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Effect of commercial mannoproteins on wine colour and tannins stability

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ABSTRACT

Commercial oenological products containing mannoproteins have the purpose of preventing tartrate salts precipitation or achieving wines with a better mouth-feeling. The evaluation of the influence of three commercial mannoproteins on colour and tannin stability of three different red wines (two from Touriga Nacional and one from Alfrocheiro and Aragonês varieties) was studied. The evolution of colour through time was similar for all modalities, resulting in an increase of polymeric pigments and colour hue and a decrease of other parameters, suggesting that there was no influence of commercial mannoproteins on colour stability. The tannin profile evolution showed a possible stabilizing effect of one of the commercial products for tannins with an mDP between 8 and 14. It is possible that the commercial mannoproteins used in this work have some influence on the tannin aggregation evolution, contributing to the delay of tannin polymerisation in red wines.

1. Introduction

Mannoproteins are one of the major polysaccharide groups present in wine (Feuillat, 2003), having their origin in the *Saccharomyces cerevisiae* yeast cell walls. Gonçalves, Heyraud, Pinho, and Rinaudo (2002) found that 32.2% of the total polysaccharides content of white wine corresponded to mannoproteins. These polysaccharides are glycoproteins and according to Klis, Mol, Hellingwerf, and Brul (2002) they represent 35–40% of the *S. cerevisiae* yeast cell wall. Mannoproteins are highly glycosylated and they are located on the cell wall external layer, where they are covalently linked to an amorphous matrix of β-1,3-glucans. Mannoproteins can be present in a wide range of molecular weights in wines that can vary between 5 and 400 kDa according to Doco, Vuchot, Cheynier, and Moutounet (2003) and even 800 kDa (Saulnier, Mercereau, & Vezinhet, 1991). Mannoproteins are composed by 10–20% of protein and about 80% of D-mannose associated to residues of D-glucose and *N*-acetylglucosamine. The release of mannoproteins to wine can occur in two different processes: release during alcoholic fermentation in the yeast growing phase and release after yeast autolysis by the action of the exogenous β-1,3-glucanase enzyme on the yeast cell walls (Feuillat, 2003). These last mannoproteins are similar to those released during fermentation but they have less protein content (Saulnier et al., 1991).

Mannoproteins are known for several important properties in wines. They have the ability to adsorb ochratoxin A (Baptista et al., 2004); to enhance malolactic bacteria growth (Guilloux-Benatier, Guerreau, & Feuillat, 1995); to inhibit tartaric salts crystallisation (Moine-Ledoux & Dubourdieu, 2002); to prevent protein haziness (Waters, Pellerin, & Brillouet, 1994); to enhance and interact with some wine aromas (Chalier, Angot, Delteil, Doco, & Gunata, 2007); to promote flocculation and yeast autolysis in sparkling wines (Nunez, Carrascosa, González, Polo, & Martinez-Rodriguez, 2006).

The interaction between mannoproteins and wine phenolic compounds is a subject of great interest as some studies show the possible impact on colour stability (Escot, Feuillat, Dulau, & Charpentier, 2001; Poncet-Legrand, Doco, Williams, & Vernhet, 2007; Riou, Vernhet, Doco, & Moutounet, 2002; Vasserot, Caillet, & Maujean, 1997) and an improvement in the sensory characteristics, namely the reduction of red wine astringency (Guadalupe, Martínez, & Ayestarán, 2010; Guadalupe, Palacios, & Ayestarán, 2007). Red wine colour is mainly due to the presence of anthocyanins existing in the coloured forms at wine pH, when wines are young, and to the formation of more stable polymeric pigments with ageing of the wine, namely by condensation of flavanoid units (Dallas, Ricardo da Silva, & Laureano, 1996; Somers, 1971). Proanthocyanidins or condensed tannins are polymeric flavanoids, resulting from the condensation between flavan-3-ol units, and are related with wine astringency, bitterness and colour. Wine tannins are composed by monomer units of (+)-catechin and (−)-epicatechin, (procyanidins) and/or monomer units of (−)-epigallocatechin (prodelphinidins), sometimes esterified by gallic acid (Fulcrand, Remy, Souquet, Cheynier, & Moutounet, 1999; Haslam, 1980; Ricardo da Silva, Rosec, Bourzeix, & Heredia, 1990).

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The interaction between colour compounds and tannins with mannoproteins is extremely important as it can influence colour stability and improve sensory qualities. Some works (Guadalupe & Ayestarán, 2008; Guadalupe et al., 2007, 2010) showed that there was no positive interaction between mannoproteins and colour compounds and that the interaction between mannoproteins and tannins resulted in a decrease of wine tannin content, suggesting the precipitation of tannin and mannoprotein aggregates and a decrease in astringency with an increase of the wine sweetness and roundness. There are several oenological products in the market that contain mannoproteins in their composition with the aim of preventing wine potassium tartrate precipitation (Moine-Ledoux, Perrin, Paladin, & Dubourdieu, 1997), and achieve wines with a better mouth-feeling (Guadalupe & Ayestarán, 2008).

Considering the fact that colour stabilisation is one of the most important objectives for some wine producers it is important to understand with which tools the winemaker can work with in order to assure the quality and evolution of the wines. Bearing this in mind, the aim of this work was to evaluate the influence of three commercial mannoproteins enriched preparations in the colour stability and tannin evolution of three different red wines (two from Touriga Nacional and other from Alfrocheiro and Aragonês (*sin.* Tempranillo) grapevine varieties).

