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Effect of Commercial Mannoprotein Addition on Polysaccharide, Polyphenolic, and Color Composition in Red Wines

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Commercially available mannoprotein preparations were tested in Tempranillo winemaking to determine their influence on polysaccharide, polyphenolic, and color composition. No effect was found in the content of grape arabinogalactans, homogalacturonans, and type II rhamnogalacturonans. In contrast, mannoprotein-treated samples showed considerably higher values of high-molecular-weight mannoproteins (bMP) than controls from the beginning of alcoholic fermentation, although these differences diminished as vinification progressed. The bMP decrease observed in the mannoprotein-treated samples coincided with a substantial reduction in their proanthocyanidin content and wine stable color, suggesting a precipitation of the coaggregates mannoprotein−tannin and mannoprotein−pigment. Contrary to what is widely described, these results revealed that at the studied conditions, mannoproteins did not act as stabilizing colloids. Mannoprotein addition did not modify the content and composition of either monomeric anthocyanins or other monomeric phenolics, and it did not affect monomeric anthocyanin color.

KEYWORDS: Tempranillo red wine; winemaking; mannoproteins; polysaccharides; monomeric polyphenols; proanthocyanidins; colloidal stability

INTRODUCTION

Wine technology is evolving continually in order to satisfy current consumer preferences. The growing trend toward the consumption of red wines aged in new barrels and with high phenolic content involves the use of enological practices that produce wines with greater body, better mouthfeel, and overall stability. Natural yeast mannoproteins, initially used for the chemical stabilization of white wines, have recently attracted the attention of enologists for the making of red wines, not only for their well-known effect on wine stability but also for their positive influence on a number of technological and quality properties of red wines. In fact, yeast cell wall mannoproteins play a very important role in the overall vinification process, and most of their technological functions have been widely described: (a) inhibition of tartrate salt crystallization (*1*−*4*), (b) reduction of protein haze (*5*−*9*), (c) stimulation of malolactic fermentation (*10*−*12*), (d) wine enrichment during autolysis of lees (*13, 14*), (e) interaction with *flor* wines (*15, 16*), (f) yeast flocculation and autolysis in sparkling wines (*17*), and (g) adsorption of toxic ochratoxin A (*18*−*21*).

More interestingly, yeast mannoproteins have been described for their positive effect on the different sensorial properties of red wines. Initially described for their interaction with aromatic compounds (*22*−*25*), recent studies relate yeast mannoproteins with other wine sensory properties, including color stabilization (*26*−*29*), reduction of astringency (*26, 30, 31*), and increased body and mouthfeel (*24, 26, 30, 31*). These properties, especially those of red wines, are very important for final wine quality, and in fact, it is due to these effects that numerous wine industries are introducing mannoprotein-based products in different stages of red winemaking. The use of these products may even be seen as an alternative to the traditional technique of wine aging on lees. It is well-known that the advantages of wine maturation on lees are due to the lysis of dead yeast cells in fine lees and mainly to mannoproteins released in this process. However, this is a very complex and slow process that may require months or years, can create microbiological and organoleptic risks, and involve an important immobilization of the wine cellar resources (*32*).

The positive effect of yeast mannoproteins on the sensorial quality of red wines does attract the wine industry, and researchers in enology are obviously showing a growing interest to better understand the effect of these polysaccharides in such sensory properties. In this sense, some studies have been developed in model systems in order to explain the interactions between mannoproteins and polyphenols (*33, 34*). To get closer to the real winemaking process, we explored different winemaking techniques to increase the concentration of this polysaccharide and studied the effects produced on the wines obtained (*31*). The following techniques were explored: (a) the addition of exogenous commercial mannoproteins directly to musts; and

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(b) the use of selected active yeast that produces high levels of mannoproteins during alcoholic fermentation. Exogenous mannoproteins clearly modified the gustative and aromatic structure of wines and seemed to have clearer effects on the analyzed parameters than yeast (*31*). Contrary to what was described in model solutions for mannoproteins purified from red wines (*26, 33, 34*), we found that the use of commercial mannoproteins in real vinification situations did not maintain the extracted polyphenols in colloidal dispersion, and neither seemed to ensure color stability. Taking into account these unexpected findings, and on the basis that they were obtained from the sensory evaluation of the wines and general enological parameters, we thought that a more detailed analytical study should be performed in order to confirm these observations.

Therefore, the aim of this article was to study the effect of the use of commercial mannoprotein-rich preparations from yeast in red winemaking on the content and profile of wine polysaccharide and polyphenolic families, and on wine color.

MATERIALS AND METHODS

Reagents and Samples. All chemicals used were of analytical reagent grade. L-Fucose, L-rhamnose, 2-*O*-methyl-D-xylose, L-arabinose, D-xylose, D-galactose, D-glucose, D-mannose, Kdo (3-deoxy octulosonic acid), vanillin, and gallic acid were supplied by Sigma (St. Louis, MO), and D-galacturonic, D-glucuronic acid, and myo-inositol were supplied by Fluka via (Sigma). D-Apiose was obtained from Omicrom (South Bend, IN), and malvidin-3-glucoside, peonidin-3-glucoside, ferulic acid, syringic acid, caffeic acid, *p*-coumaric acid, catechin, epicatechin, myricetin, quercetin, isorhamnetin, kaempferol, and rutin were purchased from Extrasynthèse (Lyon, France). Ethanol, formic acid, acetonitrile, acetone, acetyl chloride, sulfuric acid, sodium metabisulphite, and tartaric acid were supplied by Scharlab (Barcelona, Spain), and sodium hydroxide, hydrochloric acid, and phosphoric acid were supplied by Carlo Erba (Rodano, Milan, Italy). Dried methanol, disodium tetraborate, and acetaldehyde were supplied by Merck (Darmstadt, Germany), and the trimethylsilylation reagent (TriSil) was obtained from Pierce (Rockford, MA). HPLC-grade ammonium formiate and trifluoroacetic acid supplied by Fluka and Milli-Q water (Millipore, Molsheim, France) were used. A pullulan calibration kit (Shodex P-82) was obtained from Waters (Barcelona, Spain).

