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Effect of Aging on Lees and of Three Different Dry Yeast Derivative Products on Verdejo White Wine Composition and Sensorial Characteristics

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ABSTRACT: A study was made of the effect of aging on lees and of three different commercial yeast derivative products of different composition and degree of purification on the phenolic compounds, color, proteins, polysaccharides, and sensorial characteristics of white wines. The results obtained showed that the lees and yeast derivative products can interact or adsorb some of the phenolic compounds present in wines, reducing their concentration. This reduction depends on the treatment applied, the phenolic compound analyzed, and the stage of vinification or aging process. The use of lees and yeast derivative products can reduce the color intensity and the browning of the wines immediately following treatment. The monosaccharide and polysaccharide content of yeast derivative products depends on the manufacturing process and degree of purification of the product, both of which have an influence on wine treatments. After 6 months in the bottle, both the aging on lees and the treatment with commercial yeast derivative products gave rise to wines with better sensorial characteristics than in the case of the control wines.

KEYWORDS: white wine, aging on lees, yeast derivative products, phenolic compounds, polysaccharides

INTRODUCTION

The aging of white wines on lees has been a well-known vinification technique for several years. During this process yeast autolysis occurs and, as a result, different compounds are released into wines, improving their sensory quality.[1,2] Mannoproteins have been described as the most important polysaccharides released during this autolysis process due to their positive effects on the final quality of wines.[2−4] They are liberated during alcoholic fermentation[5−7] and during the aging of wines on lees.[3,8]

Mannoproteins are glycoproteins located in the yeast cell walls, and they play an important role in the whole of the vinification process.[9] They can have, then, an influence on technological characteristics such as the inhibition of tartrate salt crystallization[10,11] and the reduction of protein haze[12−14] of white wines, improving their tartaric and protein stabilities. Furthermore, these compounds can improve the sensorial characteristics of wines, because they affect aroma volatility,[15−17] reduce astringency and bitterness, and enhance the body, structure, and roundness of red wines[9,18,19] and of model wine solutions.[20−22] Some authors have also reported the influence of yeast in the browning delay of white wines as yeast can adsorb certain phenolic compounds, preventing oxidation and, therefore, the formation of browning compounds.[23,24]

Mannoproteins can have other positive effects on wines, such as the adsorption of some mycotoxins (ochratoxin A)[25] or the improvement of foaming characteristics in sparkling wines.[26] Finally, they are involved in velum formation in sherry wines.[27]

However, yeast autolysis is usually a very slow process due to the conditions of pH and temperature at which this process occurs in wines.[28] For this reason, "batonnage" is a very common technique for allowing a faster release of the yeast compounds.

On the other hand, in recent years, commercial yeast derivative preparations are being used as an alternative technique to aging wines on lees, because they permit a quicker release into the wine of yeast compounds (mainly mannoproteins and glucans). The first preparations that appeared on the market were products composed mainly of inactive yeasts, yeast autolysates, and yeast cell walls.[17] These products have a very heterogeneous composition and, in most cases, have a low solubility in wines. Currently, more hydrolyzed and purified products (such as purified mannoproteins) are being offered by commercial suppliers as completely soluble products with immediate effect on wines.

The addition of yeast mannoproteins for tartaric and protein stability was authorized by the European Community in 2005 (EC Regulation 2165/2005), and the use of yeast cell wall preparations (EC Regulation 606/2009) is also authorized to a limit of 40 g/hL in the different winemaking stages to give wines the positive characteristics mentioned above.

No studies have been found relating to the effect of different commercial yeast derivative products on the quality of white wines. For this reason, the aim of this study was to examine the effect of aging on lees and of different commercial yeast derivative products on the phenolic compounds, color, proteins, polysaccharides, and sensorial characteristics of Verdejo white wines. Three commercial yeast derivatives of different compositions and degrees of purification were used. The effect of these treatments on white wines during aging in the bottle for 6 months was also studied.

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Table 1. Characteristics of the Different Commercial Yeast Products Used in White Wines and the Doses Applied

commercial product	dose (g/hL)	expected effect (information provided by the manufacturer)	characteristics
YD 1	40	increase mouthfeel and roundness sensations; decrease astringency and increase the aromatic persistence; improve tartaric and protein stability; favor malolactic fermentation	product with polysaccharides extracted enzymatically from selected yeast walls
YD 2	40	increase aromatic complexity and persistence, improve mouthfeel and gustative balance, reduce astringency and reduction notes; increase fruity notes; improve tartaric and protein stability; prevent wine oxidation	product with parietal polysaccharides from yeast cell walls with high content in free mannoproteins
YD 3	5	improve mouthfeel and softness and persistence in mouth; improve tartaric and protein stability; increase aromatic complexity	product with polysaccharides from yeast cellular walls, highly purified and completely soluble in wine

MATERIALS AND METHODS

Winemaking Process and Treatments. The study was carried out using the Verdejo grape variety from Rueda Designation of Origin, sited in the Autonomous Community of Castilla y León in northern Spain, from the 2008 vintage. The white wines were elaborated in the research winery of the Enological Station of Castilla y León, following the traditional white winemaking process.

The grapes were harvested manually in accordance with °Brix and total acidity values (23 °Brix and 6.7 g/L of tartaric acid) and transported to the Enological Station in 15 kg plastic boxes.

The clusters of white grapes were destemmed, crushed, slightly sulfited (0.05 g/L), and pressed. The must obtained was transferred to stainless steel tanks, and a pectinolytic enzyme preparation was added (2 g/hL of Vinozym FCE, Novozymes) to favor the precipitation of colloidal substances over 24 h at 12 °C. After this period of time, the must was racked off into different stainless steel tanks and inoculated with commercial *Saccharomyces cerevisiae* yeasts (20 g/hL of IOC 18-2007 from Institut Oenologique de Champagne) to undergo alcoholic fermentation at a controlled temperature (16 ± 2 °C).

