Research Article

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Effect of selected *Saccharomyces cerevisiae* yeast strains and different aging techniques on the polysaccharide and polyphenolic composition and sensorial characteristics of Cabernet Sauvignon red wines

Rubén del Barrio-Galán,* Alejandro Cáceres-Mella, Marcela Medel-Marabolí and Álvaro Peña-Neira

Abstract

BACKGROUND: The objective of this work was to study the effect of two *Saccharomyces cerevisiae* yeast strains with different capabilities of polysaccharide liberation during alcoholic fermentation in addition to subsequent aging on lees with or without oak wood chips as well as aging with commercial inactive dry yeast on the physical, chemical and sensorial characteristics of Cabernet Sauvignon red wines.

RESULTS: The HPS (high levels of polysaccharides) yeast strain released higher amounts of polysaccharides (429 g L^{-1}) than EC1118 (390 g L^{-1}) during alcoholic fermentation, but the concentration equalized during the aging period (424 and 417 g L^{-1} respectively). All aging techniques increased the polysaccharide concentration, but the increase was dependent on the technique applied. A higher liberation of polysaccharides reduced the concentration of most of the phenolic families analyzed. Moreover, no clear effect of the different aging techniques used in this study on color stabilization was found. The HPS wines were better valued than the EC1118 wines by the panel of tasters after alcoholic fermentation.

CONCLUSION: In general, the HPS wines showed better physicochemical and sensorial characteristics than the EC1118 wines. According to the results obtained during the aging period, all aging techniques contributed to improve wine quality, but it was difficult to establish the technique that allowed the best wine to be obtained, because it depended on the aging technique used and the period of aging.

Keywords: polysaccharides; phenolic compounds; red wines; *Saccharomyces cerevisiae* yeast strains; sensory quality

INTRODUCTION

Recently, several scientific studies have focused on testing the important role that yeast cell wall polysaccharides from *Saccharomyces cerevisiae* play in the technological and sensorial characteristics of red wines. Certain studies assert that these compounds can reduce wine astringency and bitterness,[1–4] improve color stability[5–7] and enhance mouth-feel and persistence.[1,2,4,8,9] According to these studies, the most important polysaccharides of enological interest are mannoproteins, which are liberated into wines during alcoholic fermentation[10] and thereafter during yeast autolysis.[11,12] For this reason, several vinification yeast strains of *S. cerevisiae* have been commercialized by manufacturers in the last few years with the objective of releasing greater amounts of mannoproteins into wines during alcoholic fermentation.

Alternatively, for several years, winemakers have used aging on lees in white wines after alcoholic fermentation to continue the liberation of polysaccharides by autolytic processes in order to improve wine complexity. However, this technique was most recently used in red wines. The release of polysaccharides during the aging of wines on lees is too slow owing to the required temperature and pH conditions, which are not the most suitable for this process.[13] Therefore a variety of commercial products rich in yeast cell wall polysaccharides from *S. cerevisiae* have recently been developed and supplied by manufacturers to provide the same benefits as the aging of wines on lees but in a shorter period of time.[1,8] Thus wineries can put the final wines on the market in a shorter period of time but with similar quality to the wines aged on lees. In terms of structure and chemical composition, these yeast products are very heterogeneous, which is mainly due to

* *Correspondence to: Rubén del Barrio-Galán; Department of Agro-Industry and Enology, Faculty of Agronomical Sciences, University of Chile, Post Office Box 1004, Santiago, Chile. E-mail: rdelbarriogalan@gmail.com*

Department of Agro-Industry and Enology, Faculty of Agronomical Sciences, University of Chile, Post Office Box 1004, Santiago, Chile

the different inactivation (thermal or enzymatic), extraction and purification processes used in their production. Moreover, they are classified into four large groups: inactive yeasts, autolyzed yeasts, yeast walls and yeast extracts.[14]

It is also well known that aging wine in oak barrels imparts numerous benefits but entails a cost that, in some cases and given the actual global wine market, cannot currently be maintained. Therefore the addition of oak chips is used to reduce production costs and to provide greater flexibility and manageability. Additionally, these chips improve the sensory characteristics of wines, such as increasing their sweetness, structure and mouth-feel,[15,16] which is mainly due to the contribution of oak wood polysaccharides and hydrolyzed tannins. In addition, this technique can be combined with aging on lees, because the polysaccharides liberated from yeast lees can pair with oak wood tannins, reducing their astringency and bitterness.[5]

Because very few experimental and scientific trials have been conducted with these techniques, they all raise many doubts and questions for winemakers and technicians about both their use and their final effect on wines. In addition, no scientific studies have been performed that simultaneously compare the capability of different *S. cerevisiae* yeast strains to release large amounts of polysaccharides into wines as well as the subsequent application of the aging techniques mentioned above. Furthermore, the effects of these techniques have not been assessed in any scientific or experimental study on Chilean wines. However, it is important to note that most studies conducted with these techniques used shorter periods of aging.[1,8,17] Therefore it is necessary to apply longer aging periods to evaluate their effect on wines.

Thus the main objective of this work was to study the effect of two *S. cerevisiae* yeast strains with different polysaccharide liberation capabilities during alcoholic fermentation as well as subsequent aging on lees and other alternative practices on the physical, chemical and sensorial characteristics of Cabernet Sauvignon red wines. According to the last report issued by SAG,[18] the total volume of wine produced in 2013 was 12.8 million hL, 34.6% of which was Cabernet Sauvignon. Thus it is important to apply these techniques to the most important variety cultivated for wine production in Chile.

EXPERIMENTAL

Winemaking process and treatments

The study was carried out using Cabernet Sauvignon red grapes supplied by the Caliterra winery (Errazuriz group) located in Colchagua valley, Chile (34.63° S, 71.37° W). The grapes selected for this study (~65 000 kg) were harvested according to their technological maturity, based on optimal sugar content (~24.8 °Brix) and total acidity (~5.5 g L^{-1} H_2SO_4), and then transported to the winery. Once there, the traditional winemaking process for red wines was followed. Briefly, after de-stemming, crushing and subsequent sulfite addition, the must was transferred into two different 25 000 L stainless steel tanks for alcoholic fermentation. One of these tanks was inoculated with a commercial *S. cerevisiae bayanus* yeast strain (30 g hL^{-1} Lalvin EC1118), which is the conventional yeast used by the Caliterra winery. The other tank was inoculated with another commercial *S. cerevisiae* yeast strain (30 g hL^{-1} Uvaferm HPS), which produces high levels of polysaccharides during alcoholic fermentation (20–30% higher than other yeasts), according to the manufacturer's specifications. Both yeast strains were supplied by Lallemand-South America (Santiago, Chile).

The wine fermentation process strictly followed the manufacturing techniques of red wines made from Cabernet Sauvignon in the Caliterra winery. Alcoholic fermentation was carried out at a controlled temperature (21–25 °C); once fermentation was complete, the wines were kept in the tanks for 5 days to allow the sedimentation of gross lees, then they were racked off again. After this process, 600 L of each type of fermented wine was transported to the pilot plant of the Department of Agro-Industry and Enology, Faculty of Agronomical Sciences, University of Chile, Santiago, Chile Once there, the wines were kept in the tanks until malolactic fermentation, which had begun spontaneously in the winery, was complete. After that, the sulfur dioxide level was corrected to 35 μL L^{-1} free SO_2, and both types of wine were distributed into 25 L food-grade plastic tanks. The wine was kept in these tanks for 5 days to promote the sedimentation of fine lees, which were collected for use in the treatments carried out with lees, as described by Del Barrio-Galán *et al.*[1] The different treatments were carried out in triplicate and lasted 4 months. The treatments were: control wines (the wines obtained after alcoholic and malolactic fermentation without any treatment) (C); wines aged on lees (collected fine lees, 30 mL L^{-1}) (L); wines aged on lees (collected fine lees, 30 mL L^{-1}) and French oak (*Quercus petraea*) wood chips (3 g L^{-1} medium-toasted Nobile Sweet L'oenologie du bois; Laffort, France) (L + CH); and wines with commercial inactive dry yeast (CIDY) added (30 g hL^{-1} Opti-LEES). The wood chips used had a length between 7 and 20 mm and produced a sweetness sensation according to the supplier, and the CIDY was rich in low-molecular-weight polysaccharides, mainly mannoproteins, according to the supplier (Lallemand-South America). All wines were resuspended and homogenized with the lees, chips and CIDY through two batonnages per week for the first 2 months. Thereafter, only one batonnage was performed per week for the last 2 months to prevent wine oxidation and microbiological alteration.