Wine samples were produced using *Vitis Vinífera Tempranillo* grapes from the qualified origin denomination Rioja (D.O.Ca. Rioja). Commercial mannoprotein preparation and yeast (*S. cerevisiae* yeast strain RC212) and bacterial (*Oenococcus oeni* Alpha strain) strains were purchased from Lallemand (Lallemand-Inc., Montreal, Canada).

Vinification and Sample Collection. Mature Tempranillo grapes were harvested from Autol, La Rioja, Spain, at 21.9 °Brix, pH 3.56, and 6.02 g tartaric acid/L. Experimental vinifications were carried out in the wine cellar of the University of La Rioja, and wines were prepared using traditional wine technology. Grapes were destemmed, crushed, distributed into six 100 L stainless steel tanks, and inoculated with 25 g/HL *S. cerevisiae* yeast strain RC212. Thereafter, 13.5 g/HL of industrial mannoproteins were added to three of the tanks (212M), while the rest remained as control vinifications (212). The prefermentation process lasted for 6 h at 18 ± 1 °C; the fermentation−maceration process was performed at a maximum temperature of 28 ± 2 °C and went on for 10 days. Postfermentative maceration went on for 4 days at 24 ± 1 °C, and wines were runoff. Wines were then inoculated with a commercial preparation of *Oenococcus oeni* (1 g/HL) to induce malolactic fermentation, carried out at 18.5 ± 1 °C. After malolactic fermentation, all of the wines were racked and clarified by settling for 25 days at 10 °C. Wine aging was performed in new 13-L American oak barrels, which are of higher area/volume than the traditional 225-L barrels. For this reason and on the basis of organoleptic analysis, the oak aging process went on for only 45 days. Wines were then bottled and stored at 4 °C.

Samples were taken at the beginning of maceration−fermentation (0AF), during maceration−fermentation (25−30% of sugars consumed, 55−60% of sugars consumed, and 99% of sugars consumed, namely, 30AF, 60AF, and 99F, respectively), and at the beginning and end of malolactic fermentation (BMF and EMF). Sample bottles were filled completely to minimize oxygen contact and immediately frozen at −18 °C. Samples were also analyzed at the beginning and end of wine oak aging (BA and EA). All wines were analyzed for pH, ethanol concentration, titratable and volatile acidity, and free and total SO_2 according to the OIV official practices (*35*) prior freezing or cooling (**Table 1**). Polymerase chain reaction (PCR) and pulsed field gel electrophoresis (PFGE) analyses were used to ascertain the dominance of the inoculated yeast and bacterial strains (*36, 37*).

Table 1. Enological Parameters of Wines

wine	stage[a]	alcohol[b]	pH	TA[c]	VA[d]	TSO_2[e]	FSO_2[f]
212	99AF	12.6	3.82	5.81	0.19	33	2
	BMF	12.6	3.79	5.2	0.23	33	1.5
	EMF	12.6	3.95	3.71	0.24	70	25
	BA	12.5	3.97.	3.71	0.29	79	30
	EA	12.6	3.98	3.98	0.33	80	20
212M	99AF	12.6	3.75	5.63	0.21	31	5
	BMF	12.6	3.81	5.6	0.2	34	2
	EMF	12.6	3.94	3.92	0.26	68	28
	BA	12.5	3.94	3.86	0.31	74	29
	EA	12.7	3.95	3.77	0.34	73	19

[a] Vinification stage: 99AF, end of alcoholic fermentation; BMF, beginning of malolactic fermentation; EMF, end of malolactic fermentation; BA, beginning of oak aging; EA, end of oak aging. [b] Milliliter of ethanol for 100 mL of wines at 20 °C. [c] Titratable acidity as g of tartaric acid per liter. [d] Volatile acidity as g of acetic acid per liter. [e] Total sulfur dioxide (mg/L). [f] Free sulfur dioxide (mg/L).

Isolation of Soluble Polysaccharides. Samples were homogenized, and 400 mL was taken and centrifuged. The supernatants were concentrated under reduced pressure as previously described (*38*), and polysaccharides were then precipitated by adding four volumes of cold ethanol/acid and kept for 18 h at 4 °C. Thereafter, the samples were centrifuged, the supernatants discarded, and the pellets were washed several times with 96% ethanol. The precipitates were finally dissolved in ultrapure water and freeze-dried. The freeze-dried precipitates obtained (S fractions) contained the soluble polysaccharides.