Once alcoholic fermentation was completed, the wines were kept in the tanks for 4 days to allow sedimentation of the gross lees. Following this, the wines were racked off and kept in the tanks for 4−5 days to allow sedimentation of the fine lees. The base wine was again racked off, homogenized, and distributed into different 150 L tanks in which the different treatments were carried out in duplicate. The wet fine lees decanted in the bottom of the tanks were used in the experiments with lees (L) (3% v/v of fine lees).

Three different commercial yeast derivative products (YD), provided by the same commercial manufacturer (Sepsa-Enartis, Spain), were used for this study. They were selected because, according to the information provided by the commercial manufacturer, these products are rich in glucans and mannoproteins but of a different composition and/or obtained by a different extraction process (Table 1).

Wines without any additional product were used as the control wines (C).

Two batonnages per week were performed on each wine. The temperature was maintained at 15 ± 1 °C. All treatments lasted for 60 days except those that used the YD 3 preparation, which was added in the bottling process as it was recommended by its manufacturer as a result of its high degree of purity and solubility.

After the different treatments, the white wines were clarified with bentonite (100 g/hL), filtered through 0.8 μm membrane plates, and bottled.

The samples were analyzed immediately following fermentation, at the end of the treatment, and, finally, after 3 and 6 months of aging in the bottle.

Chemical Reagents. Gallic acid, D-(+)-catechin, glucose, Coomassie reactive, syringic acid, D-(+)-galacturonic acid, 3-hydroxybiphenyl, phenol, and β-D-allose were provided by Sigma-Aldrich (Steinheim, Germany); *trans*-caffeic acid, kaempferol, tyrosol, tryptophol, and acetic anhydride by Fluka (Buchs, Switzerland); bovine serum albumin, disodium tetraborate decahydrated, trifluoroacetic acid, sodium borohydrure, ethyl acetate, perchloric acid, ammonia, acetone, acetic acid, chloroform, and 1-methylimidazole by Merck (Darmstadt, Germany); and ethyl gallate, quercetin, (−)-epicatechin, and cyanidin chloride by Extrasynthèse (Lyon, France). Acetonitrile and methanol were provided by Lab Scan (Madrid, Spain). The remaining reagents were provided by Panreac (Madrid, Spain). Milli-Q water was obtained through a Millipore (Bedford, MA) system.

Analytical Methods. Enological parameters were evaluated according to official analysis methods (OIV, 1990).

The content of phenolic compounds was evaluated by the quantification of several phenolic families: total polyphenols, expressed in mg/L of gallic acid;[29] total tannins, expressed in mg/L of cyanidin chloride;[30] hydroxycinnamic acid derivatives and flavonols, expressed in mg/L of caffeic acid and quercetin, respectively.[31]

Low molecular weight phenolic compounds were also analyzed by direct injection of the samples in an Agilent Technologies LC-DAD series 1100 chromatograph, following the chromatographic conditions described by Pérez-Magariño et al.[32] The samples were previously diluted in water (1:1) and filtered through PVDF filters of 0.45 μm (Millipore).

Color intensity was evaluated by absorbance measurement at 420 nm.[33]

Proteins were determined by means of the method described by Bradford,[34] and the results were expressed in mg/L of bovine serum albumin (BSA).

Global polysaccharide content was evaluated by spectrophotometry following the method described by Segarra et al.[35] and was expressed in mg/L of galacturonic acid and glucose for acid and total polysaccharides, respectively. Neutral polysaccharides were calculated as the difference between total and acid polysaccharides.

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

Polysaccharide families were also analyzed in white wines by high-performance size exclusion chromatography (HPSEC). First, 5 mL of each wine was concentrated in a rotatory vacuum evaporator and redissolved in 2 mL of water. HPSEC was carried out by loading the previous 2 mL concentrated fraction on a Superdex 30-HR column (60 × 1.6 cm, Pharmacia, Uppsala, Sweden) with a precolumn (0.6 × 4 cm), equilibrated at 0.6 mL/min in 30 mM ammonium formate, pH 5.6. Chromatographic separation was performed with a refractive index detector (Erma-ERC 7512, Erma, Japan) coupled to Waters Baseline 810 software following the conditions described by Ducasse et al.[36] Two different fractions, containing three different polysaccharide families,

were collected according to their elution times. The first fraction contained mannoproteins and polysaccharides rich in arabinose and galactose (PRAGs) (42−53 min), and the second fraction contained mainly the rhamnogalacturonans II (RG-II) (54−61 min) but also mannoproteins and PRAGs of low molecular weight. These fractions were freeze-dried and redissolved in water. This process was repeated four times for complete removal of the ammonium salts. The quantification of polysaccharide families was carried out by quantifying neutral monosaccharide composition by means of gas chromatography (GC-FID) following their release of wine polysaccharides by hydrolysis and conversion in alditol acetates after reduction and acetylation, in accordance with the process described by Ducasse et al.[37] Allose was used as the internal standard. The content of each polysaccharide family was estimated from the concentration of individual glycosyl residues characteristic of well-defined wine polysaccharides.[38]

Estimation of polysaccharide families of commercial yeast derivative products was directly made by quantifying their neutral monosaccharides as alditol acetates by gas chromatography, in accordance with the quantity (mg) of each product used.

Sensory Analysis. Sensory analysis was carried out by a tasting panel comprising 12 persons, all expert tasters from the Regulatory Councils of various Spanish Designations of Origin and wineries. These tasters defined the descriptors used in this sensory analysis, according to the methodology described by González-Sanjosé et al.,[39] 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 an absence of sensation and 7 a very intense perception.