Chemical reagents

Methyl cellulose (1500 cP viscosity at 20 g L^{-1}), acetaldehyde, gallic acid, protocatechuic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, ellagic acid, caftaric acid, tyrosol, tryptophol, quercetin, myricetin, astilbin, (+)-catechin, (−)-epicatechin, malvidin-3-glucoside, dextran and pectin standards were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Polyethylene membranes (0.45 and 0.22 μm pore size) were acquired from EMD Millipore (Billerica, MA, USA). Sodium sulfate (anhydrous), potassium metabisulfite, vanillin (99%), ethyl acetate, diethyl ether, sodium hydroxide, acetic acid, formic acid, sulfuric acid, ethanol, hydrochloric acid and high-performance liquid chromatography (HPLC)-grade acetonitrile, methanol and ammonium formate were purchased from Merck (Darmstadt, Germany). All reagents were of analytical grade or higher.

Enological parameter analyses

Total (TA) and volatile (VA) acidity, pH (S220 SevenCompact pH/Ion, Mettler Toledo, Santiago, Chile), SO_2F (sulfur dioxide free) and SO_2T (sulfur dioxide total) and alcoholic degree (A°) were evaluated following the OIV official analytical methods.[19]

Spectrophotometric analyses

Total polyphenols were determined by UV absorbance at 280 nm and expressed as mg L^{-1} gallic acid.[20] Total anthocyanins were measured at 520 nm and expressed as mg L^{-1} malvidin-3-glucoside.[21] Total tannins, expressed as g L^{-1}

(+)-catechin, were evaluated after their precipitation with methyl cellulose and measured at 280 nm.[22] Color intensity was evaluated using the method described by Glories,[23] and CIELab parameters (with illuminant D65 and 10° observer conditions) were determined according to Pérez-Magariño and González-Sanjosé.[24] The percentages of copigmented and polymeric color were evaluated using the method described by Hermosín-Gutiérrez.[25] All measurements were performed on a UV/VIS 1700 Pharmaspec spectrophotometer (Shimadzu, Kyoto, Japan).

HPLC analyses

Anthocyanin compounds were analyzed by taking 2 mL of wine and filtering it through a 0.22 µm pore size membrane, then 150 µL aliquots of the sample were subjected to reverse phase chromatographic separation at 20 °C using a Chromolith C18 column Merck (Darmstadt, Germany). The photodiode array detector (DAD) was set to monitor from 210 to 600 nm. The two mobile phases used were (A) water/formic acid (90:10 v/v) and (B) acetonitrile. A gradient was applied at a flow rate of 1.1 mL min^{-1} from 0 to 22 min and 1.5 mL min^{-1} from 22 to 35 min as follows: 96–85% A from 0 to 22 min, 85–15% A from 12 to 22 min and 85–70% A from 22 to 35 min. Quantification was performed by peak area measurement at 520 nm. Anthocyanins were quantified and expressed as mg L^{-1} malvidin-3-glucoside. Calibration curves at 520 nm were obtained by injecting different volumes of standard solutions under the same conditions used for the samples.[26]

Low-molecular-weight phenolic compounds (non-flavonoids and flavonoids) were analyzed using the method described by Peña-Neira *et al.*[27] A 50 mL aliquot of red wine was extracted with diethyl ether (3 × 20 mL) and ethyl acetate (3 × 20 mL) to concentrate the phenolic compounds. The organic fractions were combined, dehydrated with 2.5 g of anhydrous sodium sulfate and evaporated to dryness under vacuum at 30 °C. The solid residue was dissolved in 2 mL of methanol/water (1:1 v/v) and filtered through a 0.22 µm pore size membrane. Aliquots (25 µL) of the final solution were subjected to reverse phase chromatographic separation. Compounds were analyzed with an HPLC 1100 Series system (Agilent Technologies, Santa Clara, CA, USA) consisting of a G1315B DAD, a G1311A quaternary pump, a G1379A degasser and a G1329A autosampler. A reverse phase Nova-Pak C18 column (4 µm, 3.9 mm i.d. × 300 mm; Waters (Milford, MA, USA)) was used for the HPLC-DAD analysis of individual phenolic compounds at 20 °C. Each major peak in the chromatograms of extracts was characterized by both retention time and absorption spectrum (from 210 to 360 nm). The acquisition time was 1 s. Calibration curves at 280 nm were produced by injecting standard solutions before extraction under the same conditions as the samples analyzed over the range of concentrations observed ($r^2 \geq 0.93$). Quercetin glycosides, dimeric procyanidins, gallates and stilbene glucoside, for which no standards were available, were quantified using standard curves for quercetin, (+)-catechin, gallic acid and *trans*-resveratrol respectively. All qualitative and quantitative analyses of phenolic composition (including their extraction step) were performed in triplicate.

Polysaccharides were analyzed by high-performance size exclusion chromatography with refractive index detection (HPSEC-RID) in order to determine their molecular distribution and concentration. HPSEC-RID was performed using an Agilent 1260 Infinity Series liquid chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a G1362A refractive index detector, a G1311B quaternary pump, a G1316A column oven with two Shodex columns, OHpak SB-803 HQ and SB-804 HQ, connected in series (300 mm × 8 mm i.d.; Showa Denko, Tokyo, Japan), and a G1329A autosampler. Polysaccharide fractions were quantified using calibration curves produced with dextrans and pectins (*Leuconostoc mesenteroides*).[26]

Sensory evaluation

A descriptive analysis of the wines was performed by a trained sensory panel of 12 people who were all workers and students at the Department of Agro-Industry and Enology. The wines were evaluated in individual temperature-controlled tasting booths, and water and unsalted crackers were provided for palate cleansing. Using a completely randomized order, aliquots (20 mL) of wine were served at 18–19 °C in dark wine-tasting glasses (RCristal, Mendoza, Argentina) labeled with a three-digit code. Dark wine-tasting glasses were used to prevent the interference of visual sensations and to focus the attention of panelists on gustative sensations. Between each sample, panelists chewed on a cracker and then rinsed their mouths with water. An unstructured linear 15 cm scale (where 0 = 'absence of sensation' and 15 = 'extremely high sensation') was used to evaluate eight sensory attributes (acidity, sweetness, alcohol, bitterness, red fruits, astringency, persistence, and mouth-feel).

All analyses were carried out after malolactic fermentation (AMLF) and after 2 and 4 months of treatment (2MT and 4MT respectively).

Statistical analysis

Analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test were employed for mean separation, using a significance level of 95% ($P < 0.05$). To obtain summarized and synthesized information from the large set of variables and to better understand the effect of the different treatments applied, a multivariate statistical technique, factor analysis, was utilized. All statistical analyses were conducted using Statgraphics Centurion Version 15.2 (StatPoint Technologies, Inc., Warrenton, VA, USA) and Excel 2007 Version 12.0 (Microsoft Corp., Redmond, WA, USA).

