polysaccharide contents of these preparations were also evaluated.

The purity and composition of the commercial preparations studied were very heterogeneous, as were the effects that they can produce in wines.

All the yeast derivative preparations studied increased the content of neutral polysaccharides, although those with greater mannose content reduced the absorbance values at 420 nm and acidity in white wines.

In red wines, yeast derivatives reduced green tannins increasing the softness on the palate, and managed to stabilize the color, especially those yeast derivatives that release higher neutral polysaccharides.

Keywords: Commercial dry yeast preparations, polysaccharides, phenolic compounds, wines, sensory analysis.

1. Introduction

Nowadays, one of the main targets of the wine sector is to improve wine quality, elaborating wines that satisfy consumer's demand and expanding the offer of quality wines.

Aging of wines on lees is a technique more used in white wines than in red wines. Thank to this technique, wines get rich in some compounds such as polysaccharides, fatty acids, amino acids and peptides. Mannoproteins are the main polysaccharides that are released by yeast during alcoholic fermentation (Doco, Brillouet, & Moutounet, 1996; Vidal, Williams, Doco, Moutounet, & Pellerin, 2003, Ayestarán, Guadalupe, & León, 2004) and also by the autolysis of dead yeasts during the aging of wines on lees (Doco, Vuchot, Cheynier, &, Moutounet, 2003; Gonzalez-Ramos, Cebollero, & González., 2008). These compounds seem to be those that are the most interesting in enology by their positive effects on the quality of the final wine (Doco, et al., 2003, Fournairon, Camarasa, Moutounet, & Salmon, 2002; Feuillat, 2003). Mannoproteins are proteoglycans highly glycosilated mainly composed by mannose (>90%) and glucose (Guadalupe, Martínez, & Ayestarán, 2010) and proteins (<10%) (Vidal et al., 2003). They can have a highly variable size (5-800 kDa) (Doco, et al., 2003) and constitute 25-50% of the dry weight of the *Saccharomyces cerevisiae* walls, but their release into wine depends on the yeast strain (Pozo-Bayón, Andújar-Ortiz, & Moreno-Arribas, 2009).

Different positive effects of these compounds have been described related to sensory characteristics such as stabilization of red wine color (Escot, Feuillat, Dulau, & Charpentier, 2001; Francois, Alexandre, Granes, & Feuillat, 2007), reduction of wine astringency (Escot et al., 2001; Riou, Vernhet, Doco, & Moutounet, 2002; Vidal et al., 2004, Guadalupe, Palacios, & Ayestarán, 2007; Poncet-Legrand, Doco, Williams, &

43 Vernhet, 2007) and improvement of wine aromatic profile (Lubbers, Charpentier,
44 Feuillat, & Voilley, 1994; Dufour & Bayonoue, 1999; Ramírez, Chassagne, Feuillat,
45 Voilley, & Charpentier, 2004; Bautista, Fernández, & Falqué, 2007; Chalier, Angot,
46 Delteil, Doco, Gunata, 2007). However, most of these works are carried out on model
47 wine solutions.
48 Other authors have showed that these compounds can also improve tartaric and/or
49 protein stability because they inhibit tartrate salt crystallization (Lubbers, Leger,
50 Charpentier, & Feuillat, 1993; Moine-Ledoux & Dubourdieu, 2002) and/or reduce the
51 protein haze in white wines (Moine-Ledoux & Dubourdieu, 1999, Dupin et al., 2000;
52 Waters, Dupin, & Stockdale, 2000; Lomolino & Curioni, 2007; Schmidt et al., 2009).
53 However, the release of mannoproteins during aging on lees is too slow and some
54 alternatives are being studied to obtain the positive effects above mentioned. Hence, in
55 the last years, a large variety of commercial products which are obtained from the yeast
56 cell walls are being developed to provide similar characteristics to that wines aged on
57 lees. These products are obtained by thermal or enzymatic inactivation of
58 *Saccharomyces cerevisiae* yeasts after their growth in aerobic conditions in a highly
59 concentrated sugar medium (Pozo-Bayón et al., 2009). They can be classified as
60 inactive yeasts, yeast autolysates, yeast walls and yeast extracts (mannoproteins with
61 different degree of purification) (Pozo-Bayón et al., 2009). Some of these commercial
62 products also contain β-glucanase enzymes, which can favor the hydrolysis of the cell
63 walls and the release of mannoproteins.
64 All these products can be used at different stages of the winemaking process depending
65 on the type of wine that the winemaker wants to make. However, there are different
66 kind of products in the market, with different composition, purity and solubility.
67 Therefore they can cause very different effects on wines depending on the product used.

For all these reasons, the aim of this work was to characterize several commercial dry yeast derivative preparations and to study their effect on the composition of different quality parameters of a white and a red wine.

2. Material and methods

2.1. Winemaking process and treatments

The study was carried out using the *Tempranillo* grape variety from Cigales Designation of Origin (D.O.) for red wines, and the *Verdejo* grape variety from Rueda D.O. for white wines from 2007 vintage. Both D.O.s are sited in the Autonomous Community of Castilla y León in the North of Spain.

The grapes were harvested manually on the optimum harvest date and vinifications were carried out in the experimental winery of the Enological Station, following the traditional white and red winemaking processes.

Once the alcoholic fermentation finished, white and red wines were kept in the tanks for 4 days to allow for the sedimentation of the gross lees. After this time, the wines were racked off and maintained in the tanks for 4-5 days to allow for the sedimentation of the fine lees. The base wine was then again racked off and split into different 16 L tanks in which the different commercial products were added.

The experiences carried out were the control wines, without the addition of any product (C) and wines added with six different commercial yeast derivative products (YDs). All of them were carried out by duplicate.

Table 1 shows the characteristics of the different commercial products studied: commercial supplier, and composition according to the information given by the commercial supplier. The doses applied were the maximum authorized by the European Community: 40 g/hL (EC Regulation Nº 606/2009).

During treatments, two batonnages were performed weekly, and the temperature was maintained at 15 ºC ± 1 ºC. All treatments lasted 8 weeks. After that, the white wines were filtrated and bottled and the red wines were inoculated with a commercial preparation of *O. Oeni* (Viniflora, CHR Hansen, Denmark) to induce the malolactic fermentation. Finally, the red wines were also filtrated and bottled.

Samples were taken and analyzed just after the end of the treatments and at the end of the malolactic fermentation (red wines) and after three months of aging in bottle.

2.2 Chemical reagents

Gallic acid, D-(+)-catechin, Coomassie reactive, *trans*-caffeic acid, D-galacturonic acid, D-glucuronic acid and myo-inositol, lithium nitrate of HPLC, 3-hidroxy-biphenyl, phenol, L-fucose, L-rhamnose, 2-*O*-methyl D-xylose, L-arabinose, D-xylose, D-galactose, D-glucose, D-mannose and Kdo (3-deoxy octulosonic acid) were provided by Sigma-Aldrich (Steinheim, Germany); quercetin, malvidin-3-glucoside and cyanidin chloride by Extrasynthèse (Lyon, France); bovine serum albumine, di-sodium tetraborate decahydrated, dried methanol, pyridine, hexamethyldisilazane and trimethylclorosilane by Merck (Darmstadt, Germany). Acetonitrile and methanol of HPLC grade were provided by Lab Scan (Madrid, Spain). The remaining of reagents was supplied by Panreac (Madrid, Spain) or Scharlab (Barcelona, Spain). Milli-Q water was obtained by a Millipore system (Bedford, MA).