The wines were tasted after the treatments and after 6 months in the bottle.

Statistical Analyses. All of the data were examined by the application of variance analysis (ANOVA) and the least significant difference (LSD) test, which determines statistically significant differences between the means. A 95% confidence interval or significance level of $p = 0.05$ was used.

All of the statistical analyses were carried out using the Statgraphics Plus 5.0 statistical package (Statpoint Technologies, Inc., Warranton, VA).

RESULTS AND DISCUSSION

Enological Parameters. Classic enological parameters were analyzed in white wines to study the effect of the different techniques assayed on these compounds. The data ranges of these parameters were pH between 3.1 and 3.2, total acidity between 5.8 and 6.2 g/L of tartaric acid, alcoholic degree between 12.8 and 13.4, volatile acidity average of 0.2 mg/L of acetic acid, and potassium between 590 and 660 mg/L. No statistically significant differences were found between treated and control wines, which indicates that these treatments did not modify the enological characteristics of the white wines.

Analyses of Different Phenolic Groups and Color. Table 2 shows total polyphenol, hydroxycinnamic acid derivatives, flavonol, and tannin concentrations in white wines. Statistically significant differences were found only in some cases. No statistically significant differences were found between the treated wines and the control wines in total polyphenol content following treatment. However, after 6 months in the bottle, the wines treated with the yeast derivative products showed a lower content than the control wines. The wines treated with YD 3 displayed the lowest values, followed by YD 2 and YD 1, whereas the content in the wines treated with lees was similar to that of the control wines.

Table 2. Total Polyphenols, Hydroxycinnamic Acid Derivatives, Flavonols, and Total Tannin Concentrations, Color Intensity Values, and Protein Concentration of the Elaborated White Wines[a]

	C	L	YD 1	YD 2	YD 3
			Total Polyphenols (mg/L)		
EAF[b]	179	179	179	179	179
0 MB	182	188	190	191	187
3 MB	194a	208c	202b	196ab	196a
6 MB	188d	187cd	180bc	177ab	172a
			Hydroxycinnamic Acid Derivatives (mg/L)		
EAF	42.8	42.8	42.8	42.8	42.8
0 MB	35.2	35.1	34.5	35.4	36.4
3 MB	35.3b	34.4a	34.4a	35.3b	34.3a
6 MB	35.8	36.0	35.2	35.6	36.2
			Flavonols (mg/L)		
EAF	27.0	27.0	27.0	27.0	27.0
0 MB	20.3c	19.6bc	18.5a	19.4b	19.6bc
3 MB	20.5d	18.7b	18.0a	19.8c	19.1b
6 MB	20.8c	20.1b	18.7a	20.1b	20.8c
			Total Tannins (mg/L)		
EAF	358	358	358	358	358
0 MB	314	301	303	307	310
3 MB	282d	268a	279c	278c	272b
6 MB	323b	303a	313a	313a	306a
			Color Intensity		
EAF	0.099	0.099	0.099	0.099	0.099
0 MB	0.042c	0.036a	0.037a	0.040b	0.040b
3 MB	0.043d	0.035a	0.037b	0.041c	0.038b
6 MB	0.040b	0.040b	0.037a	0.040b	0.039b
			Proteins (mg/L of BSA)[c]		
EAF	66.0	66.0	66.0	66.0	66.0
0 MB	11.4a	14.7b	14.0b	13.9b	15.2b
3 MB	10.0	10.2	11.2	11.4	14.0
6 MB	<5	<5	<5	<5	<5

[a] Values with different letters in the same row indicate statistically significant differences ($p < 0.05$), and values without letters indicate no statistically significant differences. [b] EAF, end of alcoholic fermentation; 0 MB, end of treatment; 3 MB, 3 months in bottle; 6 MB, 6 months in bottle. [c] BSA, bovine serum albumin.

Immediately subsequent to treatment, no statistically significant differences were found in the content of hydroxycinnamic acid derivatives, and, with regard to flavonols, the wines treated with YD 1 and YD 2 displayed a lower concentration than the control wines. After 6 months of aging, no statistically significant differences were seen in the content of hydroxycinnamic acid derivatives. However, all of the treated wines presented lower flavonol content than the control wines, with the exception of those treated with YD 3, which maintained a similar content to that of the control wines. The wines treated with YD 1 showed the lowest values.

After treatment, no statistically significant differences were found between the treated wines in total tannin concentration. However, at the end of bottle aging, all of the treated wines showed a lower concentration than the control wines.

Table 3. Concentrations (Milligrams per Liter) of Low Molecular Weight Phenolic Compounds in White Wines[a]