RESULTS AND DISCUSSION

Effect on enological parameters

The enological parameters were analyzed to study the effect of the different aging techniques assayed on these parameters (Table 1). No statistically significant differences were detected between the wines fermented with the EC1118 and HPS yeasts. Additionally, no statistically significant differences were found between the different aging treatments applied. These results agreed with similar published studies that used these techniques with other grape varieties.[1,8,28]

Effect on polysaccharide content

Four fractions of polysaccharides were identified, quantified and classified according to their average molecular weights: fraction I, >2000 kDa; fraction II, 200–300 kDa; fraction III, 60–80 kDa; fraction IV, ≥10 kDa. As seen in Fig. 1, fractions IV and III showed the highest polysaccharide concentration in the EC1118 and HPS wines respectively. The wines fermented with the HPS yeast strain presented, after the MLF period, a higher concentration of low-molecular-weight polysaccharides, which corresponded with fractions III and IV, and a higher total polysaccharide concentration than the wines fermented with EC1118. The polysaccharide concentration increased during the aging period in the treated

Table 1. Enological parameters (TA, g L^{-1} H_2SO_4; VA, g L^{-1} acetic acid) in Cabernet Sauvignon red wines[a]

	AMLF[b]		2MT[b]						4MT[b]					
	EC1118	HPS	EC1118			HPS			EC1118			HPS		
Parameter	C	C	C	L	L+CH	CIDY	C	L	L+CH	CIDY	C	L	L+CH	CIDY	C	L	L+CH	CIDY
A°[ns]	13.3 ± 0.1	13.7 ± 0.2	13.3 ± 0.1	13.3 ± 0.1	13.3 ± 0.1	13.3 ± 0.1	13.6 ± 0.1	13.6 ± 0.1	13.6 ± 0.1	13.6 ± 0.1	13.4 ± 0.1	14.4 ± 0.1	13.4 ± 0.1	13.3 ± 0.1	13.6 ± 0.1	14.5 ± 0.1	13.6 ± 0.1	13.5 ± 0.1
pH[ns]	3.66 ± 0.06	3.66 ± 0.05	3.65 ± 0.03	3.65 ± 0.02	3.66 ± 0.02	3.65 ± 0.01	3.70 ± 0.03	3.71 ± 0.03	3.70 ± 0.04	3.69 ± 0.02	3.65 ± 0.03	3.64 ± 0.02	3.67 ± 0.03	3.62 ± 0.04	3.66 ± 0.04	3.70 ± 0.03	3.66 ± 0.03	3.62 ± 0.03
TA[ns]	3.30 ± 0.07	3.25 ± 0.02	3.10 ± 0.02	3.12 ± 0.04	3.15 ± 0.03	3.10 ± 0.03	3.08 ± 0.05	3.05 ± 0.05	3.05 ± 0.06	3.00 ± 0.06	3.10 ± 0.03	3.10 ± 0.04	3.14 ± 0.05	3.09 ± 0.03	3.18 ± 0.06	3.10 ± 0.04	3.05 ± 0.05	3.07 ± 0.04
VA[ns]	0.37 ± 0.04	0.35 ± 0.10	0.40 ± 0.02	0.39 ± 0.03	0.40 ± 0.03	0.40 ± 0.02	0.39 ± 0.02	0.37 ± 0.03	0.39 ± 0.03	0.40 ± 0.03	0.75 ± 0.02	0.75 ± 0.03	0.80 ± 0.05	0.80 ± 0.04	0.70 ± 0.05	0.75 ± 0.03	0.75 ± 0.03	0.75 ± 0.04
SO_2F[ns]	0.000	0.000	23.0 ± 1.73	24.3 ± 1.53	24.0 ± 1.00	24.0 ± 2.00	22.3 ± 2.08	24.3 ± 1.58	25.3 ± 1.52	24.0 ± 2.04	11.0 ± 1.34	14.3 ± 1.63	10.0 ± 1.76	14.0 ± 2.65	12.7 ± 1.38	14.2 ± 1.61	15.3 ± 1.78	11.6 ± 1.84
SO_2T[ns]	5.50 ± 0.07	4.40 ± 0.15	67.7 ± 4.87	73.3 ± 1.93	71.7 ± 1.53	71.7 ± 2.74	71.3 ± 3.51	72.8 ± 1.08	70.7 ± 2.19	71.0 ± 1.73	34.2 ± 2.79	33.4 ± 1.45	29.7 ± 2.13	32.1 ± 2.72	31.0 ± 2.69	35.8 ± 1.48	33.9 ± 1.59	31.0 ± 2.33

[a] Values are expressed as mean ± standard deviation ($n = 3$).
[b] AMLF, after malolactic fermentation; 2MT, 2 months of treatment; 4MT, 4 months of treatment.
[c] No statistically significant differences.

as well as the control wines, mainly after the 2MT period. The observed increase in the polysaccharide concentration of the control wines has been explained by other authors,[1] who stated that these compounds remain in the wine in a colloidal state linked to other compounds or that they are the result of autolysis of the remaining dead yeast present in the wine. After the 4MT aging period, this concentration was maintained or decreased, but the change was dependent on the aging treatment applied. However, after the entire aging period, both types of fermented wine (EC1118 and HPS) showed a similar concentration of total polysaccharides. This suggested that the HPS yeast allowed a faster liberation of yeast polysaccharides, mainly mannoproteins, during alcoholic fermentation than the EC1118 yeast, but after the 2MT aging period, and mainly after 4MT, both types of wine were similar. Moreover, all aging techniques used (L, L + CH and CIDY) generally increased, in both EC1118 and HPS wines, the total polysaccharide concentration with respect to the control wines, but these results depended on the different molecular weight fractions analyzed and the aging technique used. These differences were more evident in the EC1118 wines than in the HPS wines. The results obtained agree with other studies performed with similar aging techniques.[1,3,8,17,29–31] Palomero *et al.*[29] studied the aging on lees of different strains of yeast over 142 days, but in a model medium, and observed a progressive increase in the concentration of polysaccharides. Guadalupe and co-workers[3,30,31] evaluated the use of a mannoprotein-overproducing yeast strain and the addition of commercial mannoproteins to red wines during or after alcoholic fermentation. They noted an increase in or maintenance of the neutral (mannoproteins) and total polysaccharides during barrel and bottle aging, although these effects were dependent on the dose and product used. Del Barrio-Galán *et al.*[1,8] evaluated the aging of wines on lees, chips and several commercial yeast derivative products rich in polysaccharides (mannoproteins) and showed that all techniques studied allowed the release of higher concentrations of total and neutral polysaccharides to the wine, although the type and content of these compounds depended on the technique used and the yeast derivative added, since each product had different purity and composition. Similar results were obtained by other authors[17] using an over-lees aging technology applied to red wines.

Effect on phenolic compounds analyzed by spectrophotometric methods

Table 2 shows the total polyphenols (TP), total tannins (TT) and total anthocyanins (TACY) analyzed in the red wines using different spectrophotometric methods. Several statistically significant differences were identified. For example, after the MLF period, the wines fermented with HPS showed a statistically significantly lower content of TP, TT and TACY than the wines fermented with EC1118. As expected, the contents of the different phenolic compounds analyzed decreased as the aging period progressed and different results were found as a function of the treatment used and the aging period analyzed. In fact, the HPS wines generally presented a lower TP content than the EC1118 wines over all aging periods. In addition, all wines treated with the different aging techniques (L, L + CH and CIDY) presented a lower TP content than the control wines for both HPS and EC1118 wines, with the wines treated with L presenting the lowest values. However, for TT, no statistically significant differences were found between the HPS and EC1118 wines. The treated wines presented a lower TT content than the control wines only after the 2MT aging period. For TACY, the HPS wines showed a lower content than the EC1118 wines after the