Fractionation of Polysaccharide Families by HRSEC. In order to separate the different polysaccharide families, the soluble S fractions were subjected to high resolution size-exclusion chromatography on a Superdex-75 HR (1.3 × 30 cm) column (Pharmacia, Sweden) equilibrated at 0.6 mL/min in 30 mM ammonium formiate, pH 5.8. Chromatographic separation was carried out at room temperature on an Agilent modular 1100 liquid chromatograph (Waldbronn, Germany) as previously described (*38*). The peaks obtained were collected in different fractions (S1, S2, and S3) according to their elution times. The isolated fractions were freeze-dried, redissolved in water, and freeze-dried again four times to remove the ammonium salt. The molecular weight distribution of the fractions S1, S2, and S3 was followed by HRSEC on two serial Shodex columns as previously described (*39*). Calibration was performed with narrow pullulan molecular weight standards (P-5, Mw = 5,900 D; P-10, Mw = 11,800 D; P-20, Mw = 22,800 D; P-50, Mw = 47,300 D; P-100, Mw = 112,000 D; P-200, Mw = 212,000 D; P-400, Mw = 404,000 D). The apparent molecular weights were deduced from the calibration equation $M_w = 11.182 - 0.405t_R$ (t_R = column retention time at peak maximum, and $r^2 = 0.997$).

Estimation of Polysaccharide Concentrations by GC and GC-MS. The carbohydrate composition of the fractions (fractions S, S1, S2, and S3) was determined by gas chromatography with a flame ionization detector and GC-MS of their trimethylsilyl-ester *O*-methyl glycosyl residues obtained after acidic methanolysis and derivatization. GC was performed with a Hewlett-Packard HP5890 chromatograph using a fused-silica capillary column (30 m × 0.25 mm × 0.25 μm, Teknokroma, Barcelona, Spain) with helium as carrier gas and the rest of the conditions as previously described (*38*). Total sugars were calculated from the sum of all monosaccharides from S fractions. The content of each polysaccharide family in fractions

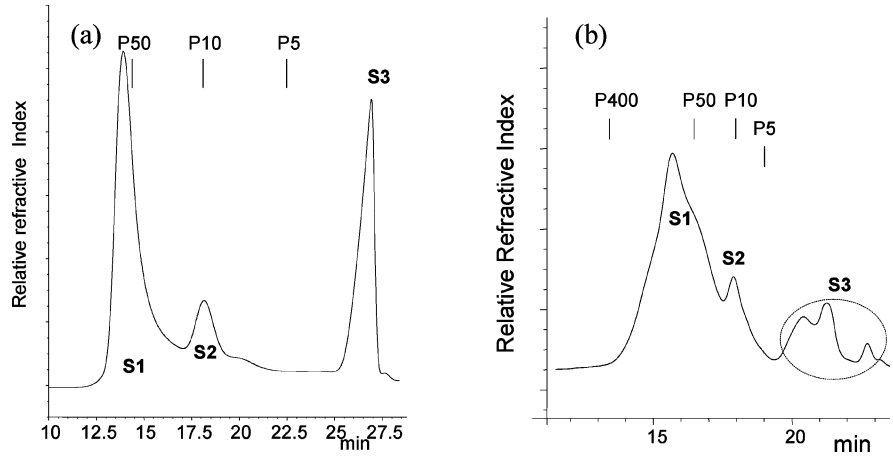

Figure 1. Molecular weight distribution of wine Fractions S1, S2, and S3 by HRSEC on a Superdex 75-HR column (**a**) and Shodex columns (**b**). Elution times of pullulan standards (P5 → P400) are also shown.

S1, S2, and S3 was estimated from the concentration of individual glycosyl residues characteristic of well-defined wine polysaccharides (*38, 40*).

Fractionation of Phenolics by GPC. Samples were directly fractionated by gel permeation chromatography on a Toyopearl gel HP-50F (Tosohaas, Montgomery-ville, PA, USA) as described by Guadalupe et al. (*38*). A first fraction (F1) was eluted with ethanol/water/trifluoroacetic acid (55:45:0.05, v/v/v), and a second fraction (F2) was recovered by elution with acetone/water (60:40, v/v). Both fractions were taken to dryness under vacuum.

Determination of Monomeric Phenolic Compounds by HPLC-DAD. F1 fractions were subjected to HPLC-DAD on a Kromasil 100-C18 reverse phase column (5 μm packing, 200 × 4.6 mm i.d.) protected with a guard column of the same material (Teknokroma, Barcelona, Spain). Chromatographic separation and quantification of monomeric phenolics was carried out on an Agilent modular 1100 liquid chromatograph (Waldbronn, Germany) as previously described (*41*).

Anthocyanin glucosides (A-Glu) were calculated as the sum of delphinidin, cyanidin, petunidin, peonidin, and malvidin-3-glucosides; acetyl-glucoside anthocyanins (A-Ac) as the sum of delphinidin, cyanidin, petunidin, and malvidin-3-(6-acetyl)-glucosides; and coumaryl-glucoside anthocyanins (A-Cm) included delphinidin, petunidin, and malvidin-3-(6-*p*-coumaryl)-glucosides. The sum of A-Glu, A-Ac, and A-Cm was referred to as total monomeric anthocyanins (TMA). Total hydroxycinnamic acids (TCin) were calculated as the sum of *trans*-caftaric (*trans*-caffeoyl-tartaric acid), *cis*-caftaric (*cis*-caffeoyl-tartaric acid), *trans*-coutaric (*trans-p*-coumaryl-tartaric acid), *cis*-coutaric (*cis-p*-coumaryl-tartaric acid), caffeic, and *trans-p*-coumaric acid. Monomeric flavanols (M-Flava) included (+)catechin, (−)epicatechin, and (−)epigallocatechin.