2.3. Analytical methods

2.3.1. Analysis of monosaccharide and polysaccharide composition

In order to characterize the different dry yeast preparations, the monosaccharide composition and their polysaccharide molecular weight distribution and content were analyzed.

116 The monosaccharide composition of the commercial preparations was determined by
117 GC-MS of their trimethylsilyl-ester O-methyl glycosyl residues obtained after acidic
118 methanolysis and derivatization (Guadalupe, Martínez-Pinilla, Garrido, Carrillo, &
119 Ayestarán, 2012).
120 A high-resolution size-exclusion chromatography (HRSEC) system (1100 Agilent
121 Technologies, Germany) with a refractive index detector (RID) was used to obtain the
122 molecular weight distributions of the polysaccharides. Two serial Shodex OHpack KB-
123 803 and KB-805 columns (0.8 x 30 cm, Showa Denko, Japan) equilibrated at 1 mL min^{-1}
124 in 0.1 M $LiNO_3$ were used. Calibration was performed with narrow pullulan molecular
125 weight standards (Shodex P-82, Waters, Barcelona, Spain): P-5, Mw = 5.9 kDa; P-10,
126 Mw = 11.8 kDa; P-20, Mw = 22.8 kDa; P-50, Mw = 47.3 kD; P-100, Mw = 112 kDa; P-
127 200, Mw = 212 kDa; P-400, Mw = 404 kDa. The apparent molecular weights were
128 deduced from the calibration equation $\log M_w = 11.188 - 0.403\ t_R$ (t_R = column retention
129 time at peak maximum, and $r^2 = 0.999$).
130 Polysaccharide contents were estimated using calibration curves constructed from the
131 pullulan standards P-10, P-50, P-100 and P-200, which were chosen because their peaks
132 properly matched with those obtained for the commercial samples.
133 2.3.2. Analyses in wines
134 Oenological parameters were evaluated following the OIV official analysis methods
135 (OIV, 1990).
136 The content of phenolic compounds was evaluated by quantification of several phenolic
137 families: total polyphenols, total anthocyanins, catechins, total tannins, tartaric esters of
138 phenolic acids, flavonols, and polymeric anthocyanins (Del Barrio-Galán, Pérez-
139 Magariño & Ortega-Heras, 2011).

The content of individual anthocyanins and their derivatives were determined by direct injection of the wines previously filtrated through PVDF filters of 0.45 μm (Millipore, Bedford, MA) in a chromatograph Agilent-Tecnologies LC-DAD 1100, following the method described by Pérez-Magariño, Ortega-Heras, Cano-Mozo, & González-Sanjosé (2009). The compounds identified in this study were grouped as it is indicated in Sánchez-Iglesias, González-Sanjosé, Pérez-Magariño, Ortega-Heras, & González-Huerta (2009).

The color of wines was evaluated using the Glories parameters (Glories, 1984).

Acid and total polysaccharides were quantified by the colorimetric method described by Segarra, Lao, López-Tamames, & De La Torre-Boronat (1995). Neutral polysaccharides were calculated as the difference between total and acid polysaccharides.

Proteins were determined using the method described by Bradford (1976).

All spectrophotometric measurements were carried out in a UV-vis spectrophotometer (Shimadzu series UV-1700 pharmaspec, China).

2.4. Sensory analysis

The sensory analysis was carried out by a tasting panel made up of twelve persons, all of them expert tasters from the Regulatory Councils of different Spanish D.O. and wineries. These tasters defined the descriptors used in this sensory analysis, according to the methodology described in González-Sanjosé, Ortega-Heras, & Pérez-Magariño (2008), and were trained to quantify them using structured numerical scales. This training was carried out in accordance with UNE-87-020-93 Norm (ISO 4121:1987).

A structured numerical scale of seven points was used, with 1 representing absence of sensation and 7 a very high intense perception. All wines were tasted after the treatment.

2.5. Statistical analyses

All the data were treated applying the variance analysis (ANOVA), and the Least Significant Difference test. Confidence intervals of 95% or significant level of $\alpha = 0.05$ were used. All the statistical analyses were carried out using the Statgraphics Plus 5.0 statistical package.

3. Results and discussion

3.1. Monosaccharide and polysaccharide contents in the commercial yeast products

Table 2 shows the monosaccharide composition of the commercial products evaluated. The YD-1, YD-3, and YD-4 showed very similar monosaccharide compositions. The proportion of mannoproteins in these yeast preparations, estimated directly from their proportion of mannose, was 41%-43%. The percentage of glucose, used to estimate the glucan content, was about 60%, which indicates that during the process to obtain these products more glucans are extracted than mannoproteins. In the case of YD-2, the glucan/mannoprotein relationship was higher (65% vs. 34%). On the other hand, the mannoprotein content in YD-5 and YD-6W was much higher than that of glucan (72% vs. 28% and 44% vs. 25%, respectively). Finally, it is important to note that the YD-6W and YD-6R products showed a high percentage of other monosaccharides, mainly galactose, which are not constituents of parietal polysaccharides from yeasts. This could indicate the presence of some polysaccharide or other glycoside compounds that do not come from yeast. It should be pointed out that both products were provided by the same supplier.

Table 2 also shows the polysaccharide purity of the commercial products evaluated. This purity was expressed as the total amount of monosaccharides in relation to the weight of the product analyzed. It is interesting to point out that only two products (YD-3 and YD-6R) showed a purity above 80%.

189 The percentage of different molecular weights of polysaccharide fractions was
190 estimated using HRSEC-RID (**Table 2**). With the exception of YD-2, all the products
191 showed a content of high molecular weight polysaccharides significantly higher than
192 that of low molecular weight polysaccharides. In contrast, in both YD-2 and YD-3 the
193 percentage of low molecular weight polysaccharides was similar to or even higher than
194 that of larger polysaccharides. This is in good agreement with the commercial
195 description as both products were extracted enzymatically from the selected yeast walls.
196 3.2. White wines
197 3.2.1. Enological parameters
198 Enological parameters were analyzed in white wines to study the effect of the different
199 techniques assayed on these compounds. The data ranges of these parameters were: pH
200 between 3.2-3.3, total acidity between 6.1-6.2 g/L of tartaric acid, alcoholic degree
201 between 11.8-12.3, volatile acidity average of 0.18 g/L of acetic acid and potassium
202 between 590-630 mg/L. No statistically significant differences were found between the
203 treated wines and the control wines, which indicate that the commercial yeast
204 preparations used did not have an effect on the enological characteristics of wines.
205 3.2.2. Analysis of phenolic compounds
206 **Table 3** shows the content of some phenolic families analyzed in white wines.
207 Statistically significant differences were only found in some cases. Only YD-4 and YD-
208 5 wines showed a lower concentration of total polyphenols, tartaric esters of phenolic
209 acids, and flavonols than control wines and the other treated wines at the end of
210 treatment (0 MB). However, the analysis of the tannins did not show any statistically
211 significant differences between treated wines and control wines at the end of treatment.
212 After three months in bottle, the wines treated with yeast derivatives presented higher
213 concentrations of total polyphenols than control wines that are probable due to the

mannoproteins can prevent the phenolic precipitation. On the other hand, wines treated with YD-4 and YD-5 showed a significantly lower concentration of tannins, tartaric esters of phenolic acids, and flavonols than control wines. These results are probably due to the adsorption of some polyphenols on the yeast cell walls (Razmkhab et al., 2002; Márquez, Millán, Souquet, & Salmon, 2009) or to the interaction of some polyphenols with the compounds released to the wine, such as mannoproteins and glucans from yeast derivative products (Riou et al., 2002; Poncet-Legrand et al., 2007). This interaction depends on the type of phenols. In addition, the decrease in these compounds also seems to depend on the type of yeast preparations, the high molecular weight polysaccharides being responsible for this interaction **(Table 2)**. The effect of yeast derivative products was also observed in the color of white wines **(Table 3)**. The YD-4 and YD-5 preparations with 100% of high molecular weight polysaccharides produced a greater decrease in wine color after 3 months in bottle. These results agree with those obtained by Razmkhab et al. (2002), who proposed using yeast cell walls as fining agents for the correction of browning in white wines.