	EAF[b]	0 MB (end of treatment)					3 MB (3 months in bottle)					6 MB (6 months in bottle)				
compound	C	C	L	YD 1	YD 2	YD 3	C	L	YD 1	YD 2	YD 3	C	L	YD 1	YD 2	YD 3
hydroxybenzoic acids																
gallic acid	3.03	3.54a	3.60a	3.80b	3.88b	3.47a	0.51b	0.59c	0.58c	0.56c	0.42a	0.33a	0.46 cd	0.48d	0.44c	0.39b
protocatechuic acid	0.39	0.51a	0.47a	0.61bc	0.62c	0.52a	0.43	0.39	0.40	0.40	0.36	0.40a	0.44bc	0.52d	0.47c	0.42b
syringic acid	0.40	0.39	0.41	0.46	0.53	0.41	0.34d	0.24b	0.16a	0.29c	0.24b	0.14b	0.17b	0.07a	0.18b	0.18b
ethyl gallate	1.04	0.99b	1.00b	0.83a	1.04b	0.98b	0.93c	0.88b	0.72a	0.87b	0.90bc	0.84b	0.85b	0.65a	0.86b	0.86b
total	4.86	5.45a	5.48a	5.69a	6.06b	5.38a	2.21c	2.10b	1.87a	2.11bc	1.93a	1.71a	1.93c	1.72a	1.95c	1.85b
hydroxycinnamic acids																
trans-caffeic acid	0.60	0.83b	0.87c	1.48d	0.87c	0.74a	0.79b	0.80b	1.35d	0.83c	0.74a	0.77a	0.85b	1.37c	0.84b	0.79a
trans-*p*-coumaric acid	0.08	0.29a	0.29a	0.56c	0.29a	0.36b	0.52a	0.54a	0.88b	0.52a	0.53a	0.60b	0.62c	0.99d	0.59b	0.57a
total	0.68	1.12ab	1.16b	2.04c	1.16b	1.10a	1.31ab	1.34b	2.24c	1.35b	1.27a	1.37a	1.47c	2.36d	1.43b	1.37a
hydroxycinnamic acid esters																
trans-caftaric acid	7.55	7.50a	9.59d	8.91b	9.17c	7.31a	7.36b	9.26e	8.64c	8.77d	7.29a	7.47a	9.39d	8.76b	8.87c	7.47a
cis-coutaric acid	0.95	0.87c	0.83bc	0.78b	0.84bc	0.64a	0.49c	0.31a	0.31a	0.49c	0.35b	0.41c	0.38b	0.35a	0.43d	0.43d
trans-coutaric acid	0.78	0.65b	0.67bc	0.42a	0.68c	0.73d	0.78a	1.02d	0.83b	0.79a	0.99c	0.84b	0.90d	0.70a	0.83b	0.86c
trans-fertaric acid	1.57	1.76c	1.71b	1.70b	1.70b	1.63a	1.93c	1.77b	1.73a	1.89c	1.75ab	1.92b	1.91b	1.84a	1.90b	1.91b
total	10.8	10.8b	12.8e	11.8c	12.4d	10.3a	10.6b	12.4e	11.5c	11.9d	10.4a	10.6a	12.6d	11.6b	12.0c	10.7a
flavanol monomers																
(+)-catechin	3.10	3.72bc	3.99 cd	4.24d	3.22a	3.63b	2.93b	2.35a	2.39a	2.29a	2.20a	1.91b	1.92b	1.67a	1.89b	1.90b
(−)-epicatechin	0.64	0.97c	0.75ab	0.73ab	0.78b	0.61a	0.81d	0.72c	0.43a	0.63b	0.62b	0.69ab	0.81bc	0.63a	0.88c	0.82bc
total	3.74	4.69bc	4.74bc	4.97c	4.00a	4.24ab	3.74b	3.07a	2.81a	2.92a	2.82a	2.61b	2.74b	2.30a	2.77b	2.72b
flavanol dimers																
proanthocyanidin B1	2.11	2.61b	2.52b	2.59b	2.10a	2.77b	nd[c]	nd	nd	nd	nd	nd	nd	nd	nd	nd
proanthocyanidin B2	0.679	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
alcohols																
tyrosol	18.64	19.21b	19.76bc	20.62d	20.27 cd	18.52a	16.77	16.33	16.06	16.34	16.77	16.05	16.19	15.95	16.11	16.76
tryptophol	0.62	0.54d	0.51 cd	0.46c	0.31a	0.39b	0.38b	0.24a	0.29a	0.26a	0.25a	0.20	0.20	0.21	0.20	0.19
total	19.26	19.75b	20.27bc	21.08d	20.58 cd	18.91a	17.15	16.57	16.35	16.60	17.12	16.25	16.39	16.16	16.31	16.95

[a] Values with different letters in the same row indicate statistically significant differences ($p < 0.05$), and values without letters indicate no statistically significant differences in each sampling. [b] EAF, end of alcoholic fermentation. [c] nd, not detected.

These results seem to indicate that lees and certain yeast derivative products can reduce the content of some phenolic compounds, such as total polyphenols, flavonols, and tannins. This fact has been pointed out by several studies carried out on model wine solutions,[40−42] in white wines,[23,24] and in red wines,[18] and it may be due to the capacity of yeast or yeast compounds, such as mannoproteins and/or glucans, to adsorb or interact with different wine phenolic compounds.[20,22] The results encountered also suggest that this interaction does not occur immediately after the treatment but over time (Table 2).

The different treatments produced some changes in the color of the white wines (Table 2). The differences found between the wines just after alcoholic fermentation (EAF) and 0 MB were due to the fact that following treatment the wines were clarified with bentonite and filtered, which can reduce the color by adsorption of colored compounds. Subsequent to treatment, all of the treated wines displayed a lower color intensity than the control wines, with the wines treated with lees and YD 1 presenting the lowest color intensity values. These results are in agreement with those found by some authors[23,24] who proposed the use of yeast cell walls as fining agents for the correction of browning in white wines. However, after 6 months of aging, this effect was observed only in wines treated with YD 1.

Analysis of Low Molecular Weight Phenolic Compounds. Table 3 shows the low molecular weight phenolic compounds identified and quantified in white wines. Hydroxycinnamic acids represented 28.2% of total low molecular weight phenolic compounds after treatment and 38.5% after 6 months in the bottle, whereas tyrosol represented 43.1% following treatment and 47.7% after 6 months in the bottle (average values). These compounds were the main phenolic groups in Verdejo wines, as was also observed in other varietal white wines.[43,44]

In general, gallic and protocatechuic acid concentrations increased after the treatments. The wines treated with YD 1 and YD 2 presented a higher concentration of both acids than the control wines after treatment. During bottle aging, the concentration of both acids decreased in all of the wines, and after 6 months, all of the treated wines showed higher concentrations than the control wines, the wines treated with YD 1 being the ones with the highest values.