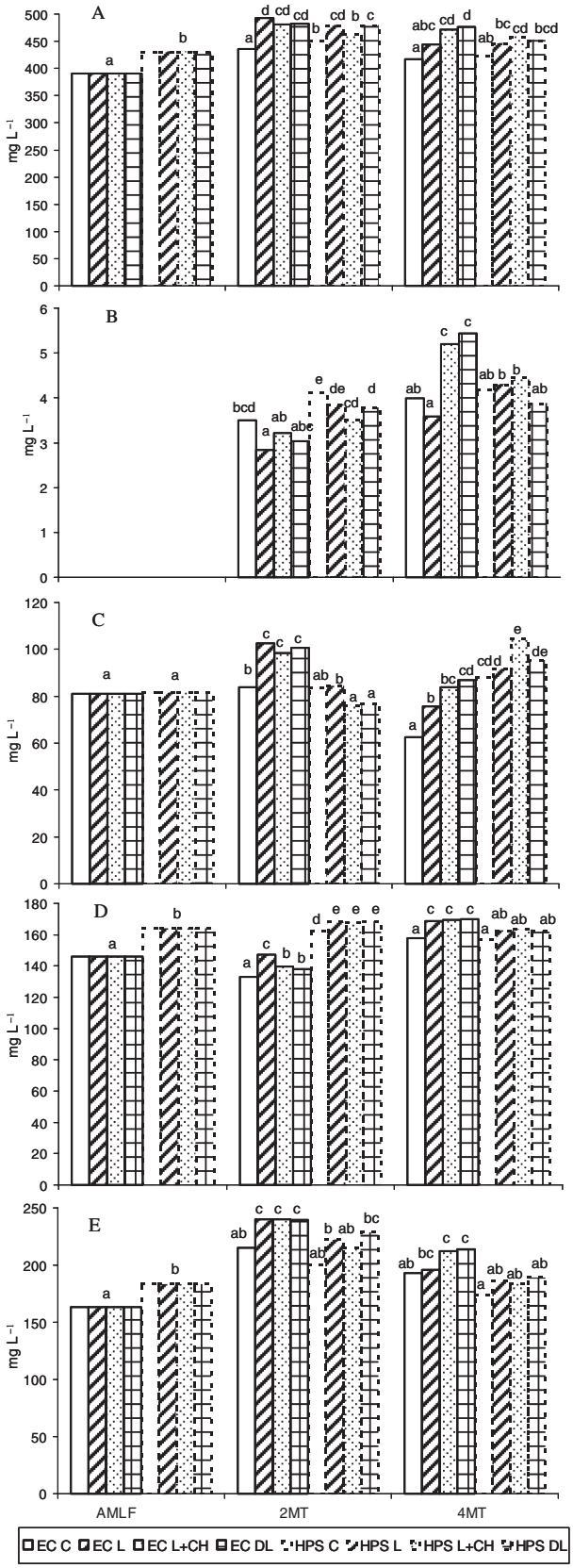

Figure 1. Concentrations of (A) total polysaccharides and (B–E) polysaccharide fractions I–IV respectively in wines. AMLF, after malolactic fermentation; 2MT, 2 months of treatment; 4MT, 4 months of treatment. For each period (AMLF, 2MT or 4MT), different letters indicate statistically significant differences ($P < 0.05$) between values.

2MT aging period, and this content was lower in all treated wines compared with the control. However, different results were found after 4MT; while the EC1118 treated wines presented a lower content than the controls, the HPS treated wines exhibited opposite results, with a higher TACY content being observed. These results agree with those obtained by other authors who used techniques similar to those mentioned above over 2 months.[1,8]

The lower contents of the different phenolic compounds observed for the HPS and EC1118 wines and the difference between the different treated wines with respect to the controls could be explained by a higher liberation of polysaccharides by the HPS yeast compared with the EC1118 yeast after alcoholic fermentation. The greater amount of these polysaccharides liberated by the different aging techniques, compared with the control wines, could interact with the different phenolic compounds, reducing their concentration in wines. These results agree with those obtained by other authors, which revealed the capability of the mannoprotein-overproducing yeast strain, yeast lees and several commercial products rich in mannoproteins to retain or adsorb different wine phenolic compounds.[3,8,30–33] The smaller differences found between the two types of fermented wine after the 4MT aging period for certain compounds were most likely due to the content of polysaccharides in the EC1118 wines, which continued to increase, reaching similar values to the HPS wines. However, these results depended on the treatment used and the compound analyzed.

Effect on phenolic compounds analyzed by HPLC methods

The monomeric anthocyanins identified and quantified in this study were grouped into the glucosylated anthocyanins (delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside and malvidin-3-glucoside), the acetylated anthocyanins (delphinidin-3-(6-acetyl)-glucoside, cyanidin-3-(6-acetyl)-glucoside, petunidin-3-(6-acetyl)-glucoside, peonidin-3-(6-acetyl)-glucoside and malvidin-3-(6-acetyl)-glucoside) and the coumaroylated anthocyanins (delphinidin-3-(6-*p*-coumaroyl)-glucoside, cyanidin-3-(6-*p*-coumaroyl)-glucoside, petunidin-3-(6-*p*-coumaroyl)-glucoside, peonidin-3-(6-*p*-coumaroyl)-glucoside and malvidin-3-(6-*p*-coumaroyl)-glucoside). As seen in Table 2, the group of glucosylated anthocyanins presented the highest concentration.

The TACY content, analyzed by spectrophotometric methods, and the content of monomeric anthocyanins decreased during the aging period, most likely owing to the interaction of these compounds with other phenolic compounds, such as tannins or other anthocyanins, by polymerization and copigmentation reactions as explained by several authors,[34–37] which can form more complex molecular structures.

The wines fermented with HPS yeast contained lower monomeric anthocyanin concentrations than those fermented with EC1118 yeast. The most significant differences were found in malvidin-3-glucoside, malvidin-3-(6-acetyl)-glucoside and malvidin-3-(6-*p*-coumaroyl)-glucoside (data not shown), which were the compounds with higher concentrations in both types of wine. As discussed above, the polysaccharides (mainly mannoproteins) liberated from the yeast cell walls can interact or adsorb these phenolic compounds. This could lead to the formation of more complex structures that can remain stable in wine as colloids or precipitate owing to their higher molecular weight. These results oppose those obtained by some authors,[30,31] because no statistically significant differences in the monomeric anthocyanin content were found between the

Table 2. Color parameters (CI, color intensity; *L*, *C*, *H*, *a* and *b*, CIELab parameters; CC, copigmentated color; PC, polymeric color), total polyphenols (TP, mg L^{-1} gallic acid), total tannins (TT, g L^{-1} catechin), total anthocyanins (TACY, mg L^{-1} malvidin-3-glucoside) and monomeric anthocyanins (mg L^{-1} malvidin-3-glucoside) in Cabernet Sauvignon red wines[a]

	AMLF[b]		2MT[b]								4MT[b]							
	EC1118	HPS	EC1118				HPS				EC1118				HPS			
Parameter	C	C	C	L	L + CH	CIDY	C	L	L + CH	CIDY	C	L	L + CH	CIDY	C	L	L + CH	CIDY
CI	11.4b	9.13a	14.1f	13.0 cd	13.1d	13.5e	12.8c	12.1b	12.2b	11.8a	16.3 g	16.0ef	15.9e	16.2 fg	13.0d	11.4a	11.8b	12.3c
L	49.8a	56.9b	44.5a	45.8b	45.5b	44.7a	46.7c	48.3d	48.1d	49.2e	37.8a	38.2a	37.7a	38.0a	46.3b	50.5e	49.2d	47.9c
C	52.0b	46.1a	57.1b	55.4a	55.5a	56.4a	54.4e	53.1c	53.1c	52.9d	54.0f	53.1e	52.0c	54.5 g	52.1 cd	49.4a	50.5b	52.3d
H	6.30a	8.6b	9.0b	8.5a	9.4c	9.4c	11.8e	11.4d	12.2f	11.3d	16.1 cd	15.6c	14.4a	16.2d	16.0 cd	15.0b	14.7ab	14.7ab
a	51.7b	45.6a	56.4e	54.8c	54.8c	55.7d	53.3b	52.1a	51.9a	51.9a	51.9f	51.2e	50.3 cd	52.3 g	50.1c	47.7a	48.9b	50.6d
b	5.7a	6.9b	8.9b	8.2a	9.0bc	9.2c	11.1e	10.5d	11.2e	10.4d	15.0c	14.3b	12.9a	15.3c	14.4b	12.8a	12.8a	13.3a
%CC	33.2b	30.2a	32.4c	30.9b	30.2b	34.4d	25.6a	25.6a	25.6a	25.6a	23.9c	23.4b	23.1a	24.1d	28.4 g	28.1f	26.7e	28.5 g
%PC	23.7a	25.6b	26.2a	26.5a	28.1b	26.7a	37.9d	37.4d	37.6d	36.4c	50.7e	52.4f	56.2 g	50.8e	43.2a	46.6d	44.1b	45.4c
TP	1612b	1416a	1588 g	1552e	1559f	1592 g	1418c	1407b	1429d	1397a	1519f	1465d	1478e	1524f	1401c	1344a	1385b	1382b
TT	1.40b	1.21a	1.14c	0.808a	0.973b	0.959b	1.13c	0.950b	1.02b	0.924b	1.02abc	0.772ab	1.02abc	1.00abc	1.06bc	0.901abc	1.10c	0.813a
TACY	647b	567a	653 h	615f	607e	627 g	515d	489b	476a	504c	283c	270b	253a	276bc	349d	385f	372e	370e
Monomeric anthocyanins																		
Glucosylated ACY	531b	418a	440e	471f	385d	390d	359c	336b	317a	363c	140de	106bc	59a	97b	123 cd	162ef	149e	179f
Acetylated ACY	156b	136a	151f	131e	115d	110d	92.0c	83.0b	86.9bc	58.3a	49.5f	40.8de	30.8bc	27.3ab	36.3 cd	24.7a	37.6de	42.7e
Coumaroylated ACY	39.2b	34.6a	34.2d	31.4c	39.2e	30.8c	30.1bc	23.7ab	21.2a	27.2b	28.7d	22.5c	13.2ab	14.4b	21.7c	10.5a	11.8ab	12.4ab
TMACY[b]	727b	589a	623e	634e	539d	531d	481c	443ab	425a	451b	218ef	169c	103a	139b	181 cd	197de	198de	234 g