3.2.3. Analysis of proteins and polysaccharides

As expected, at the end of the treatment, the wines treated with commercial yeast derivative products presented statistically significant higher protein concentrations than control wines **(Table 3)**, except for the wines treated with YD-2, which showed a similar content to the control wines. The wines treated with YD-4, YD-5, and YD-6 products showed the highest content. However, after three months in bottle only the wines treated with YD-5 showed statistically significant higher concentration of proteins than the control wines. These results suggest that at the beginning of the treatment the commercial yeast derivatives obtained from autolyzed yeasts or polysaccharides extracted from the yeast cell wall (YD-1, YD-2, and YD-3) release to

239 wine a lower amount of protein compounds than the other commercial yeast derivatives
240 (YD-4, YD-5, and YD-6) that are theoretically products with higher cell wall
241 degradation.
242 Polysaccharide concentrations in the wines were also evaluated **(Table 3)**. A significant
243 increase in total and neutral polysaccharides in all white wines treated with the
244 commercial yeast derivatives was found at the end of treatment and after three months
245 in bottle. This increase depended on the commercial yeast product used; statistically
246 significant differences were observed among the different treatments. The wines treated
247 with YD-1 and YD-4 showed the lowest concentrations of neutral and total
248 polysaccharides. However, it was also observed that total and neutral polysaccharides
249 increased during the bottle aging in all the white wines studied, even in the control
250 wines. This increase was more important in wines treated with YD-2 and YD-3 than in
251 the other treated wines showing the highest content after three months in bottle. In
252 addition, the wines treated with the yeast preparation with the highest mannose content
253 (YD-5) showed the highest concentration of neutral polysaccharides after treatment,
254 while only an 8% increase was observed during bottle aging. These results suggest that
255 the addition of commercial yeast products does not produce an immediate release of
256 these compounds and that this release continues during wine aging. This is probably due
257 to the presence of endogenous β-glucanase enzymes in the wines, either released from
258 the yeast added to carry out the alcoholic fermentation or present in the commercial
259 products. These enzymes are active and continue working over time, allowing for the
260 release of neutral polysaccharides from more complex soluble compounds or from the
261 autolyzed yeast and/or cell wall extracts added. Consequently, the purer the yeast
262 preparations and the higher their mannose content, the higher the amount of neutral
263 polysaccharides released to wine.

As expected, the concentration of acid polysaccharides was more or less stable in all wines, although slight differences were found among the treatments.

3.2.4. Sensory analysis

Some differences were found in the color parameters between the treated wines and control wines at the end of treatment, although they were not statistically significant. All treated wines showed higher values of color intensity and yellow tones and lower green tones than control wines **(Figure 1A)**.

In the olfactory phase **(Figure 1A)**, all treated wines showed less olfactory intensity than control wines, but no statistically significant differences were found. However, the tasters found less fruity aromas in all the wines treated with commercial yeast derivatives than in control wines. This was probably due to the interaction of the aromatic compounds with some compounds released from commercial yeast derivatives, such as glucans and mannoproteins, which can produce a decrease in the volatility of these aromatic compounds but that improve the aromatic perception over time. These interactions have been observed by other authors in model wine solutions (Voilley, Beghin, Charpentier, & Peyron, 1991; Chalier et al., 2007) and in red wines (Rodríguez-Bencomo, Ortega-Heras, & Pérez-Magariño, 2010). On the other hand, the tasters found more exotic fruity notes in treated wines than in control wines, especially in YD-1 and YD-2 wines.

In the gustative phase **(Figure 1B)**, all treated wines showed less acidity than control wines. However, the tasters found no statistically significant differences in balance and overall scores between wines.

3.3. Red wines

3.3.1. Enological parameters

The data ranges of the enological parameters were: pH between 3.5-3.6, total acidity between 4.8-5.1 g/L of tartaric acid, alcoholic degree between 12.4-12.7, volatile acidity average of 0.40 g/L of acetic acid and potassium between 1100-1200 mg/L. As in white wines, no statistically significant differences between the treated and control wines were found in the enological parameters. Other studies published on the use of different commercial products rich in mannoproteins showed that applying them did not affect these parameters either (Guadalupe et al., 2007; Guadalupe et al., 2010).

3.3.2. Analyses of phenolic compounds

Total polyphenol content, tannins, tartaric esters of phenolic acids, and flavonols showed similar or higher concentrations in treated wines than in control wines **(Table 4)**. The wines treated with YD-2, YD-3, YD-5, and YD-6 were richer in total polyphenols, tannins and catechins than the control wines. Commercial yeast preparations can not release this type of compounds; therefore the higher presence of these compounds in the wines treated with these yeast derivatives could indicate that their use could prevent the precipitation and loss of polyphenols, tannins and catechins. On the other hand, some of the yeast preparations (such as YD-1, YD-4, and YD-5 after treatment and YD-2, YD-4, and YD-6 after three months in bottle) reduced anthocyanin content. These results agree with those described by some authors, who found adsorption of this type of compounds in the yeast (Guadalupe et al., 2007; Mazauric & Salmon, 2005; Mazauric & Salmon, 2006; Lizama, Rodríguez, Álvarez, García, & Aleixandre, 2006). However, this could be also due to the fact that compounds released from the yeast preparations such as mannoproteins can interact with tannins and anthocyanins, preventing their aggregation and precipitation and then contributing to maintaining and stabilizing the color in red wines (De Freitas, Carvalho, & Mateus, 2003). The polymeric anthocyanin results **(Table 4)** confirm this hypothesis, since the