Determination of Total Proanthocyanidin Content. Total proanthocyanidins (PAs) were quantified in F2 fractions by the vanillin assay according to the method described by Sun et al. (*42*) but with few modifications (*41*). The spectrophotometric measurements were performed on a Cary 300 Scan UV−vis spectrophotometer (Varian Inc., Madrid, Spain).

Determination of Color Parameters and Total Polyphenol Index. Wine color, monomeric anthocyanin color, copigmentation color, and bisulfite-stable color were determined by the method proposed by Levengood and Boulton (*43*). The CIE tristimulus values (*X*, *Y*, *Z*) and CIELAB rectangular (*L**, *a**, and *b**) parameters (illuminant D65 and 10° observer conditions) were determined according to Ayala et al. (*44*). Color intensity was calculated as the sum of absorbances at 420, 520, and 620 nm, and visual color was analyzed by sensory analysis of the wines as previously described (*31*). The total polyphenol index (TPI) was determined by absorbance at 280 nm of diluted wine with synthetic wine (12% alcohol, 5 g/L of tartaric acid in water, pH 3.6).

Statistical Procedures. Vinifications and analyses were performed in triplicate. Significant differences between samples were analyzed using the SPSS 12.0 program for Microsoft Windows (SPSS Inc., Chicago, IL). The values of polysaccharide and polyphenolic content, and color composition were analyzed by a two-way analysis of variance (ANOVA) with repeated measurements in one factor (vinification stage) to test the effect of mannoprotein addition. If the data did not meet normality assumptions, a Kruskal−Wallis test was used. In this article, differences between samples always refer to significant differences with at least $p < 0.05$.

RESULTS AND DISCUSSION

Characterization of the Commercial Mannoproteins Used. A commercial mannoprotein-rich preparation was purchased from Lallemand and characterized with regard to glycosyl-residue composition. This commercial preparation was actually a yeast derivative product obtained by a specific refining process that produces a high level of reactive yeast cell wall polysaccharides, mainly mannoproteins (information supplied by the manufacturer). The moment of addition and the dose used were those recommended by the manufacturer.

The glycosyl-residue analysis revealed a composition of 73% polysaccharides and 2% proteins, mannose being the main sugar (91%) with glucose far behind (9%), thus confirming the prevalence of parietal mannoproteins.

Effect of Commercial Mannoprotein Addition on Polysaccharide Composition during Vinification and Oak Aging. Must and wine polysaccharides were fractionated by HRSEC on a Superdex 75-HR column in order to analyze the specific polysaccharide families. Wine samples revealed a fractionation of compounds into three peaks, S1, S2 and S3, similar to that previously described (*41*), while must refractometric profiles showed only two of these peaks, S1 and S3. Molecular weight distribution HRSEC on Shodex columns revealed a fractionation of compounds similar to that obtained with the Superdex column (**Figure 1**). Higher-molecular-weight polysaccharides, eluting in fraction S1, corresponded to molecules with a molecular weight higher than 50 kD, and, according to their glycosyl composition and to previously published data, it corresponded to a complex mixture of high-molecular-weight arabinogalactans and arabinogalactan-proteins (bAGP) from grape berries and high-molecular-weight mannoproteins (bMP) from yeasts (*39*). Polysaccharides with an average molecular weight of 12 kD, fractionated in the second fraction (S2), corresponded mainly to grape rhamnogalacturonan-II dimers (dRG-II) but also to low-molecular-weight arabinogalactan-proteins and mannoproteins (sAGP and sMP, respectively). Fraction S3 displayed a molecular weight of less than 6 kD, and it was composed of homo and rhamnogalacturonan oligomers (GL), although glycosyl