[a] For each period (AMLF, 2MT or 4MT), different letters indicate statistically significant differences ($P < 0.05$) between values within a row.
[b] AMLF, after malolactic fermentation; 2MT, 2 months of treatment; 4MT, 4 months of treatment; TMACY, total monomeric anthocyanins.

wines fermented with the mannoprotein-overproducing yeast strain and those fermented with the use of commercial mannoproteins during alcoholic fermentation. In general, during the aging period, all HPS wines showed lower monomeric anthocyanin concentrations than the EC1118 wines. As occurred in the AMLF period, the most significant differences were found in malvidin-3-glucoside, malvidin-3-(6-acetyl)-glucoside and malvidin-3-(6-*p*-coumaroyl)-glucoside (data not shown). Furthermore, both the HPS and EC1118 treated wines presented lower concentrations of these compounds after the 2MT period, with some exceptions, because this effect was different depending on the treatment and the compound analyzed. Thus all treated wines presented lower concentrations of acetylated anthocyanins than the control wines, with the wines treated with CIDY showing the lowest content. Similar results were obtained for the glycosylated anthocyanins, but the HPS wines treated with L showed a higher content than the control and the other treated wines, and the EC1118 wines treated with CIDY showed a similar content to the control wines. Finally, all treated wines presented lower coumaroylated anthocyanin contents than the control wines in this period, with the exception of the EC1118 wines treated with L + CH, which presented a higher content. After the 4MT aging period, the results obtained for the total monomeric anthocyanin (TMACY) content, and particularly for the glycosylated anthocyanins, correlated well with those obtained for TACY by spectrophotometric methods. Moreover, all EC1118 treated wines showed lower TMACY contents than the control wines, while the opposite was obtained for all HPS treated wines, with exception of the wines treated with L, which were lower in TMACY. However, both EC1118 and HPS treated wines showed lower contents of coumaroylated anthocyanins than the control wines. As with TACY, these results agree with those obtained by other authors.[1,8]

Table 3 shows the low-molecular-weight phenolic compounds identified and quantified in the wines. These compounds were also grouped into phenolic families to more clearly understand both the results and the statistically significant differences obtained. Tyrosol, gallic acid and (+)-catechin were the compounds present in the highest concentrations. After the MLF period, the wines fermented with HPS yeast had lower contents of all phenolic families than the EC1118 wines, with the exception of hydroxycinnamic acids (HCA), hydroxycinnamic acid tartaric esters (HCATE) and flavonol glycosides. As explained previously, the lower concentration of these compounds in the HPS wines could be due to higher interaction and/or adsorption between the mannoproteins and/or the polysaccharides released in higher concentration than with the EC1118 yeast during alcoholic fermentation. Several statistically significant differences were also found during the aging period, but these differences depended on the treatment applied, the aging period studied and the type of phenolic compounds analyzed. For hydroxybenzoic acids (HBA), the most significant differences were found at the end of the aging period, where all HPS wines had lower HBA contents than EC1118 wines. In both cases the treated wines had lower HBA contents than the control wines, with the exception of the HPS wines treated with L + CH that were higher in HBA, most likely owing to the liberation of gallic and ellagic acids from the oak wood chips, as explained by the authors of a comparable previous study.[38] Similar results were found for HCATE, mainly in the EC1118 wines. These results were also shown by several authors who used aging on lees, chips and commercial mannoproteins in red wines.[1,8,30,39,40] For HCA, the most important differences between the HPS and EC1118 wines were found after the 2MT aging period, with the concentration being higher in the latter. The concentration of HCA increased during the aging period of the wines, which was explained by several authors to be a result of the enzymatic hydrolysis of their esterified forms or the hydrolysis of coumaroylated anthocyanins.[41–43] Several authors also noted that some HCA such as caffeic, *p*-coumaric and ferulic acids can increase in wines aged in oak wood by hydroalcoholysis;[38,44] however, under the conditions used in the present study, this effect not was observed. Finally, the concentrations of HCA and HCATE are generally well correlated during the aging period.

A similar trend in the concentration of stilbenes and phenolic alcohols was found during the aging period, which increased at the end of this period. The HPS wines had a lower content of both types of these phenolic compounds than the EC1118 wines, but certain differences were found between the aging treatments. After the 2MT period, all treated wines had a lower stilbene content than the control wines, with the wines treated with L + CH, CIDY and L showing lower values in the order specified. Similar results were found after the 4MT aging period, but the order of the lower values was L + CH, L and CIDY. However, for the phenolic alcohols, the EC1118 treated wines had a higher concentration than the control wines, and no significant differences between the EC1118 and HPS treated wines were found after the 2MT aging period. After the 4MT period, all treated wines had a lower content than the control wines for both HPS and EC1118 wines, but it was dependent on the treatment used. This lower phenolic alcohol content agrees with that obtained in previous studies carried out with the same or similar aging techniques.[39,40]

Moreover, a similar trend was observed in the total flavanol monomers ((+)-catechin and (−)-epicatechin) and the total procyanidins (B3, B4 and B2); as seen in Table 3, the concentration decreased during the aging period. After the 2MT period, the concentration of total flavonol monomers was significantly lower in the HPS wines than in the EC1118 wines, but a smaller difference between the treated and control wines was found. Only the EC1118 wines treated with CIDY had a significantly lower flavanol concentration than the control wines. After the 4MT period, fewer significant differences were found between the wines developed with both yeast strains, and only the HPS control wines had a lower concentration than the EC1118 controls. In contrast, the HPS wines treated with CIDY presented a higher concentration than the same wines fermented with EC1118 yeast. However, the EC1118 and HPS wines treated with L + CH and CIDY showed lower contents of total procyanidins than the control wines after the 2MT period. Several authors have shown that the interaction between commercial mannoproteins from *S. cerevisiae*, mannoprotein-overproducing yeast strain (during or after alcoholic fermentation) and yeast lees with procyanidins resulted in a significantly lower content in wines treated with these products.[30,31,39,40] No significant differences after the 4MT aging period were found between the HPS and EC1118 wines, and only procyanidin B4 was detected.

As expected, the flavonol glycosides and the aglycones showed a similar trend to HCA and HCATE during the aging period. The concentration of flavonol glycosides decreased and that of aglycones increased, mainly owing to the hydrolysis of glycosides, which allowed the liberation of aglycones into wines. After the 2MT period, all HPS wines had a lower concentration of flavonol glycosides than the EC1118 wines, with the exception of the wines treated with L. The EC1118 wines treated with L had higher aglycone contents than the respective HPS wines. The applied treatments seemed to affect the EC1118 wines more than the HPS wines for these phenolic compounds. Thus all treated wines presented lower contents of both glycoside and aglycone forms, with

Table 3. Low-molecular-weight phenolic compounds quantified (mg L^{-1}) in Cabernet Sauvignon red wines[a]