313 wines treated with YD-2, YD-4, and YD-5 showed higher percentages of these
314 compounds than control wines, and they showed lower content of total anthocyanins.
315 Only wines treated with YD-1 presented lower total anthocyanin and lower polymeric
316 anthocyanin levels than control wines, which can indicate that this yeast preparation
317 really produced a reduction of monomeric anthocyanins by adsorption.
318 Just after treatment, the detailed analysis of the monomeric anthocyanins only showed
319 statistically significant differences between the different treatments for the cinnamic
320 anthocyanins. However, higher differences were found between treatments after bottle
321 aging. In general, the wines treated with YD-1, YD-2, YD-4, and YD-5 showed lower
322 concentrations of monomeric anthocyanins than the control wines with the exception of
323 YD-2 and YD-5 wines that showed lower concentration of cinnamic anthocyanins.
324 These results agree with those found for the total anthocyanins. In addition, the wines
325 treated with YD-1, YD-2, YD-4, and YD-5 presented higher values of new anthocyanin
326 pigment content than the control wines **(Table 4)**; these compounds are more stable and
327 are partially responsible for wine color stability. The wines treated with these yeast
328 preparations also showed the highest color intensity values both after treatment and
329 after bottle aging. These results are well correlated with the higher percentage of
330 polymeric anthocyanins obtained in these wines. They suggest that these yeast
331 preparations favored the formation of new polymeric pigments, which are more stable
332 and resistant to pH changes and oxidation reactions (Asenstorfer, Hayasaka, & Jones,
333 2001) and, thus, contribute to color stability. It can consequently be said that only some
334 of the commercial yeast derivative products used seem to have a positive effect on color
335 stability, probably due to their different composition. The positive effects of
336 mannoproteins and other polysaccharides on color stability have been reported by some
337 authors (Escot et al., 2001; Francois et al., 2007). However, some recent studies did not

find an improvement of wine color intensity and color stability using mannoproteins, in some cases, they even found a loss of color in the wines analyzed (Guadalupe & Ayestarán, 2008; Guadalupe et al., 2010).

3.3.3. Analysis of proteins and polysaccharides

After bottle aging the wines treated with YD-2, YD-5, and YD-6 had higher protein content than the control wines and the remaining treated wines **(Table 4)**.

At the end of treatment, all treated wines showed higher concentrations of neutral polysaccharides than the control wines. The wines treated with YD-5 presented the highest concentration of these compounds, while those treated with YD-1 showed the lowest **(Table 4)**. After bottle aging, all treated wines also showed higher neutral polysaccharide content than control wines, with the only exception of wines treated with YD-2. The wines treated with YD-4, YD-5, and YD-6 showed the highest concentration and those treated with YD-2, the lowest. These results agree with those obtained by other authors (Guadalupe et al., 2007; Guadalupe & Ayestarán, 2008), who pointed out that the addition of commercial mannoprotein products to red wines before alcoholic fermentation increased or remained constant the concentration of neutral (mannoproteins) and total polysaccharides during the barrel and bottle aging. It can therefore be said that all yeast derivatives release neutral polysaccharides, but in different amounts and probably with different composition of polysaccharides. This could produce different effects on the sensorial characteristics and the quality of wines.

3.3.4. Sensory analysis

In red wines, the sensory analysis showed smaller differences than in white wines. No statistically significant differences were found in color between the treated wines and the control wines just after treatment **(Figure 2A)**.

In the olfactory phase **(Figure 2A)**, all wines treated with the commercial yeast derivatives presented lower olfactory intensity values than the control wines. However, no statistically significant differences were found for any of the olfactory attributes studied.

In the gustative phase **(Figure 2B)**, statistically significant differences were only found in green tannin values, which were lower in all treated wines than in control wines. This type of tannins produces negative sensations including intense astringent and acid sensations with strong green or herbaceous notes. Consequently, these results can indicate that adding yeast derivatives can reduce aggressive green tannins of red wines, probably due to the interactions between these products and the tannins, increasing roundness and softness on the palate (Escot et al., 2001; Riou et al., 2002; Guadalupe et al., 2007; Poncet-Legrand et al., 2007). The wines treated with YD-4, YD-5, and YD-6 presented the lowest green tannin values, which coincides with their greater overall rating values.

4. Conclusion

In general, the use of commercial dry yeast preparations improves some sensorial characteristics of white and red wines, probably due to the increase of neutral polysaccharides. In white wines, some dry yeast preparations reduced acidity and the absorbance values at 420 nm, while in red wines, dry yeast preparations mainly reduced green tannins increasing the softness on the palate. Therefore, they could be useful especially in young wines that are more astringent and/or acid in order to improve the roundness and softness in mouth. However, all the commercial yeast products did not produce these positive effects that can be due to their different purity and composition.

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References

Asenstorfer, R. E., Hayasaka, Y., & Jones, G. P. (2001). Isolation and structures of oligomeric wine pigments by bisulfite-mediated ionexchanges chromatography. *Journal of Agricultural and Food Chemistry, 49*, 5957-5963.

Ayestarán, B., Guadalupe, Z., & León, D. (2004). Quantification of major grape polysaccharides (Tempranillo v.) released by maceration enzymes during the fermentation process. *Analytica Chimica Acta*, *513*(1), 29-39.

Bautista, R., Fernández, E., & Falqué, E. (2007). Effect of the contact with fermentation-lees or commercial-lees on the volatile composition of white wines. *European Food Research and Technology*, *224*, 405–413.

Bradford, M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248-254.

Chalier P., Angot, B., Delteil, D., Doco, T., & Gunata, Z. (2007). Interactions between aroma compounds and whole mannoprotein isolated from Saccharomyces cerevisiae strains. *Food Chemistry*, *100*, 22-30.

De Freitas, V., Carvalho, E., & Mateus, N. (2003). Study of carbohydrate influence on protein-tannin aggregation by nephelometry. *Food Chemistry*, *81*(4), 503-509.

409 Del-Barrio-Galán, R., Pérez-Magariño, S., & Ortega-Heras, M. (2011). Techniques for
410 improving or replacing ageing on lees of oak aged red wines: The effects on
411 polysaccharides and the phenolic composition. *Food Chemistry*, *127*, 528–540.
412 Doco, T., Brillouet, J. M., & Moutounet, M. (1996). Evolution of grape (Carignan noir
413 cv) and yeast polysaccharides during fermentation and post-maceration. *American*
414 *Journal of Enology and Viticulture*, *47*(1), 108-110.
415 Doco, T., Vuchot, P., Cheynier, V., & Moutounet, M. (2003). Structural modification of
416 wine arabinogalactans during aging on lees. *American Journal of Enology and*
417 *Viticulture*, *54*, 150-157.
418 Dufour, C., & Bayonoue, C. L. (1999). Influence of wine structurally different
419 polysaccharides on the volatility of aroma substances in a model solution. *Journal of*
420 *Agricultural and Food Chemistry*, *47*, 671-677.
421 Dupin, I. V. S., McKinnon, B. M., Ryan, C., Boulay, M., Markides, A. J., Jones, G. P.,
422 Williams, P. J., & Waters, E. J. (2000). Saccharomyces cerevisiae mannoproteins
423 that protect wine from protein haze: their release during fermentation and lees
424 contact and a proposal for their mechanism of action. *Journal of Agricultural and*
425 *Food Chemistry*, *48*, 3098–3105.
426 Escot, S., Feuillat, M., Dulau, L., & Charpentier, C. (2001). Release of polysaccharides
427 by yeast and the influence of released polysaccharides on color stability and wine
428 astringency. *Australian Journal of Grape and Wine Research*, *7*, 153-159.
429 Feuillat, M. (2003). Yeast macromolecules: origin, composition, and enological interest.
430 *American Journal of Enology and Viticulture*, *54*(3), 211-213.
431 Fournairon, C., Camarasa, C., Moutounet, M., & Salmon, J. M. (2002). New trends on
432 yeast autolysis and wine ageing on lees. *Journal International de la Vigne et du Vin*,
433 *36*, 49-69.