No statistically significant differences were found in the concentration of syringic acid after treatment. The concentration of this compound decreased during bottle aging, with a greater loss in wines treated with YD 1 after 6 months in the bottle. Similar results were obtained for ethyl gallate, for which the wines treated with YD 1 presented lower concentration than the control wines following treatment and after 3 and 6 months of aging.

The hydroxycinnamic acids evaluated, *trans*-caffeic and *trans*-*p*-coumaric acids, increased in all of the wines after treatment. However, during bottle aging, *trans*-*p*-coumaric acid continued to increase, whereas *trans*-caffeic acid remained relatively constant. After 6 months in the bottle, the *trans*-caffeic acid concentration was higher in all of the treated wines than in the control wines, with the exception of the wines treated with YD 3. The wines treated with YD 1 showed the highest concentration (78% higher than the control wines). As for *trans*-*p*-coumaric acid, the wines treated with lees and YD 1 displayed a higher concentration than the control wines, whereas the wines treated with YD 3 presented lower contents than the control wines.

trans-Caftaric acid was the most abundant tartaric ester quantified, contributing 80% of the total tartaric esters evaluated. This concentration increased slightly in wines after treatment, except in the wines treated with YD 3, which showed the same concentration as the controls. *trans*-Caftaric acid concentration remained stable in all of the wines during bottle aging, and the differences between treatments stayed the same. The wines treated with lees, YD 1, and YD 2 showed a higher concentration of *trans*-caftaric acid than the control wines, with the wines treated with lees presenting the highest concentrations.

cis and *trans*-coutaric acid concentrations decreased in all of the wines after treatment. At this particular stage, only the wines treated with YD 1 and YD 3 showed a lower concentration of *cis*-coutaric acid than the control wines, and those treated with YD 1 also displayed a lower concentration of *trans*-coutaric acid than the control wines. During bottle aging, these compounds showed different trends. Whereas *cis*-coutaric acid continued decreasing, *trans*-coutaric acid increased in all wines up to 3 months, remaining constant during the last 3 months. The wines treated with YD 1 also had the lowest concentrations of both acids after 6 months in the bottle.

trans-Fertaric acid increased in all wines after treatment, and all of the treated wines showed a lower concentration than the control wines. This concentration continued to augment in all of the wines during bottle aging, although only the wines treated with YD 1 had a statistically significant lower concentration than the control wines after 6 months in the bottle.

The flavanol monomers, (+)-catechin and (−)-epicatechin, and proanthocyanidins B1 and B2 were detected and quantified after alcoholic fermentation (Table 3). However, proanthocyanidin B2 was not detected either after treatment or during aging in the bottle. Both flavanol monomers increased in the wines after treatment, and some statistically significant differences were found. Thus, only the wines treated with YD 2 showed a lower (+)-catechin concentration than the control wines, whereas the concentration of (−)-epicatechin was statistically significant, lower in all of the treated wines than in the control wines, with those treated with YD 3 showing the lowest concentration. The concentration of proanthocyanidin B1 also increased after treatment in all of the wines, with the exception of those treated with YD 2, which showed concentrations similar to the ones found at the end of alcoholic fermentation. These wines also showed a statistically significant lower concentration than the other wines.

During bottle aging, (+)-catechin concentration decreased in all of the wines. After 6 months of bottle aging, the wines treated with YD 1 were the only ones that had lower concentrations of this compound than the control wines (a reduction of 12.5%). During bottle aging, the concentration of (−)-epicatechin followed different trends, and after 6 months all of the treated wines displayed concentrations similar to those of the control wines, with the exception of the wines treated with YD 2, which showed the highest concentration.

Proanthocyanidin B1 was not detected during bottle aging.

No studies have been found relating to the effect of commercial yeast products on the concentration of low molecular weight phenols in white wines. Only Razmkhab et al.[23] and López-Toledano et al.[24] have examined the use of inactive yeast or yeast cell walls in white wines. Both studies found a reduction of brown polymers. However, contradictory results were obtained regarding the concentration of hydroxycinnamic acids and flavanols. Razmkhab et al.[23] observed that the addition of yeast reduced the concentrations of *trans*-caftaric acid, catechin, epicatechin, and procyanidins, whereas López-Toledano et al.[24] reported higher caftaric acid and catechin concentrations in wines with added yeast than in wines without yeast. Moreover, these authors found no effect from the addition of yeast on procyanidin content. Generally speaking, in this study the wines treated with lees showed a higher content of hydroxycinnamic acids, in both free and esterified forms, than in the case of the control wines. This effect was also observed in the wines treated with YD 1 and YD 2. However, no clear effect was detected for flavanol compounds. These differing results could be due to several causes. On the one hand, each yeast or commercial product may give rise to different compounds or fragments of variable size, with different active sites for retaining phenols.[23] On the other hand, the concentration of certain phenolic compounds depends on the balance between the oxidation and polymerization reactions that will produce a decrease in the concentration of these compounds, as well as on the hydrolysis of higher oligomers that will increase the presence of these flavanols in wines.[45]

Kaempferol was the most important flavonol detected after alcoholic fermentation (0.421 mg/L). Other flavonols such as quercetin (0.031 mg/L) and quercetin-3-*O*-glycosides (0.020 mg/L) were also detected, albeit at low concentrations (Table 3). However, after treatment and during aging in the bottle, these flavonol compounds were detected below the quantification limit of the method used.

Neither myricetin nor its 3-*O*-glycoside derivatives were found in the white wines. As was reported by other authors, this type of flavonol is considered to be exclusively of red grape varieties.[46] Castillo-Muñoz et al.[47] determined the different flavonol types present in several *Vitis vinifera* white grape varieties, myricetin and its 3-*O*-glycoside derivatives being undetected in any of them.