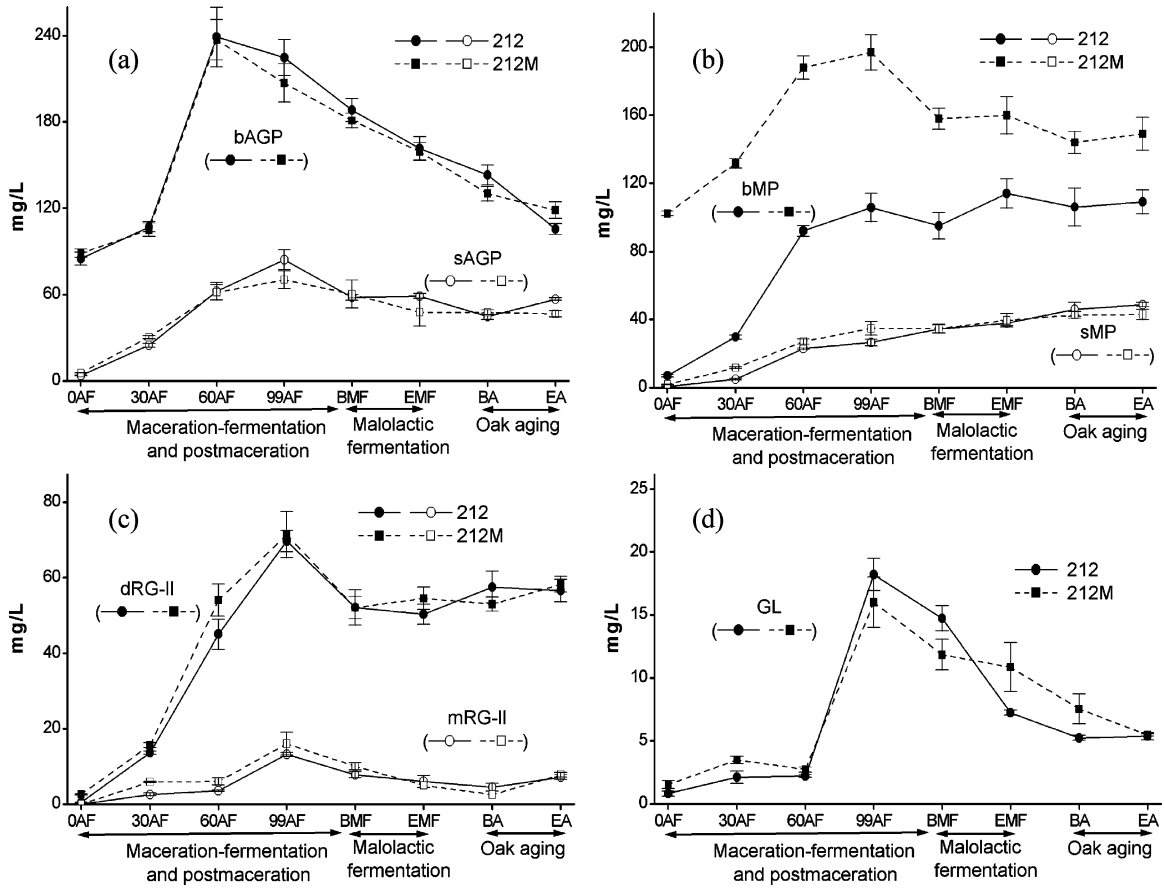

Figure 2. Effect of commercial mannoprotein addition on the evolution of major polysaccharide families during vinification and oak aging: (**a**) high-molecular-weight arabinogalactan-proteins (bAGP) and low-molecular-weight arabinogalactan-proteins (sAGP); (**b**) high-molecular-weight mannoproteins (bMP) and low-molecular-weight mannoproteins (sMP); (**c**) rhamnogalacturonan-II dimers (dRG-II) and rhamnogalacturonan-II monomers (mRG-II); (**d**) oligomers of homo- and rhamnogalacturonans (GL). See text for conditions and calculations.

Table 2. Mannose Content (mg/L) of Must and Wine Fractions S3 Obtained by HRSEC on a Superdex-75 HR Column and Determined by GC and GC-MS of Their TMS Derivatives

	vinification stages[a]							
samples	0AF	30AF	60AF	99AF	BMF	EMF	BA	EA
212	3.0 ± 0.1	3.58 ± 0.04	5.8 ± 0.3	25.7 ± 0.5	20.8 ± 0.6	20.7 ± 0.4	17.1 ± 1.4	18.9 ± 1.4
212M	2.63 ± 0.09	2.7 ± 0.2	3.7 ± 0.3	26.2 ± 0.8	22.8 ± 0.1	19.6 ± 0.4	22.3 ± 1.2	18.7 ± 0.9

[a] Vinification stages: 0AF, 30AF, 60AF, 99AF, alcoholic fermentation; BMF, beginning of malolactic fermentation; EMF, end of malolactic fermentation; BA, beginning of oak aging; EA, end of oak aging.

residues characteristics of AGP, MP, and RG-II polysaccharides were also detected. The former were attributed to small fragments of larger AGP and MP, and the presence of rare sugars was attributed to monomeric RG-II (mRG-II), previously detected in must and wine samples by our workgroup (*39*).

Figure 2 shows the concentration of sample polysaccharide families during winemaking and oak aging. Except for bMP in 212M samples, polysaccharides evolved in a relatively similar manner to that observed in previous studies performed by our workgroup (*39*). When comparing the control vinification with the mannoprotein-treated one, no significant differences could be observed in either high-molecular-weight grape polysaccharide content, such as bAGP or dRG-II, or in the case of smaller sAGP, mRG-II, or GL. With regard to yeast mannoprotein content, there was obviously a great difference between both vinifications. Mannoprotein-treated musts presented around 100 mg/L more high-molecular-weight mannoproteins (bMP) than their respective controls, but no significant differences were observed for the smaller molecules (sMP), indicating that more than 75% of the added mannoproteins remained in solution after their addition and that they were large compounds, with an average size of around 105 kD. No differences were observed in the evolution of bMP during alcoholic fermentation between both vinifications, but the postmaceration period accounted for a significant reduction of more than 20% in the amount of bMP in the treated 212M samples. Therefore, the differences in bMP content observed between both musts were substantially reduced, although there were still significant differences between the wines after malolactic fermentation and oak aging, the treated-wines showing 40−50 mg/L more bMP than the controls. No noteworthy differences were observed between both vinifications in the mannose content of must and wine S3 fractions (**Table 2**), which is attributed to small fragments of large mannoproteins. Consequently, the bMP decrease observed in 212M samples during postmaceration was attributed to molecule precipitation rather than enzymatic fragmentation, confirming our previous results showing that MP precipitation is probably the major phenomenon influencing the polysaccharidic balance during postmaceration and malolactic fermentation (*39*).