Francois, J. M., Alexandre, H., Granes, D., & Feuillat, M. (2007). Vers une meilleure connaissance des produits dérivés de levures. *La Revue des Oenologues*, *122*, 9–12.

Glories, Y. (1984). La couleur des vins rouges. 2éme partie. Mesure, origine et interprétation. *Connaissance de la Vigne et du Vin*, *18*, 253–271.

Gonzalez-Ramos, D., Cebollero, E., & González, R. (2008). Recombinant *Saccharomyces cerevisiae* strain overproducing mannoproteins stabilizes wine against protein haze. *Applied and Environmental Microbiology*, *74*, 5533-5540.

González-Sanjosé, M. L., Ortega-Heras, M., & Pérez-Magariño, S. (2008). Micro-oxygenation treatment and sensory properties of young red wines. *Food Science and Technology International*, *14*(5), 123-130.

Guadalupe, Z., & Ayestarán, B. (2008). Effect of commercial mannoprotein addition on polysaccharide, polyphenolic, and color composition in red wines. *Journal of Agricultural and Food Chemistry*, *56*, 9022-9029.

Guadalupe, Z., Martínez-Pinilla, O., Garrido, A., Carrillo, J.D., & Ayestarán, B. (2012). Quantitative determination of wine polysaccharides by gas chromatography-mass spectrometry (GC-MS) and size exclusion chromatography (SEC). *Food Chemistry*, *131*, 367-374.

Guadalupe, Z., Martínez, L., & Ayestarán, B. (2010). Yeast Mannoproteins in red winemaking: Effect on polysaccharide, polyphenolic, and color composition. *American Journal of Enology and Viticulture*, *61*(2), 191-200.

Guadalupe, Z., Palacios, A., & Ayestarán, B. (2007). Maceration enzymes and mannoproteins: A possible strategy to increase colloidal stability and color extraction in red wines. *Journal of Agricultural and Food Chemistry*, *55*, 4854-4862.

Lizama, V., Rodríguez, M. L., Álvarez, I., García, M. J., & Aleixandre, J. L. (2006). Influencia de la adición de manoproteínas sobre la estabilidad de vinos tintos de

variedades Monastrell y Tempranillo. In 29º Congreso mundial de la viña y el vino, Logroño (Spain).

Lomolino, G., & Curioni, A. (2007). Protein haze formation in white wines: effect of *Saccharomyces cerevisiae* cell wall components prepared with different procedures. *Journal of Agricultural and Food Chemistry*, *55*, 8737–8744.

Lubbers, S., Charpentier, C., Feuillat, M., & Voilley, A. (1994). Influence of yeast cell walls on the behaviour of aroma compounds in a model wine. *American Journal of Enology and Viticulture*, *45*, 29-33.

Lubbers, S., Leger, B., Charpentier, C., & Feuillat, M. (1993). Effect Colloide-protecteur extracts de parois de levures sur la stabilite tartrique d'une solution hydro-alcoholique modele. *Journal International des Sciences de la Vigne et du Vin*, *27*, 13–22.

Márquez, T., Millán, C., Souquet, J. M., & Salmon, J. M. (2009). Effect of different yeast strains and their culture conditions on the prevention of wine model solution browning by yeast lees. *Journal of Agricultural and Food Chemistry*, *57*, 3771–3779.

Mazauric, J. P., & Salmon, J. M. (2005). Interactions between yeast lees and wine polyphenols during simulation of wine aging: I. Analysis of remnant polyphenolic compounds in the resulting wines. *Journal of Agricultural and Food Chemistry*, *53*, 5647-5653.

Mazauric, J. P., & Salmon, J. M. (2006). Interactions between yeast lees and wine polyphenols during simulation of wine aging: II. Analysis of desorbed polyphenol compounds from yeast lees. *Journal of Agricultural and Food Chemistry*, *54*, 3876-3881.

483 Moine-Ledoux, V., & Dubourdieu, D. (1999). An invertase fragment responsible for
484 improving the protein stability of dry white wines. *Journal of the Science of Food*
485 *and Agriculture*, *79*, 537-543.
486 Moine-Ledoux, V., & Dubourdieu, D. (2002). Role yeast mannoproteins with regard to
487 tartaric stabilisation of wines. *Bulletin OIV*, *75*(857,858), 471-482.
488 Office International de la Vigne et du Vin. International Analysis Methods of Wines
489 and Must, OIV: Paris, France, 1990.
490 Pérez-Magariño, S., Ortega-Heras, M., Cano-Mozo, E., & González-Sanjosé, M. L.
491 (2009). The influence of oak wood chips, micro-oxygenation treatment, and grape
492 variety on colour, and anthocyanin and phenolic composition of red wines. *Journal*
493 *of Food Composition and Analysis*, *22*, 204–211.
494 Poncet-Legrand, C., Doco, T., Williams, P., & Vernhet, A. (2007). Inhibition of grape
495 seed tannin aggregation by wine mannoproteins: Effect of polysaccharide molecular
496 weight. *American Journal of Enology and Viticulture*, *58*, 87-91.
497 Pozo-Bayón, M. A., Andújar-Ortiz, I., & Moreno-Arribas, M. V. (2009). Scientific
498 evidences beyond the application of inactive dry yeast preparations in winemaking.
499 *Food Research International*, *42*, 754-761.
500 Ramírez, G., Chassagne, D., Feuillat, M., Voilley, A., & Charpentier, C. (2004). Effect
501 of wine constituents on aroma compound sorption by oak wood in a model system.
502 *American Journal of Enology and Viticulture*, *55*, 22-26.
503 Razmkhab, S., López-Toledano, A., Ortega, J. M., Mayen, M., Mérida, J., & Medina,
504 M. (2002). Adsorption of phenolic compounds and browning products in white wines
505 by yeasts and their cell walls**.** *Journal of Agricultural and Food Chemistry*, *50*, 7432-
506 7437.

507 Riou, V., Vernhet, A., Doco, T., & Moutounet, M. (2002). Aggregation of grape seed
508 tannins in model wine-effect of wine polysaccharides. *Food Hydrocolloids*, *16*, 17-
509 23.
510 Rodríguez-Bencomo, J. J., Ortega-Heras, M.,& Pérez-Magariño, S. (2010). Effect of
511 alternative techniques to ageing on lees and use of non-toasted oak chips in alcoholic
512 fermentation on the aromatic composition of a red wine. *European Food Research*
513 *and Technology*, *230*, 485-496.
514 Rosi, I., Gheri, A., Domizio, P., &Fia, G. (2000). Production de macromolécules
515 pariétales de *Saccharomyces cerevisiae* au cours de la fermentation et leur influence
516 sur la fermentation malolactique. *La Revue des Oenologues*, *94*, 18–20.
517 Sánchez-Iglesias, M., González-Sanjose, M. L., Pérez-Magariño, S., Ortega-Heras, M.,
518 & González-Huerta, C. (2009). Effect of micro-Oxygenation and wood type on the
519 phenolic composition and color of an aged red wine. *Journal of Agricultural and*
520 *Food Chemistry*, *57*, 11498–11509.
521 Schmidt, S. A., Tan, E. L., Brown, S., Nasution, U. L., Pettolino, F., MacIntyre, O. J.,
522 de Barros Lopes, M., Waters, E. J., & Anderson, P.A. (2009). Hpf2 glycan structure
523 is critical for protection against protein haze formation in white wine. *Journal of*
524 *Agricultural and Food Chemistry*, *57*, 3308–3315.
525 Segarra, I., Lao, C., López-Tamames, E., & De La Torre-Boronat, M. C. (1995).
526 Spectrophotometric methods for the analysis of polysaccharide levels in winemaking
527 products. *American Journal of Enology and Viticulture*, *4*(46), 564-570.
528 Vidal, S., Courcoux, P., Francis, L., Kwiatkowski, M., Gawel,R., Williams, P., Waters,
529 E., & Cheynier, V. (2004). Use of an experimental design approach for evaluation of
530 key wine components on mouth-feel perception. *Food Quality and Preference*, *15*,
531 209-217.