	AMLF[b]		2MT[b]								4MT[b]							
	EC1118	HPS	EC1118				HPS				EC1118				HPS			
Compound	C	C	C	L	L + CH	CIDY	C	L	L + CH	CIDY	C	L	L + CH	CIDY	C	L	L + CH	CIDY
Non-flavonoid phenolic compounds																		
Gallic acid	16.3b	12.1a	18.4bc	17.7bc	19.8c	18.3bc	15.6ab	13.7a	19.0bc	13.7a	23.7f	17.5de	18.1e	16.0d	12.3c	9.60a	11.6bc	10.5ab
Protocatechuic acid	0.810b	0.747a	1.31ab	0.926a	0.781a	0.983a	2.24c	2.11c	1.66bc	2.21c	2.25c	1.62a	1.65a	1.78ab	2.11bc	1.84ab	1.65a	1.92abc
Methyl gallate	0.718b	0.559a	0.819a	0.797a	0.751a	0.819a	0.809a	0.809a	0.679a	0.755a	0.772ab	0.653a	0.930b	0.832b	0.825b	0.867b	0.891b	0.804b
Vanillic acid	0.855a	0.840a	0.835ab	0.763a	0.807a	0.823ab	0.789a	0.789a	0.781a	1.003b	1.07bc	0.923ab	1.18c	0.883a	0.962ab	1.01abc	1.074bc	0.975ab
Syringic acid	1.07a	1.10a	1.74ab	1.54a	1.75ab	1.76ab	1.71ab	1.71ab	2.18c	2.03bc	1.74c	1.37ab	1.46bc	1.24ab	1.30ab	1.17a	1.35ab	1.27ab
Ethyl gallate	3.38b	2.46a	2.12ab	1.65ab	0.97a	2.03ab	2.04ab	2.46b	1.35ab	1.52ab	2.38c	2.36c	2.19c	2.10bc	1.71a	1.46a	1.63a	1.73ab
Ellagic acid	5.10b	4.49a	5.11abc	3.95a	4.68ab	5.10abc	4.30a	5.67bc	6.13c	4.47ab	2.03ab	2.47ab	3.01abc	1.63a	2.69ab	3.05abc	4.39c	3.27bc
Total HBA[b]	28.2b	22.4a	30.4bc	27.3ab	29.5abc	29.8abc	27.5abc	28.2abc	31.8c	25.7a	34.0f	26.9de	28.5e	24.5 cd	21.9b	19.0a	22.6bc	20.4ab
t-Caffeic acid	2.84a	2.99b	1.98a	2.27a	2.26a	2.13a	3.71c	3.71c	3.05b	2.87b	4.58b	4.44ab	4.42ab	3.77a	4.21ab	4.64b	4.35ab	4.70b
t-*p*-Coumaric acid	2.30a	2.29a	2.61a	2.49a	2.33a	2.78a	4.30c	4.32c	3.80bc	3.57b	5.26ab	5.16ab	4.98a	4.99a	5.29ab	4.73a	5.12ab	5.77b
t-Ferulic acid	0.743a	0.702a	ND	ND	ND	ND	ND	ND	ND	ND	1.45b	0.736a	0.710a	0.808a	ND	ND	ND	ND
Total HCA[b]	5.88a	5.99a	4.59a	4.76a	4.59a	4.90a	8.01c	8.84d	6.85b	6.44b	9.48ab	9.61ab	9.40ab	8.77a	9.50ab	9.36ab	9.47ab	10.5b
t-Caftaric acid	1.84a	1.92a	1.87ab	1.45a	1.51a	1.54a	2.10b	2.10b	1.76ab	2.22b	2.02d	1.78c	1.52b	1.60b	1.57b	1.37a	1.39a	1.56b
c-Coutaric acid	0.625a	0.722a	1.40c	1.07b	0.96ab	1.06b	0.935ab	1.07b	0.722a	0.750a	0.824b	0.748ab	0.802b	0.643a	0.749ab	0.720ab	0.744ab	0.753ab
t-Coutaric acid	0.784b	0.687a	0.700ab	0.678ab	0.451a	0.621ab	0.781ab	0.862b	0.514ab	0.535ab	1.17c	1.14bc	0.913ab	0.864a	0.910ab	0.775a	0.825a	0.851a
t-Fertaric acid	0.260a	0.276a	0.426ab	0.288a	0.289a	0.294a	0.576ab	0.680b	0.727b	0.551ab	ND	ND	ND	ND	ND	ND	ND	ND
t-*p*-Coumaric acid hexose ester	0.231b	0.214a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total HCATE[b]	3.73a	3.82a	4.39bc	3.48ab	3.21a	3.51ab	4.40bc	4.75c	3.72abc	4.06abc	4.01d	3.67c	3.24b	3.11ab	3.23ab	2.86a	2.96ab	3.17ab
t-Resveratrol -3-glucoside	0.434b	0.392a	0.707d	0.653 cd	0.569bc	0.543bc	0.513b	0.569c	0.283a	0.311a	0.253abc	0.292c	0.284bc	0.220a	0.235abc	0.256abc	0.237abc	0.212a
c-Resveratrol -3-glucoside	0.377b	0.329a	0.369b	0.221ab	0.145a	0.223ab	0.363b	0.340b	0.292ab	0.301ab	0.461c	0.331ab	0.255a	0.273a	0.363abc	0.390bc	0.292ab	0.261a
t-Resveratrol	ND	ND	0.656d	0.593c	0.443ab	0.466abc	0.610 cd	0.447ab	0.317a	0.368a	0.607d	0.520c	0.387b	0.438bc	0.354ab	0.308a	0.293a	0.323ab
c-Resveratrol	0.142a	0.148a	0.307c	0.268bc	0.159a	0.188ab	0.250bc	0.227b	0.150a	0.215ab	0.801 cd	0.646ab	0.606ab	0.667b	0.901d	0.505a	0.512a	0.630ab
Total stilbenes	0.953b	0.869a	2.04d	1.74c	1.32b	1.42b	1.74c	1.58b	1.04a	1.20a	2.12e	1.79d	1.53bcd	1.60bc	1.72 cd	1.31ab	1.26a	1.42ab
Tyrosol	25.7b	20.0a	28.6b	37.1c	35.3c	33.1bc	20.3a	20.2a	17.2a	19.3a	40.7e	33.9 cd	31.3bcd	34.3d	31.6bcd	26.2ab	27.7abc	24.1a
Tryptophol	2.97b	2.28a	3.74bc	3.32abc	3.02ab	4.15c	2.97ab	2.60a	2.54a	3.18abc	0.660bc	0.512ab	0.686bc	0.313a	0.783bc	0.765bc	0.924c	0.748bc
Total phenolic alcohols	28.7b	22.3a	32.3b	40.4c	38.3c	37.2c	23.2a	22.8a	19.7a	22.5a	41.4d	34.4c	32.0bc	34.6c	32.4bc	26.9ab	28.6ab	24.8a
Flavonoid phenolic compounds																		
Catechin	17.1b	10.6a	10.6de	11.5e	10.2de	9.93d	7.21bc	7.85c	5.39a	6.01ab	6.15e	5.16 cd	3.87a	4.30ab	4.82bc	5.64de	4.77bc	5.71de
Epicatechin	5.81b	4.37a	9.82d	7.38bc	8.41 cd	5.85ab	4.84a	6.11abc	5.07ab	6.00abc	4.65c	3.42b	2.47a	2.53a	2.74a	3.51b	2.51a	2.97ab
Total flavanol monomers	22.9b	15.0a	20.4d	18.9 cd	18.7 cd	15.8bc	12.1a	14.0ab	10.5a	12.0a	10.8e	8.58 cd	6.34a	6.83ab	7.56b	9.15 cd	7.28ab	8.68d
Procyanidin B3	1.17b	1.02a	0.657d	0.392c	ND.	0.154a	0.273ab	0.369 cd	0.163abc	0.293bcd	ND	ND	ND	ND	ND	ND	ND	ND
Procyanidin B4	0.990b	0.908a	0.555d	0.262b	0.144a	0.362c	ND	ND	ND	ND	1.06ab	0.924a	0.987a	0.894a	1.07ab	1.38b	1.22ab	1.10ab
Procyanidin B2	3.97a	3.86a	3.44d	3.39d	3.60d	3.25 cd	2.25b	2.60bc	1.36a	1.38a	ND	ND	ND	ND	ND	ND	ND	ND

Table 3. Continued

Compound	AMLF[b]		2MT[b]						4MT[b]					
	EC1118	HPS	EC1118			HPS			EC1118			HPS		
	C	C	C	L	L + CH	CIDY	C	L	L + CH	CIDY	C	L	L + CH	CIDY	C	L	L + CH	CIDY
Total procyanidins	6.13b	5.78a	4.65e	4.04de	3.74 cd	3.76 cd	2.52b	2.96bc	1.53a	1.68a	1.06ab	0.92a	0.99a	0.89a	1.07ab	1.38b	1.22ab	1.10ab
Quercetin	5.51b	4.91a	5.85de	5.26 cd	4.44ab	5.12abc	6.03e	5.46cde	4.35a	4.82abc	7.55d	6.89 cd	5.29a	5.85ab	6.44bc	6.56bc	5.85ab	7.06 cd
Myricetin	4.83a	4.88a	5.28bcd	4.30ab	3.74a	4.40ab	5.87d	5.61 cd	4.01a	4.71abc	6.59c	5.81bc	4.72a	5.67b	6.07bc	6.12bc	5.61b	6.67c
Astilbin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.13ab	2.10ab	2.05ab	1.92ab	1.64a	2.24b	1.92ab	2.38b
Total flavonol aglycones	10.3b	9.79a	11.1b	9.56a	8.18a	9.51a	11.9b	11.1b	8.36a	9.52a	16.3c	14.8bc	12.1a	13.4ab	14.1ab	14.9bc	13.4ab	16.1c
∑Flavonol glycosides	14.2a	14.0a	16.9c	13.7b	11.4ab	13.2b	12.1ab	10.0a	11.6ab	11.5ab	13.2b	12.0ab	10.5a	10.6a	11.0a	12.1ab	11.9ab	13.2b