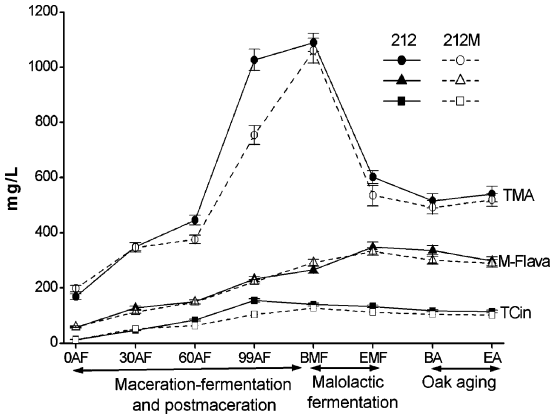

Figure 3. Effect of commercial mannoprotein addition on the evolution of total monomeric anthocyanins (TMA), total monomeric flavanols (M-Flava), and total hydroxycinnamic acids (TCin) during vinification and oak aging. See text for conditions and calculations.

The results showed that control wines, both young and aged ones, were essentially composed of grape cell wall AGP, followed by yeast MP, and dRG-II, in similar proportions to those described in ref *45*. However, mannoprotein-treated wines had larger concentrations of MP, and these compounds were therefore proportionally relatively similar in wines compared with AGP polysaccharides. The content of mRG-II and GL was less than 2% of the total polysaccharide families in both control and treated wines.

Effect of Commercial Mannoprotein Addition on Monomeric Phenolics during Vinification and Oak Aging. Figure 3 shows the content of total monomeric anthocyanins (TMA), hydroxycinnamic acids (TCin), and monomeric flavanols (M-Flava) in samples taken during winemaking and wine oak aging. Control and mannoprotein-treated samples presented comparable values of these phenolics throughout the vinification process except for the values of TMA and TCin during late maceration−fermentation. Thus, mannoprotein-treated samples showed a slight delay in the extraction of pomace anthocyanins and hydroxycinnamic acids between 60AF and 99AF, coinciding with the period of the maximum concentration of high-molecular-weight mannoproteins (**Figure 2**). Establishing any relationship between the presence of big MP and the slower diffusion rate of anthocyanins or hydroxycinnamic acids was dismissed because during postmaceration, the 212M wine still presented higher amounts of these colloids and a higher extraction rate than the control. As a result, the differences observed during maceration−fermentation disappeared completely after this stage. The identical TMA and TCin contents observed for both wines during the later stages of malolactic fermentation and oak aging confirmed that high-molecular-weight mannoproteins had no influence on the evolution of these compounds.

As expected, we also did not observe any significant difference in the different forms of the monomeric anthocyanins between controls and 212M wines after the postmaceration period (**Figure 4a**). Surprisingly, the slower TMA extraction observed for 212M samples at the end of alcoholic fermentation was seen to be caused by slower extraction in all of the anthocyanic forms, the majority of nonacylated forms and the minority of coumarated and acetylated forms (**Figure 4a**). A more detailed analysis revealed that the compounds responsible for these differences were malvidin-3-glucoside together with their respective coumarated and acetylated forms (data not shown). With regard to monomeric flavanols, no remarkable differences were observed in the extraction and evolution of