532 Vidal, S., Williams, P., Doco, T., Moutounet, M., & Pellerin, P. (2003). The
533 polysaccharides of red wine: total fractionation and characterization. *Carbohydrate*
534 *Polymers*, *54*, 439-447.
535 Voilley, A., Beghin, V., Charpentier, C., & Peyron, D. (1991). Interaction between
536 aroma substances and macromolecules in a model wine. *LWT-Food Science and*
537 *Technology*, *24*, 469-472.
538 Waters, E., Dupin, I., & Stockdale, V. (2000). A review of current knowledge on
539 polysaccharides which 'protect' against protein haze in white wine. *Australian*
540 *Grapegrower and Winemaker*, *438*(13), 15-16.
541

FIGURE CAPTIONS

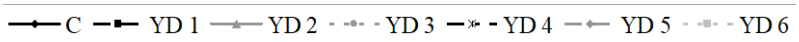
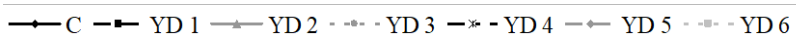
Figure 1. Sensory diagrams of color and olfactory phase (A) and gustative phase (B) in white wines at the end of treatment. The asterisk indicates statistically significant differences for $\alpha < 0.05$. 

Figure 2. Sensory diagrams of color and olfactory phase (A) and gustative phase (B) in red wines at the end of treatment and malolactic fermentation. The asterisk indicates statistically significant differences for $\alpha < 0.05$.



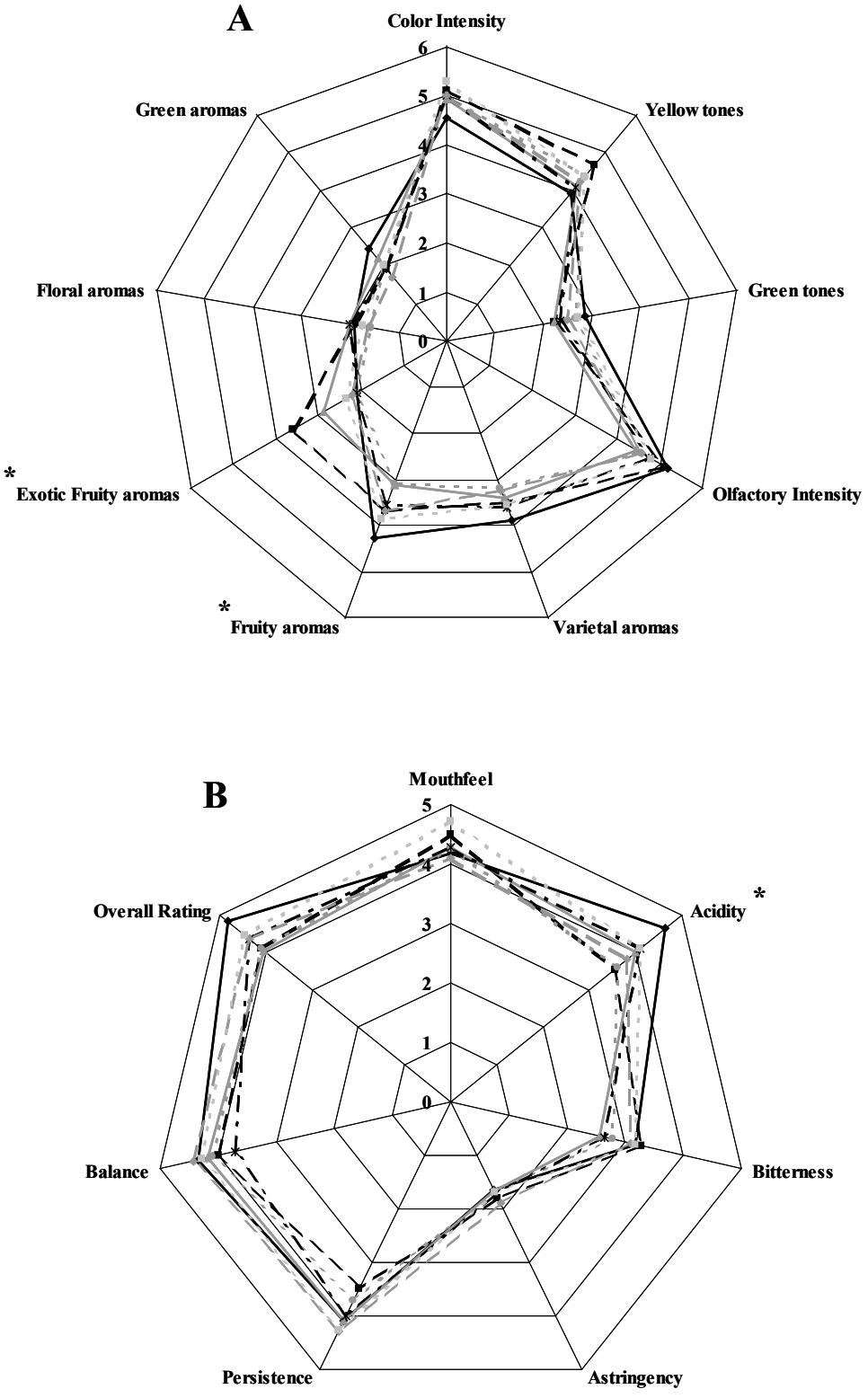

A
Color Intensity
6
5
4
3
2
1
0
Yellow tones
Green tones
Olfactory Intensity
Varietal aromas
Fruity aromas
*
Exotic Fruity aromas
*
Floral aromas
Green aromas
B
Mouthfeel
5
4
3
2
1
0
Acidity
*
Bitterness
Astringency
Persistence
Balance
Overall Rating