Tyrosol and tryptophol are alcohols that are formed from deamination and decarboxylation reactions of tyrosine and tryptophan amino acids, respectively, during yeast fermentation.[48] Tyrosol was the most abundant and represented about 97% of total alcohols. In general, the content of this compound increased slightly in all of the wines after treatment, and several statistically significant differences were found. For instance, the wines treated with YD 1 and YD 2 manifested a higher concentration of tyrosol than in the case of the control wines, with the wines treated with YD 3 representing the poorest in this regard. However, during bottle aging, this compound decreased in all of the wines, and no statistically significant differences were encountered between the treated and control wines.

Tryptophol concentration decreased after treatment and throughout aging in the bottle. In general, statistically significant differences were undetected, and only after treatment did the wines treated with the commercial yeast derivative products reveal a lower concentration of tryptophol than in the case of the control wines and those treated with lees.

Table 4. Monosaccharide Percentage and Polysaccharide Concentration (Milligrams per Gram) in the Different Commercial Yeast Derivative Products[a]

	YD 1	YD 2	YD 3
monosaccharides			
2-*O*-methylfucose	nd[b]	nd	nd
rhamnose	0.057	0.092	0.073
fucose	nd	nd	nd
2-*O*-methylxylose	nd	nd	nd
arabinose	0.475a	1.72b	0.336a
apiose	nd	nd	nd
xylose	0.145	0.102	0.099
mannose	29.3a	59.8b	77.1c
galactose	0.477	0.154	0.220
glucose	69.5c	38.1b	22.2a
polysaccharides[c]			
MPs	99.9a	186.9b	407.5c
RG-II	nd	nd	nd
PRAGs	3.02	4.82	2.66
total	103.0a	191.7b	410.1c

[a] Values with different letters in the same row indicate statistically significant differences ($p < 0.05$), and values without letters indicate no statistically significant differences. [b] nd, not detected. [c] MPs, mannoproteins; RG-II, rhamnogalacturonans II; PRAGs, polysaccharides rich in arabinose and galactose.

Table 5. Monosaccharide and Estimated Polysaccharide Concentrations (Milligrams per Liter) in the White Wines after 6 Months[a]

	C	L	YD 1	YD 2	YD 3
monosaccharides					
2-*O*-methylfucose	0.25	0.27	0.25	0.27	0.25
rhamnose	3.35b	1.40a	1.41a	1.41a	1.50a
fucose	0.23	0.31	0.15	0.15	0.11
2-*O*-methylxylose	0.18	0.18	0.20	0.20	0.27
arabinose	3.47	2.85	2.33	3.11	2.57
apiose	0.31	0.36	0.39	0.40	0.54
xylose	0.61	0.30	0.16	0.16	0.10
mannose	58.7a	48.0a	82.7b	90.2b	59.0a
galactose	17.9c	15.6bc	7.7a	18.0c	14.0b
glucose	3.50	2.23	2.22	2.98	2.30
polysaccharides					
MPs	73.3a	59.9a	103.3b	112.7b	73.8a
RG-II	27.7	29.0	28.8	30.4	33.4
PRAGs	25.9b	22.1b	11.1a	25.5b	19.4b
total	126.9ab	111.0a	143.2b	168.6c	126.6ab

[a] Values with different letters in the same row indicate statistically significant differences ($p < 0.05$), and values without letters indicate no statistically significant differences.

Analyses of Polysaccharides and Proteins. *Monosaccharide and Polysaccharide Content in the Commercial Yeast Derivative Products.* Table 4 shows the monosaccharide percentage of each commercial yeast derivative preparation. Mannose and glucose were the main monosaccharides quantified in these products, as was expected due to their being the main components of microbial polysaccharides.[49] However, differences in the relationship between glucans and mannoproteins were found. The percentage of glucose, used to estimate glucan content, was highest (69.5%) in YD 1, which indicates that during the process to obtain this product more glucans are extracted than mannoproteins. On the other hand, YD 2 and, especially, YD 3 showed higher mannose contents, 59.8 and 77.1%, respectively; this may indicate a greater purification process. These results agree with the information provided by the manufacturer (Table 1), who points out that YD 2 and YD 3 have high contents of free and highly purified mannoproteins.

The concentrations of the different polysaccharide families were estimated from the monosaccharide concentration (Table 4). Thus, mannoprotein concentration was calculated directly from the concentration of mannose, and it was observed that YD 3 preparations showed the highest concentration, approximately 2 and 4 times higher than the concentrations in YD 2 and YD 1, respectively. RG-II was calculated from the concentration of apiose, 2-*O*-methylfucose, and 2-*O*-methylxylose, which were not detected in the commercial products; this was expected because this type of polysaccharide results from the enzymatic degradation of grape pectins.[49]

Finally, it is important to point out the presence in these products of other monosaccharides such as galactose and arabinose, which are constituents of arabinogalactan-proteins (PRAGs), a type of polysaccharide that originates from the pectocellulosic cell walls of grape berries.[6] Consequently, these results seem to indicate the presence of some polysaccharides that do not come from yeast, despite the fact that the concentrations found were low, representing between 0.6 and 3.0% of total polysaccharide concentration. The presence of these compounds could be related to the manufacturing process of the yeast derivative commercial products.

Monosaccharide and Polysaccharide Contents in White Wines. Table 5 shows the monosaccharide concentration of white wines and the estimated polysaccharide concentration at the end of bottle aging. Statistically significant differences in only the rhamnose, mannose, and galactose monosaccharide concentrations were found. Thus, mannoprotein concentration estimated from mannose was higher in the wines treated with YD 1 and YD 2 than in the control and the other treated wines. This indicates that these commercial yeast derivative products release more polysaccharides into the wines than the lees or the YD 3 product. These results demonstrated that lees did not release neutral polysaccharides or mannoproteins from yeast cell walls during autolysis, probably due to the short period of time involved in this treatment.