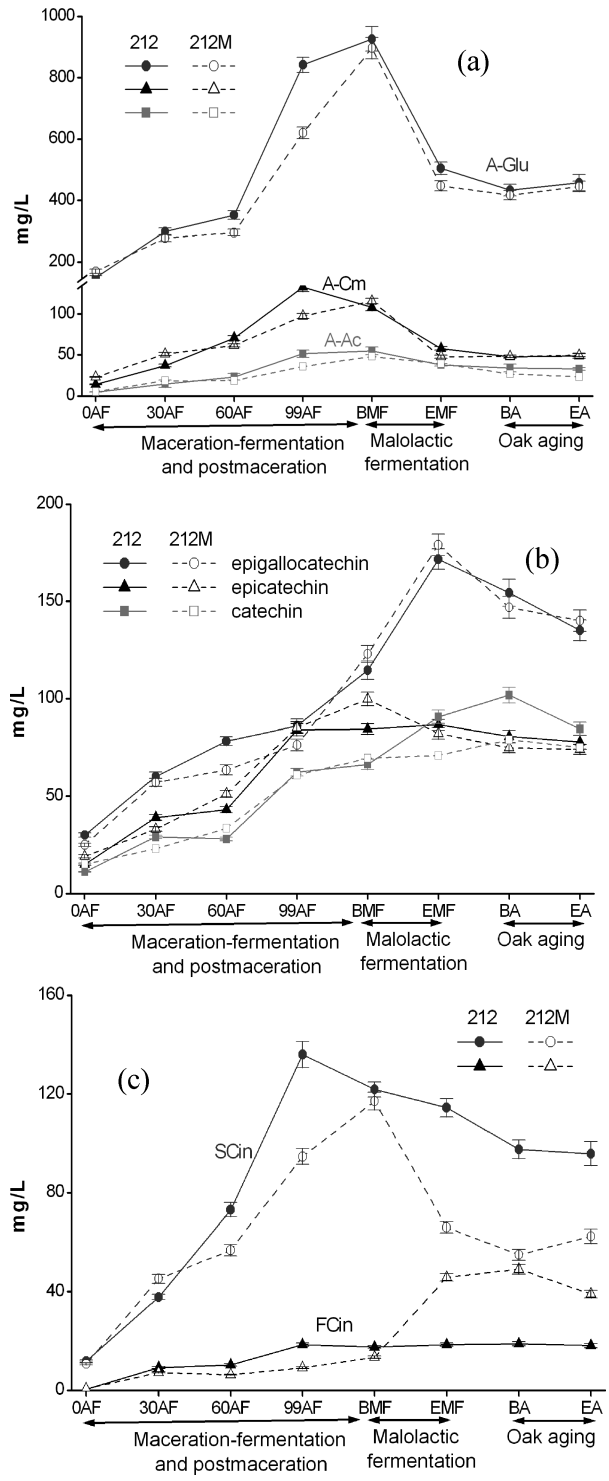

Figure 4. Effect of commercial mannoprotein addition on the evolution of (**a**) anthocyanin glucosides (A-Glu), coumaryl-glucoside anthocyanins (A-Cm), and acetyl-glucoside anthocyanins (A-Ac); (**b**) monomeric flavanols; and (**c**) sterified hydroxycinnamic acids (SCin) and free hydroxycinnamic acids (FCin). See text for conditions and calculations.

(+)catechin, (−)epicatechin, and (−)epigallocatechin between control and 212M samples (**Figure 4b**). However, hydroxycinnamic acids showed quite different behavior in both vinifications (**Figure 4c**). As in the case of anthocyanins, samples with MP addition displayed slower extraction of hydroxycinnamic acids during late maceration−fermentation, but the greatest differences between both vinifications occurred during malolactic fermentation. In the case of 212M wines, this stage produced a substantial decrease in the sterified hydroxycinnamic acids (SCin) in favor

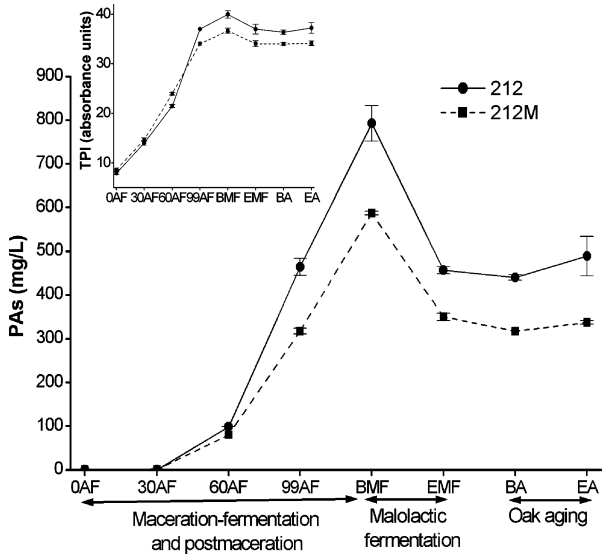

Figure 5. Effect of commercial mannoprotein addition on the evolution of total proanthocyanidins (PAs) and total polyphenol index (TPI) during vinification and oak aging. See text for conditions and calculations.

of an increase in their respective free acids (FCin), which was seen to be due to a reduction in *trans*-hydroxycinnamate derivatives, especially *trans*-caftaric and *trans*-coutaric acid, because the *cis* forms remained stable (data not shown). Some authors have observed more or less intense changes in *trans*-hydroxycinnamates during malolactic fermentation (*46*) and aging in oak barrels (*47*) because of hydrolysis of the tartaric esters. However, this hydrolysis phenomenon was only observed in the MP-enriched wines.

Effect of Commercial Mannoprotein Addition on Proanthocyanidins during Vinification and Oak Aging. Contrary to what was observed with monomeric phenolics, the amount of wine proanthocyanidins (PAs) was significantly affected by the addition of commercial mannoproteins (**Figure 5**). Thus, mannoprotein-added samples presented significantly lower proanthocyanidin contents from the end of maceration−fermentation. These differences were maintained throughout malolactic fermentation and oak aging, where the PA content of the controls was around 1.5 times higher than that of the treated wines.

In year 2000, Saucier et al. (*26*) proposed a model that could possibly explain the polysaccharide−tannin interactions. Basically, this model suggests that wine proanthocyanidins alone are highly reactive toward salivary proteins with the subsequent increase in wine astringency. However, the tannin−mannoprotein combination would produce stable structures that are not reactive toward proteins, explaining why wine tannins are less astringent in the presence of mannoproteins (*27*). Additionally, more recent studies have shown that adding polysaccharides to a model tannin suspension has a strong impact on tannin particle size evolution. Thus, it was observed that mannoproteins did not prevent initial tannin aggregation but that they strongly inhibited it at wine concentrations (*33*). A more detailed study suggested that the tannin−mannoprotein combination could be steric rather than electrostatic (*34*).