Figure 1

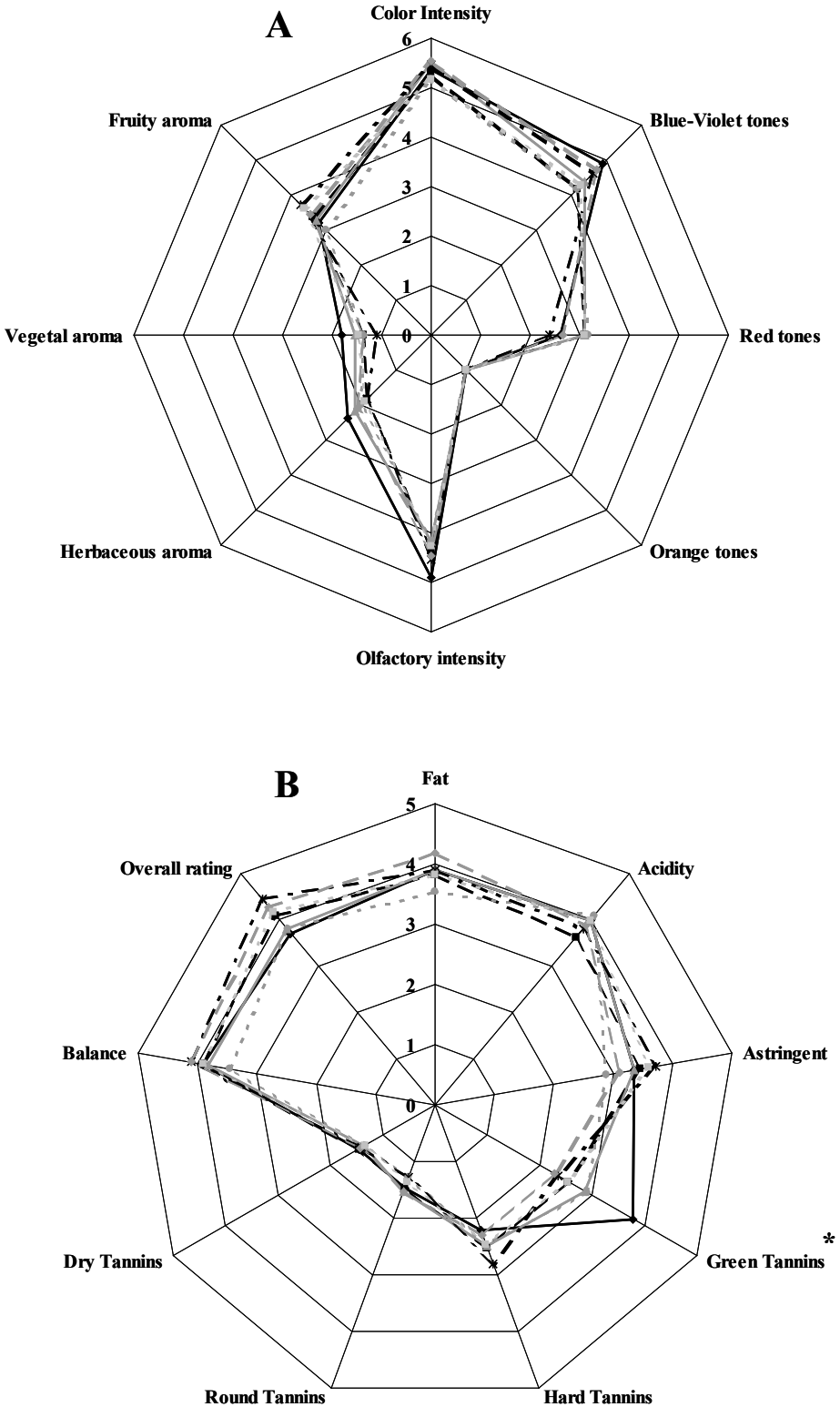

A
Color Intensity
6
5
4
3
2
1
0
Blue-Violet tones
Red tones
Orange tones
Olfactory intensity
Herbaceous aroma
Vegetal aroma
Fruity aroma
B
Fat
5
4
3
2
1
0
Acidity
Astringent
Green Tannins
*
Hard Tannins
Round Tannins
Dry Tannins
Balance
Overall rating

Figure 2

Table 1. Commercial yeast derivative composition and characteristics.

Yeast derivative	Comercial supplier	Composition and characteristics
YD-1	Agrovin	Product with autolysed yeast enriched in polysaccharides.
YD-2	Agrovin	Product with autolysed yeast enriched in polysaccharides and with β-glucanase activity.
YD-3	Sepsa	Product with polysaccharides extracted enzymatically of selected yeast walls.
YD-4	Laffort	Contain a peptide fraction found in the yeast which has sweeter power.
YD-5	Bio Springer	Constituted exclusively for polysaccharides from the yeast cell wall. It contains 25 % of free highly soluble mannoproteins.
[a]YD-6W	AEB	Product with yeast cellular walls rich in mannoproteins and nucleotides.
[b]YD-6R	AEB	Product with yeast cellular walls rich in mannoproteins and nucleotides. Mannoproteins with a medium molecular weight.

[a] Yeast derivative product used in white wines, [b] yeast derivative product used in red wines

Table 2. Monosaccharide composition, percentage of polysaccharide purity and percentage of different molecular weights of polysaccharide fractions estimated using high-resolution size-exclusion chromatography (HRSEC) (% ± sd) of the different commercial products[a].

Monosaccharides	Commercial products						
	YD1	**YD2**	**YD3**	**YD4**	**YD5**	**YD6R**	**YD6W**
Apiose	nd[c]	nd	nd	nd	nd	nd	1.08±0.13
Arabinose	0.34±0.05a	0.28±0.25a	nd	nd	nd	3.7±0.60b	0.88±0.80a
Rhamnose	nd	nd	nd	nd	nd	0.72±0.34a	2.9±0.50b
Xylose	0.15±0.05a	0.33±0.29a	nd	nd	nd	0.25±0.22a	0.29±0.25a
Mannose	41.5±3.6a	34.4±9.6a	42.9±3.9a	40.4±5.4a	72.4±12.5b	33.7±3.6a	43.8±7.7a
Dha[b]	nd	nd	nd	nd	nd	nd	nd
Galactose	0.20±0.34a	0.28±0.30a	nd	nd	nd	11.5±4.8b	11.5±2.9b
Gal. Acid[b]	nd	nd	nd	nd	nd	1.4±0.86a	3.0±0.10b
Glucose	57.8±6.1ab	64.7±7.6b	57.1±6.0ab	59.6±6.9ab	27.6±5.6c	47.9±2.5a	25.5±3.2c
Gluc. Acid[b]	nd	nd	nd	nd	nd	nd	10.9±0.22
% polysaccharide purity	58.3±5.7ab	75.9±9.1bc	82.7±10.9c	56.6±7.9ab	42.7±6.3a	98.3±5.7c	54.4±12.2ab
% ∑ (P400-P50)[d]	77.30±0.71d	35.92±2.92a	55.62±0.38b	100.00±4.30e	100.00±0.07e	65.00±2.68c	100.00±2.88e
% P10[d]	22.70±1.02a	64.08±3.30d	44.38±3.62c			35.00±1.33b	

[a] The data shown are the average and standard deviation of three analysis of each product. Values with different letter indicate statistically significant differences at $\alpha < 0.05$.
[b] Dha: 3-deoxy-D-*lyxo*-heptulosaric acid, Gal. Acid: galacturonic acid, Gluc. Acid: glucuronic acid.
[c] nd: no detected (≤0.05%).
[d] Σ(P400-P50): polysaccharides with an average molecular weight between 47.3 kDa and 404 kDa, P10: polysaccharides with an average molecular weight of 11.8 kDa.