Although the YD 3 product was the richest in mannoprotein content (Table 4), the wines treated with YD 3 showed a similar mannoprotein concentration to that of the control wines. This could be due to the fact that, in line with the manufacturer's instructions, the maximum recommended doses of YD 3 were added (5 g/hL). This amount was 8 times lower than the added doses of YD 1 and YD 2 (40 g/hL), which was also the maximum dose recommended by the manufacturer. Therefore, although the latter indicates that YD 3 contains highly purified mannoproteins which are completely soluble in wines, the maximum doses recommended are not enough to observe certain effects in the polysaccharide contents of wines.

The wines treated with YD 1 showed a statistically significant lower concentration of PRAGs than the control wines and the other treated wines. This was mainly due to the lower content in galactose encountered in the wines treated with YD 1.

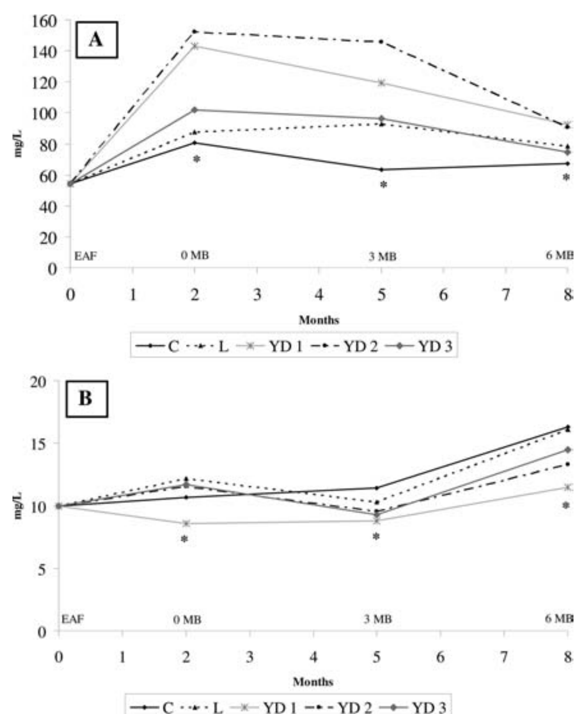

Figure 1. Neutral (A) and acid (B) polysaccharide concentrations in white wines. EAF, end of alcoholic fermentation; 0 MB, end of treatment; 3 MB, 3 months in bottle; 6 MB, 6 months in bottle. The asterisk indicates statistically significant differences for $p = 0.05$.

No statistically significant differences were found in the concentration of RG-II.

Polysaccharides by UV–Vis Spectrophotometry. Figure 1 shows the evolution of neutral (A) and acid (B) polysaccharides in elaborated white wines, showing certain statistically significant differences. Total polysaccharides revealed a trend similar to that of neutral polysaccharides. Total (TPS) and neutral polysaccharides increased in all of the wines, including the control wines (Figure 1A), from the end of alcoholic fermentation until the end of the treatment. This fact could indicate that these compounds remain in wine in a colloidal state linked to other compounds or that they originate from the autolysis of the remaining dead yeasts present in the wine. However, this increase was statistically significantly higher in the wines treated with the different commercial yeast derivative products than in the control wines and the wines treated with lees. The wines treated with YD 2 showed the highest content of neutral polysaccharides, followed by the wines treated with YD 1 and YD 3. During bottle aging, all of the wines showed a decrease of TPS and NPS, more noticeable in the wines treated with the yeast derivative products, especially in the wines treated with YD 1 and YD 2. However, after 6 months in the bottle, the wines treated with YD 1 and YD 2 continued to show the highest values for neutral polysaccharides compared with the control wines and then the wines treated with lees. This decrease could be due to the formation of unstable complexes between the polysaccharides and other phenolic compounds, which, as has been pointed out by other authors with regard to red wines,[18,50] might precipitate.

As expected, the APS concentration remained relatively constant throughout the whole vinification and aging process in all of the wines, and only slight differences were found between the different treatments (Figure 1B).

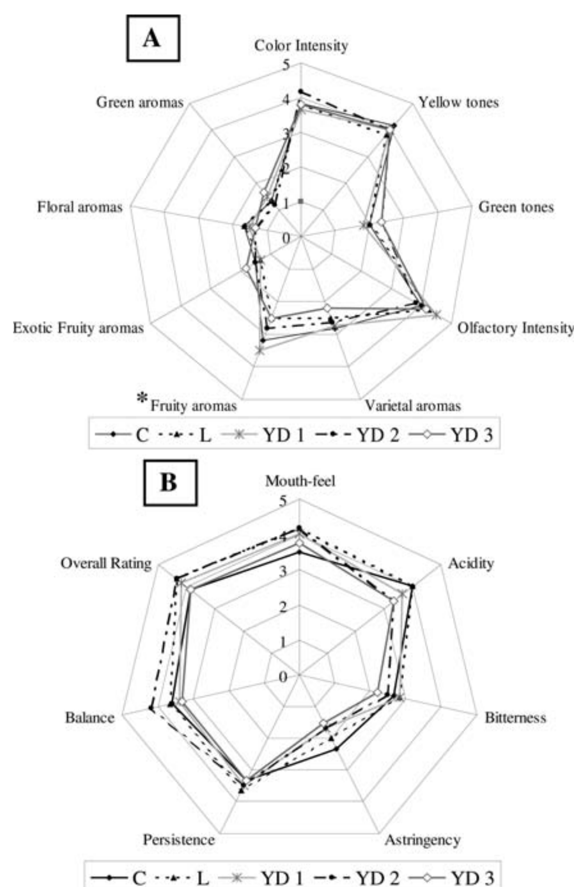

Figure 2. Sensory diagrams of the color and the olfactory phase (A) and the gustative phase (B) in white wines at the end of treatment. The asterisk indicates statistically significant differences for $p = 0.05$.