As reported in refs *26, 27, 30, 33* and (*34*), we found that mannoproteins had a strong impact on tannin colloids and thus on wine astringency. This effect would imply a combination/adsorption of mannoproteins and tannins, confirming the widely accepted hypothesis proposed by Saucier. However, and contrary to what is described, our results suggested that mannoproteins did not have any protective effect toward tannins but quite the contrary. In order to be able to explain it, two hypotheses may be proposed: (i) at the studied conditions, the combination tannin−mannoproteins could result in high-molecular-weight structures that would be unstable and precipitate, leading to a decrease in total PA content; (ii) at the studied conditions, mannoproteins could act as flocculating polymers instead of stabilizers. Flocculation is usually explained by bridging, the same mannoprotein molecules would bind to different tannins, leading to the formation of bigger aggregates and further precipitation. Although studies in synthetic media seem to indicate the opposite (*33, 34*), it must be noted that authors have obtained different results depending on the conditions of the medium, such as ethanol content and ionic strength, and the concentration and type of the studied polysaccharide. Thus, only the smallest mannoproteins, with an average molecular size of 50−60 kD, limited tannin aggregation under standard wine conditions (pH 3.4, 12% ethanol, and 2 g/L tartaric acid), while larger mannoproteins did not show any effect (*34*), and other wine polysaccharides enhanced it (*33*). To the best of our knowledge, our study is the first to analyze the protective role of mannoproteins toward tannins in real vinification situations, with real winemaking conditions and with real must and wine matrix, and well-dissolved seed and skin grape tannins rather than purified aggregates. A previous study carried out in Pinot Noir wines, with ethanol and gelatin indexes, suggested that yeast mannoproteins can combine with tannins and produce less astringent tannins (*27*), but they did not analyze the effect on tannin content.

In conclusion and regardless of the mechanism involved, the precipitation of the mannoprotein−tannin coaggregate occurring at the end of maceration−fermentation could explain the high extent of bMP precipitation observed in the mannoprotein-treated samples during this period. The lower PA content observed for the 212M wines, which was also in good correlation with their lower values in the total polyphenol index (**Figure 5**), resulted in wines with decreased astringency and tannicity, and enhanced sweetness and roundness (*31*).

Effect of Commercial Mannoprotein Addition on Wine Color. For some time now, there has been speculation that mannoproteins may bind with other phenolic compounds besides proanthocyanidins and by doing so, stabilize red wine color. Although no studies have been conducted on the effect of mannoproteins on wine pigments, it is hypothesized that mannoproteins are adsorbed by the colloidal molecules of anthocyanin−tannin, copigmented anthocyanins, and so forth, completely covering the surface of these colloids and thus avoiding their degradation and precipitation (*48*).

On the basis of this hypothesis, the protective colloid role of mannoproteins toward colorant material precipitation was studied. **Figure 6** shows the evolution of wine color (WC), monomeric anthocyanin color (MAC), and stable color (SC) in the samples throughout the period studied, the latter being considered the sum of copigmented and bisulfite-stable color.

As previously described by our workgroup (*31*), young wines produced with mannoprotein addition presented considerably lower values of wine color at the end of maceration−fermentation, and these differences were maintained during malolactic fermentation and oak aging. The results of the present study showed that wine color differences were due to the stable color component, and no significant differences were found between both vinifications in the monomeric anthocyanin color throughout the period studied, which confirmed that mannoproteins had no effect on the monomeric anthocyanins (see previous section on monomeric phenolics). On the contrary, mannoprotein-treated

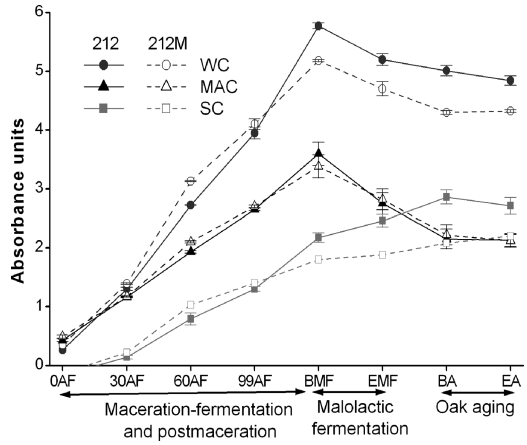

Figure 6. Effect of commercial mannoprotein addition on the evolution of wine color (WC), monomeric anthocyanin color (MAC), and stable color (SC) during vinification and oak aging. See text for conditions and calculations.

Table 3. Color Attributes in Wines After Oak Aging[a]

wine	CI	hue	*a**	*b**	*L**
212	5.008 ± 0.004[a]	0.62[a]	26.8 ± 0.5[a]	0.90 ± 0.03[a]	72.2 ± 0.4[a]
212M	4.831 ± 0.005[b]	0.68[b]	25.6 ± 0.5[a]	0.94 ± 0.01[a]	69.4 ± 1.5[a]

[a] CI, color intensity as sum of absorbances at 420, 520, and 620. Hue, A_{420}/A_{520}. *a**, from green to red; *b**, from blue to yellow; *L**, lightness. Different labels (a−b) indicate that means between 212 and 212M significantly differ with at least $p < 0.05$.

wines showed significantly lower values of stable color than controls at the end of maceration−fermentation, and these differences became even more evident during malolactic fermentation. Thus, 212M wines presented less than 20% of stable color compared with the control wines both before and after wine oak aging, resulting in wines with lower color intensity values (**Table 3**) and visually weaker color intensity (*31*). Control and 212M wines did not show any noteworthy difference in the CIELAB parameters *a** (from green to red), *b** (from blue to yellow), and *L** (lightness) at the end of oak aging (**Table 3**).

On the basis of our results, the stabilizing effect of mannoproteins toward color was also questioned. As in the case of tannins, our observations showed that at the studied conditions, mannoproteins did not have any protective influence on colorant colloidal material, but they could act as colloidal-destabilization or precipitation species. Thus, bearing in mind that stable color is of utmost importance in red winemaking, this effect should be studied in greater detail.

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