Table 3. Total polyphenols (mg/L of gallic acid), tannins (mg/L of cyanidin chloride), tartaric esters of phenolic acids (mg/L of caffeic acid), flavonols (mg/L of quercetin), total, neutral and acid polysaccharides (mg/L), absorbance at 420 nm, and proteins (mg/L of Bovine Serum Albumine) in white wines [a]

				End of treatment			
Compounds	**C**	**YD1**	**YD2**	**YD3**	**YD4**	**YD5**	**YD6**
Total Polyphenols	195bc	194bc	195c	196c	187a	186a	189ab
Tannins	215ab	211ab	215ab	207a	205a	206a	218b
Tartaric esters	35.8c	34.9c	34.6c	34.1bc	32.0a	33.1ab	34.0bc
Flavonols	22.9c	22.3c	21.6bc	21.4bc	19.3a	20.4ab	21.4bc
Total polysaccharides	43.1a	59.9b	75.2d	85.0e	54.9b	89.1e	68.5c
Neutral polysaccharides	32.8a	49.4b	63.8d	69.0d	46.0b	84.9e	56.5c
Acid polysaccharides	11.1bc	11.7bc	13.6d	12.6c	9.3a	8.8a	10.9b
Absorbance at 420 nm	0.570a	0.570a	0.605ab	0.585a	0.590a	0.590a	0.630b
Proteins	52.7a	61.2b	57.0ab	59.3b	72.3c	75.5c	80.6d
				Three months in bottle			
Compounds	**C**	**YD1**	**YD2**	**YD3**	**YD4**	**YD5**	**YD6**
Total Polyphenols	176a	187bc	191c	189c	184b	184b	191c
Tannins	214b	213b	213b	208b	197a	193a	191a
Tartaric esters	36.6d	34.7bc	35.0c	34.9c	33.6a	34.1ab	35.5c
Flavonols	23.2d	21.9b	22.0b	22.1bc	20.8a	21.6b	22.7cd
Total polysaccharides	62.8a	70.9ab	119e	119e	76.9b	111d	99.5c
Neutral polysaccharides	40.0a	58.6b	107e	111e	69.9c	92.0d	88.2d
Acid polysaccharides	11.0bc	10.1b	14.1e	15.5f	8.5a	11.9cd	12.6d
Absorbance at 420 nm	0.606bc	0.620c	0.625c	0.595bc	0.560ab	0.538a	0.615c
Proteins	67.2ab	68.3abc	65.0a	65.4a	72.4bc	73.1c	78.2b

[a] Values with different letter indicate statistically significant differences at $\alpha < 0.05$.

Table 4. Total polyphenols (mg/L of gallic acid), tannins (mg/L of cyanidin chloride), total anthocyanins (mg/L of malvidin-3-glucoside), catechins (mg/L of D-(+)-catechin), tartaric esters of phenolic acids (mg/L of caffeic acid), flavonols (mg/L of quercetin), polymeric anthocyanins (%), glucoside, acetic and cinnamic anthocyanins (mg/L of malvidin-3-glucoside), new pigments (%), color parameters, total, neutral and acid polysaccharides (mg/L), and proteins (mg/L of Bovine Serum Albumine) in red wines [a]

	End of treatment and malolactic fermentation						
Compounds	**C**	**YD1**	**YD2**	**YD3**	**YD4**	**YD5**	**YD6**
Total Polyphenols	2180ab	2143a	2395e	2284d	2201b	2246c	2282d
Tannins	2245ab	2165a	2407c	2320bc	2218a	2383c	2329bc
Total Anthocyanins	586d	540a	583cd	598d	563bc	561b	583d
Catechins	853b	832a	939e	899d	890c	892cd	898d
Tartaric esters	233abc	217ab	233cd	236d	215a	224bc	224bc
Flavonols	134b	120a	141cd	144d	125a	130b	135bc
Polymeric anthocyanins	39.1a	40.7ab	47.4d	41.4ab	43.6bc	45.4cd	40.8a
Glucoside Anthocyanins	269	257	256	273	258	255	272
Acetic Anthocyanins	8.76	8.73	8.26	9.02	8.48	8.44	8.99
Cinnamic Anthocyanins	23.1c	21.4a	23.0c	24.7d	22.5b	25.5e	24.8d
New pigments	1.79ab	1.60a	2.23d	1.86b	1.96bc	2.09cd	1.89b
Color intensity	8.94a	8.83a	11.43c	9.77b	10.26b	10.99c	9.81b
% Blue	10.9b	10.5a	11.0bc	11.0bc	10.9b	11.1c	10.9b
% Red	54.7abc	54.5a	55.1abc	54.8ab	55.8d	55.3c	55.1bc
Total polysaccharides	469a	470a	533c	507bc	497ab	610e	580d
Neutral polysaccharides	310a	353b	416d	384c	376bc	482e	439d
Acid polysaccharides	139b	136b	118a	121a	119a	117a	137b
Proteins	1232ab	1232a	1556c	1293ab	1357ab	1395bc	1311ab

[a] Values with different letter indicate statistically significant differences at $\alpha < 0.05$.

Table 4 (continued). Total polyphenols (mg/L of gallic acid), tannins (mg/L of cyanidin chloride), total anthocyanins (mg/L of malvidin-3-glucoside), catechins (mg/L of D-(+)-catechin), tartaric esters of phenolic acids (mg/L of caffeic acid), flavonols (mg/L of quercetin), polymeric anthocyanins (%), glucoside, acetic and cinnamic anthocyanins (mg/L of malvidin-3-glucoside), new pigments (%), color parameters, total, neutral and acid polysaccharides (mg/L), and proteins (mg/L of Bovine Serum Albumine) in red wines [a]

	Three months in bottle						
Compounds	**C**	**YD1**	**YD2**	**YD3**	**YD4**	**YD5**	**YD6**
Total Polyphenols	2178ab	2070a	2267b	2248b	2189b	2265b	2265b
Tannins	2205bc	2040a	2301c	2233bc	2151ab	2238bc	2266c
Total Anthocyanins	557d	509bcd	476ab	541cd	529cd	463a	511bc
Catechins	857abc	822a	935e	897de	862b	895cd	889bcd
Tartaric esters	219ab	215a	239c	225b	214a	235c	234c
Flavonols	118a	125ab	141c	131b	123a	139c	139c
Polymeric anthocyanins	43.8a	46.9abc	49.9bcd	47.0ab	50.3cd	51.0d	47.3abc
Glucoside Anthocyanins	237c	208ab	217abc	229c	209ab	203a	222bc
Acetic Anthocyanins	7.72d	7.05bc	7.10bc	7.59d	6.89b	6.52a	7.29cd
Cinnamic Anthocyanins	20.7c	16.8a	19.9c	20.5c	17.6b	20.5c	20.0c
New pigments	2.05a	2.42bc	2.71c	2.20ab	2.68c	2.54c	2.14a
Color intensity	8.60a	8.86a	10.52c	9.51ab	9.91bc	10.62c	9.67bc
% Blue	11.1ab	10.8a	11.3b	11.3b	11.4b	11.5b	11.2ab
% Red	53.7ab	53.9ab	53.9ab	53.7a	54.3b	54.0ab	53.9ab
Total polysaccharides	475a	515ab	521b	571c	618d	647e	621d
Neutral polysaccharides	315a	361b	355ab	418c	486d	485d	470d
Acid polysaccharides	154bc	159c	155bc	150b	129a	162c	149b
Proteins	998ab	935a	1282e	1051bc	958a	1151d	1087c

[a] Values with different letter indicate statistically significant differences at $\alpha < 0.05$.