[a] For each period (AMLF, 2MT or 4MT), different letters indicate statistically significant differences ($P < 0.05$) between values within a row. ND, not detected.
[b] AMLF, after malolactic fermentation; 2MT, 2 months of treatment; 4MT, 4 months of treatment; HBA, hydroxybenzoic acids; HCA, hydroxycinnamic acids; HCATE, hydroxycinnamic acid tartaric esters.

the wines treated with L + CH showing the lowest content. Fewer differences were found in HPS wines, and only those treated with L + CH and CIDY showed lower contents of aglycones than the control wines and those treated with L. No statistically significant differences were found in the flavonol glycosides, and these results agree with those obtained by other authors.[39] Fewer differences between the EC1118 and HPS wines were found after the 4MT aging period. Moreover, the HPS wines treated with CIDY showed a higher concentration of glycosides and aglycones than the respective EC1118 treated wines. On the other hand, a different effect was also found between the aging treatments after this period. In general, all EC1118 treated wines had lower concentrations of both types of flavonols than the control wines, with the exception of the wines treated with L, which maintained similar contents. Some authors noted that these compound can act as copigments because of their higher ability to interact with anthocyanins,[45–47] which could explain the lower flavonol content in these treatments. This effect can also be explained by the interaction of flavonols with the polysaccharides liberated during these aging treatments. However, in HPS wines, only those treated with CIDY presented a higher content than the controls, while the rest of the treated wines maintained a concentration similar to the controls. These results agreed with those obtained by some authors.[39,40]

Effect on color of wines

Table 2 shows several color parameters evaluated to observe possible differences in the color evolution of the wines fermented with the two yeast strains and the subsequent evolution during aging on lees and the other accelerated aging techniques used in this study. The wines fermented with EC1118 yeast presented a higher color intensity (CI) than the wines fermented with HPS yeast after the MLF period and the aging period. The HPS wines showed higher values of the *L* (lightness), *H* (hue) and *b* (chromatic coordinate of blue/yellow colors) CIELab parameters than the EC1118 wines after the MLF period. These results were maintained during the aging period, with some exceptions, because after the 4MT aging period, some EC1118 treated wines showed *H* and *b* values similar to or higher than those of the HPS wines. As explained in the literature,[48] the aging of wines entails an increase in the *L*, *H* and *b* parameters, and there is an inverse relationship between these parameters and CI. Therefore the results obtained for these parameters, as shown in Table 2, are well correlated after the MLF period and during the aging period, with the exception of the *H* and *b* parameters after 4MT. This trend was maintained for both EC1118 and HPS treated wines, because, in general, all treated wines presented lower CI and higher *L* than the control wines, with the exception of the EC1118 treated wines, which had a similar *L* to the control wines. However, all treated wines generally presented lower values of *H* and *b* than the control wines. These results indicate that the higher liberation of polysaccharides by the HPS yeast and the different aging treatments did not produce color stabilization, but color loss may have occurred owing to polymerization of the polysaccharides with the phenolic compounds responsible for the color of wines.

The trends of the percentages of copigmented color (%CC) and polymeric color (%PC) are typically opposite during the aging process, because copigmentation reactions occur more in young wines while polymeric reactions usually occur in aging wines. These values are well correlated because, while %CC decreased during aging, %PC increased. Furthermore, the EC1118 wines showed higher %CC values than the HPS wines after the MLF period, and opposite results were found for the %PC values. These

results were maintained after the 2MT aging period but were opposite after 4MT. This trend can be explained because, as noted by some authors,[49] the reactions that form copigmented complexes occur very easily and are reversible over time, but the complexes also disassociate easily and are unstable. Fewer significant differences in %CC between treatments were found during the aging period, and only the EC1118 wines treated with CIDY showed higher values than the control and the remaining treated wines. In contrast, all treated wines presented higher values of %PC than the control wines after the 4MT aging period, with the exception of the EC1118 wines treated with CIDY, which maintained a similar content to the control wines. Higher %PC was observed in the EC1118 wines than in the HPS wines after the aging period, which could explained by a higher contribution of these treated wines to color stabilization. More polymerization reactions could occur between the anthocyanins and other phenolic compounds, which could contribute to color stabilization. In addition, the %PC values are well correlated with TACY, TMACY and CI. However, the higher values of %PC observed in both EC1118 and HPS treated wines (with respect to the controls) were not well correlated with the CI values, because the expected hypothesis was that higher %PC values contributed to the increase in CI values, which would explain the improvement in color stabilization. Several authors suggest that polysaccharides and mannoproteins can interact with anthocyanins and tannins, thus preventing their precipitation and improving their color stability.[5–8,50] Meanwhile, other authors[4,51] indicate that polysaccharides can act as color stabilizers or enhance the precipitation of phenolic polymers to cause a loss of color, depending on their molecular weight, adsorbent character, charge and structure. However, other recent studies did not find an improvement in wine color intensity and color stability using mannoproteins.[1,30,31]

Multivariate analysis

Factor analysis was applied on polysaccharide, polyphenolic and color values to study the association of different variables. To minimize the number of variables and better understand the results obtained, only the grouped values were used for the low-molecular-weight phenolic compounds and monomeric anthocyanins. The varimax rotation criterion was applied and only factors with eigenvalues greater than unity were selected. All data were standardized before the factor analysis.

The factor analysis selected five factors with an eigenvalue greater than 1, which explained 91.2% of the total variance. Table 4 shows the factor loadings for each variable of the selected factors. The variables with higher loading values contribute most significantly to the explanatory meaning of the factors. As seen in Table 4, most variables used for this multivariate analysis were associated with factor 1, which explained 51.8% of the total variance. Figure 2 shows the distribution of the different wines studied in the plane defined by factors 1 and 2, which explained 68.6% of the total variance. These factors allowed the separation of both types of fermented wine (EC1118 and HPS) during the aging period as well as the separation of the wines treated with the different aging techniques assayed. This effect was most evident at the end of the aging period (4MT).

Sensory analysis

The sensory analysis was focused on the gustative attributes (Fig. 3) described above. Various statistically significant differences were found in the AMLF, 2MT and 4MT periods, depending on the yeast strain used, the aging treatment used and the period analyzed. Moreover, the wines fermented with the HPS yeast strain showed lower values of acidity, bitterness and astringency and higher values of red fruits and persistence than the wines fermented with the EC1118 yeast. Conversely, no significant differences were found between the EC1118 and HPS wines during the entire aging period, but some differences were found between the aging treatments and the control. Thus the taster panel indicated that, in general, certain treated wines presented higher values of red fruits, persistence and mouth-feel than the control wines

Table 4. Factor loadings after varimax rotation of treated wines

Item	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Eigenvalue	15.0	4.86	2.96	2.40	1.21
Cumulative variance	51.8	68.6	78.8	87.1	91.2
Total polyphenols	**0.568**	**0.784**			
Total tannins					**0.969**
Total anthocyanins	**0.969**				
L	0.339	**−0.919**			
C	**0.757**	**0.565**			
H	**−0.963**				
a	**0.857**	0.446			
b	**−0.931**				
% copigmented color	0.258	0.252	**−0.862**	0.296	
% polymeric color	**−0.959**				
Color intensity	−0.296	**0.928**			
Polysaccharide fraction I	**−0.755**				0.418
Polysaccharide fraction II			**−0.807**	−0.270	
Polysaccharide fraction III	**−0.754**		0.460	−0.324	
Polysaccharide fraction IV	**0.721**	0.284		**−0.572**	
Total polysaccharides	0.360			**−0.848**	
Total monomeric anthocyanins	**0.986**				
Glucosylated anthocyanins	**0.969**				
Acetylated anthocyanins	**0.968**				
Coumarylated anthocyanins	**0.815**	0.290			
Total hydroxybenzoic acids	0.470	**0.557**	0.483		
Total hydroxycinnamic acids	**−0.905**				
Total hydroxycinnamic acid tartaric esters			**0.767**		
Total monomers	**0.955**				
Total procyanidins	**0.946**				
∑Flavonol glycosides	**0.515**			**0.655**	
Total flavonol aglycones	**−0.722**			**0.599**	
Total stilbenes		0.475		**0.719**	
Total alcohols		**0.767**	−0.435		

[a] Loadings lower than an absolute value of ±0.250 are not shown. Bold numbers indicate the higher loading values that contribute to each factor.