The results of neutral polysaccharides found by spectrophotometry are in agreement with those found by HPSEC and GC, with the wines treated with YD 1 and YD 2 showing the highest concentrations. Therefore, the spectrophotometric method, which can be carried out more quickly and easily, might be used by winemakers to estimate the neutral polysaccharide concentration that a certain commercial product could release into a wine.

Proteins. Table 2 shows the protein concentration of the different wines, which decreased strongly in all wines after treatment; this was due to their clarification with bentonite immediately following treatment and prior to being bottled. At this moment, all of the treated wines showed a higher concentration of proteins than the control wines. This concentration continued decreasing in all wines during bottle aging, and after 6 months, all of the wines showed a protein concentration lower than 5 mg/L, with no statistically significant differences being detected between them.

Sensory Analysis. No statistically significant differences were found in the color parameters between the treated and control wines after the treatment (Figure 2A) or after the aging period (Figure 3A).

In the olfactory phase, some statistically significant differences were seen after treatment (Figure 2A) and the bottle aging period (Figure 3A). Following treatment, all of the treated wines presented lower fruity aromas than the control wines, except the wines treated with YD 1. This could be due to the interaction between volatile compounds and other metabolites such as mannoproteins and/or other polysaccharides released by lees

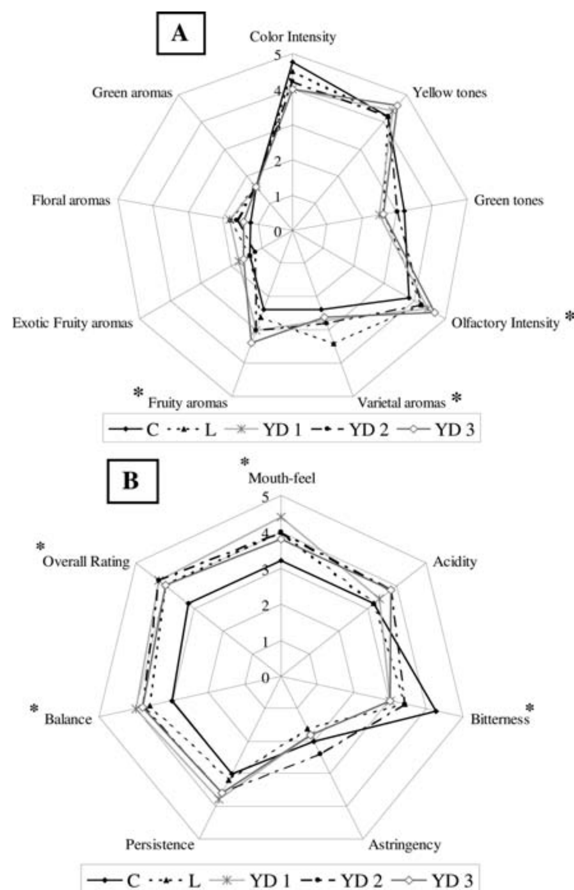

Figure 3. Sensory diagrams of the color and the olfactory phase (A) and the gustative phase (B) in white wines after 6 months in bottle. The asterisk indicates statistically significant differences for $p = 0.05$.

and yeast derivatives, which can reduce the volatility of some aromatic wine compounds. Similar interactions have been observed by other authors in model wine solutions[15,16,51] and in previous studies in white and red young wines using other commercial products.[52,53] In general, after 6 months in the bottle (Figure 3A), all of the wines treated displayed stronger varietal, fruity, and floral aromas and higher olfactory intensity than the control wines. This might indicate that these initially retained aromatic compounds are released over time, increasing aroma intensities.

In the gustative phase, all of the treated wines showed, generally speaking, higher values of mouthfeel and overall rating and lower values of acidity and astringency than the control wines after treatment (Figure 2B); this was especially the case for wines treated with the commercial yeast derivative products, although no statistically significant differences were detected. However, after 6 months in the bottle (Figure 3B), statistically significant differences were found. All of the wines treated with commercial yeast derivative products and those wines treated with lees showed less bitterness and stronger mouthfeel, persistence, balance, and overall rating values than the control wines. This indicates that the treated white wines evolved better than the control wines throughout the aging period.

To summarize, the results found in this study have indicated that lees and yeast derivative products can interact or adsorb some of the phenolic compounds present in wines, reducing their concentration. This reduction depends on the treatment applied, the phenolic compound analyzed, and the stage of vinification or aging process.

The use of lees and yeast derivative products can give rise to a reduction in the color intensity of wines immediately after treatment, so they can be used as agents for reducing browning in white wines.

The monosaccharide and polysaccharide contents of the commercial yeast derivative products depends on the manufacturing process and the product's degree of purification.

The results obtained for total, neutral, and acid polysaccharides in white wines by means of HPSEC-GC agreed with those obtained by spectrophotometric analysis. Therefore, the spectrophotometric method could be used as a fast and easy enological method to determine the concentration of total, neutral, and acid polysaccharides of a wine. However, a larger number of samples should be analyzed, and correlation studies between the results obtained with the two methods should be carried out to corroborate this.

The effects on the chemical composition and sensory characteristics of the wines depended on the YD product used, although in general it can be said that YD 3 does not improve the quality of the wine. The other two YD products and aging on lees gave rise to wines with better sensorial characteristics than the control wines, especially after 6 months in the bottle, which means it is difficult to establish which one produces the best quality wine.

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