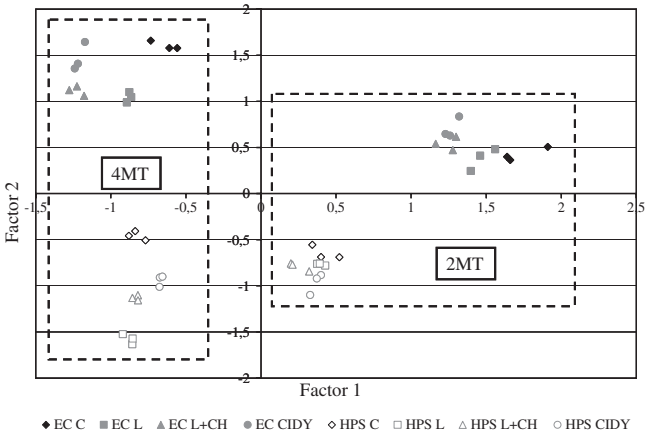

Figure 2. Distribution of different wines defined by factor 1 and factor 2. 2MT, 2 months of treatment; 4MT, 4 months of treatment.

after the 2MT aging period, but statistically significant differences were only found in a few cases. For example, only the HPS wines treated with L + CH presented higher values of red fruits than the control wines. In addition, the EC1118 and HPS wines treated with L shower higher persistence and lower astringency than the control wines. Furthermore, both EC1118 and HPS wines treated with L + CH and CIDY showed higher values of mouth-feel than the control wines. A similar trend was observed after the 4MT aging period, where all treated wines showed lower values of bitterness and astringency and higher persistence and mouth-feel than the control wines, but significant differences were not found in all cases. Additionally, the EC1118 and HPS wines treated with L showed significantly lower values of bitterness than the control wines, and the EC111 wines treated with L and CIDY as well as the HPS wines treated with L presented lower values of astringency. The EC1118 wines treated with L + CH and CIDY and the HPS wines treated with L presented higher values of persistence than the control wines. Finally, only the EC1118 wines treated with L + CH showed higher values of mouth-feel than the control wines.

Del Barrio-Galán *et al.*[1] also studied the effect of aging on lees, chips and commercial yeast derivatives rich in mannoproteins on several gustative attributes of red wines; in general, they discovered lower values of astringency and 'green' tannins and higher values of grassy, balance and overall punctuation than in the control wines. In another study carried out by Del Barrio-Galán *et al.*[8] regarding the effect of six different commercial yeast derivatives rich in mannproteins and with different purification degrees of red wines, they noted that all treated wines presented lower values of 'green' tannins than the control wines. According to their explanation, these types of tannins produce negative sensations, including intense astringent and acidic sensations with strong 'green' or herbaceous notes. Other authors note that the interactions between these products and the tannins can increase roundness and softness on the palate.[2,4,5] Therefore the use of these aging techniques can improve the gustative sensations of red wines, likely owing to the increased liberation of polysaccharides and/or mannoproteins into the wines.

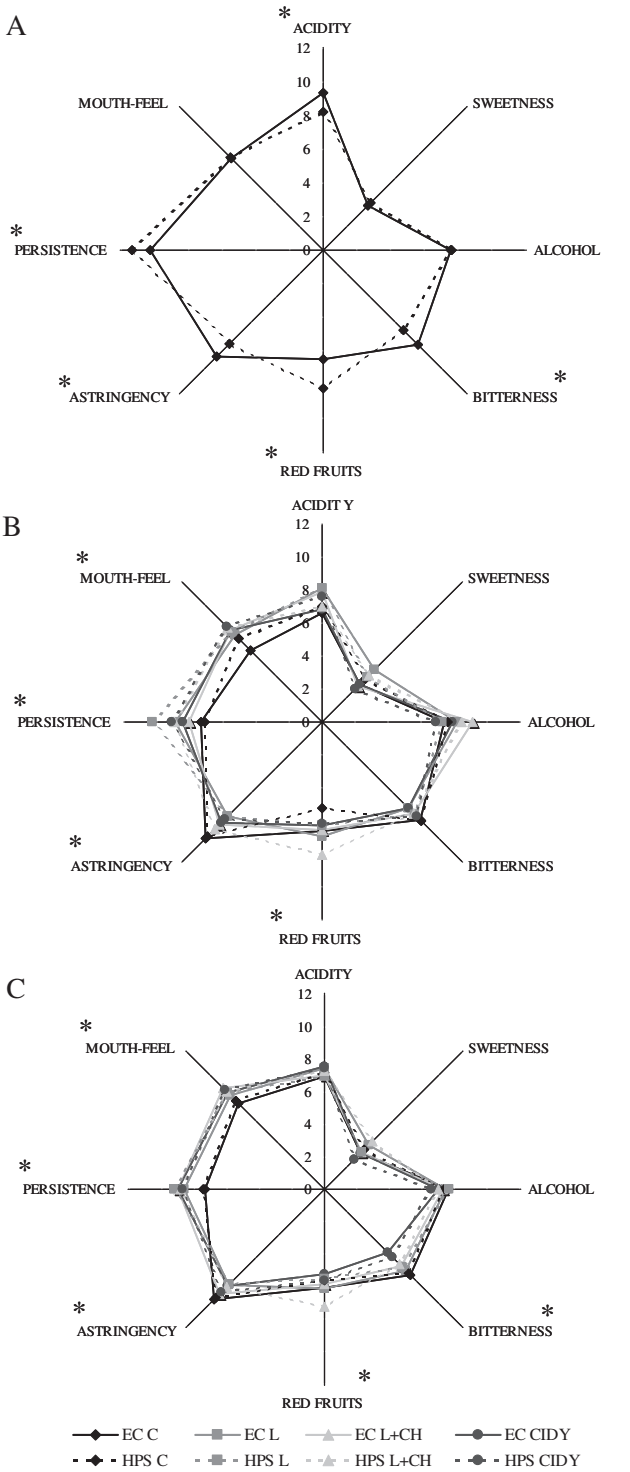

Figure 3. Sensory analysis graphics of different wines after (A) malolactic fermentation, (B) 2 months of treatment and (C) 4 months of treatment. *Statistically significant differences ($P < 0.1$).

CONCLUSIONS

The use of HPS yeast allowed a quicker release of higher amounts of polysaccharides (mainly mannoproteins) with low molecular weights during alcoholic fermentation than the EC1118 yeast. This concentration generally equalized during the aging period, indicating that the EC1118 yeast released these polysaccharides more slowly. The different assayed aging techniques increased the polysaccharide concentration but depended on the technique applied. However, these aging techniques seemed to have more of an effect on the EC1118 wines.

In general, the wines fermented with the HPS yeast strain had lower concentrations of most of the phenolic families analyzed in this study, which could indicate a higher interaction between the polysaccharides released by this yeast and the phenolic compounds of the wines. The different aging techniques assayed in this study also reduced the concentration of several phenolic families analyzed, but this effect depended on the aging technique used, the period of aging and the compound analyzed.

The EC1118 wines seemed to have more positive color stabilization than the HPS wines after the aging period, but no clear effect was found for the different aging techniques used in this study on color stabilization.

The HPS wines were better valued than the EC1118 wines according to the panel of tasters, but no statistically significant differences were found between the two types of wine during the aging period. However, in general, the wines developed with different aging techniques obtained better sensorial values than the control wines, but it was difficult to establish which technique had a better contribution to the improvement of the sensorial quality of the wines. Therefore further research should performed to determine the effect of these aging techniques on the physical, chemical and sensorial characteristics of red wines.

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