2. Materials and methods

2.1. Chemicals

All chemicals used were of analytical reagent grade. All solvents were of HPLC grade. (+)-Catechin, (−)-epicatechin, (−)-epicatechin-3-O-gallate and (−)-epigallocatechin were purchased from Extrasynthese (Genay, France). Toluene-α-thiol (phenylmethanethiol) was purchased from Fluka (Buchs, Switzerland).

2.2. Wines

Grapes from Touriga Nacional, Aragonês and Alfrocheiro were harvested and vinified at Dão Sul winery (Dão Region, Portugal) in order to prepare three different red wines: wine A: Touriga Nacional variety harvested in 2007 (9000 kg); wine B: Alfrocheiro + Aragonês varieties harvested in 2007 (9000 kg); wine C: Touriga Nacional variety harvested in 2009 (9000 kg). The grapes were de-stemmed and crushed into a stainless steel vessel and a preparation of commercial maceration enzymes was added to each vessel. After 24 h of cold pre-fermentative maceration at 15 °C, the musts were inoculated with a commercial activated *S. cerevisiae* preparation. The alcoholic fermentation occurred for ten days at 20–25 °C. After the beginning of the alcoholic fermentation, the musts were punched down for 20 min every three hours, and they were submitted to a rack and return program for half an hour every day, until the end of alcoholic fermentation. After the end of alcoholic fermentation, the free-run wines were transferred to another stainless steel vessel in order to perform malolactic fermentation. The three wines were analysed for ethanol content, pH, volatile acidity, titrable acidity and free and total SO_2 according to the Organisation International de la Vigne et du Vin official methods (OIV, 2006). The wines chemical parameters were the following: wine A: alcohol content 13.3% v/v, titrable acidity 5.3 g/l expressed in tartaric acid, volatile acidity 0.5 g/l expressed in acetic acid, pH 3.82, 21 mg/l of free SO_2, 40 mg/l of total SO_2; wine B: alcohol content 13.6% v/v, titrable acidity 5.5 g/l expressed in tartaric acid, volatile acidity 0.5 g/l expressed in acetic acid, pH 3.82, 12 mg/l of free SO_2, 27 mg/l of total SO_2; wine C: alcohol content 13.6% v/v, titrable acidity 7.8 g/l expressed in tartaric acid, volatile acidity 0.3 g/l expressed in acetic acid, pH 4.05, 15 mg/l of free SO_2, 32 mg/l of total SO_2. At the end of malolactic fermentation, two different experiments were prepared.

2.3. Mannoprotein commercial preparations trials in red wines

Two different commercial preparations containing yeast mannoproteins (MP1 and MP2) were added to wines A and B in concentrations of 0.2 g/l (C1) and 0.4 g/l (C2) after malolactic fermentation according to the manufacturer's indications, followed by bottling of the resulting wines. T samples refer to wine with no addition of commercial mannoprotein preparations. The bottled wines were kept at winery temperatures (20 ± 3 °C) for twenty-one months. Samples of each wine were analysed for their colour characterisation and proanthocyanidins composition at 0, 28, 91, 122, 164, 256 and 644 days. Two different commercial preparations containing yeast mannoproteins (MP2 and MP3) were added to wine C in concentration of 0.3 g/l after malolactic fermentation according to the manufacturer's indications. The wines were kept in amber flasks, at 35 °C, for sixty days. Samples of each wine were analysed for their colour characterisation and proanthocyanidins composition according to their degree of polymerisation at 0, 6, 13, 19, 26, 47 and 60 days.

2.4. Colour characterisation

In order to characterise the wine colour compounds it was used the spectrophotometrical method described by Somers and Evans (1977). The wines were centrifuged for 10 min at 3.500 r.p.m. and the absorbances were measured using a Unicam UV–Vis UV4 spectrophotometer (Unicam, Cambridge, UK).

2.5. Proanthocyanidins analysis

2.5.1. Isolation of oligomeric and polymeric proanthocyanidins

The isolation of wine proanthocyanidins was made according to the methods described by Sun, Leandro, Ricardo da Silva, and Spranger (1998) and Labarbe, Cheynier, Brossaud, Souquet, and Moutounet (1999). Four millilitres of wine were injected onto a Toyopearl TSK HW-40F (Tosoh Corp., Tokyo, Japan) packed column (100 × 10 mm) and first washed with a solution of ethanol:water:TFA (55:45:0.05 v/v/v) in order to remove small molecules and flavan-3-ols. The wine oligomeric and polymeric proanthocyanidins were eluted with a solution of acetone:water (40:60 v/v). After evaporation of the tannin fraction at 30 °C under vacuum, the proanthocyanidins were resuspended in 1 ml of methanol to be used in further analysis.

2.5.2. Fractionation of wine proanthocyanidins according to their degree of polymerisation using a sequential dissolving procedure on an inert glass powder column

The wine C proanthocyanidins were separated according to their degree of polymerisation as described by Labarbe et al. (1999) during the experiment time. One millilitre of proanthocyanidins methanol solution was precipitated by chloroform on the top of a glass powder column (50 × 10 mm). The elution gradient (chloroform/methanol) was applied as following: FI – 75:25 (v/v); FII – 70:30 (v/v); FIII – 65:35 (v/v); FIV – 60:40 (v/v); FV – 55:45 (v/v); FVI – 50:50 (v/v); FVII – 45:55 (v/v); FVIII – 0:100 (v/v). The eight fractions were analysed by HPLC after thiolysis in order to determine their structural characteristics.

2.5.3. Characterisation of wine proanthocyanidins by acid-catalysed depolymerisation in the presence of toluene-α-thiol followed by reversed-phase HPLC analysis

The proanthocyanidins were submitted to depolymerisation in the presence of toluene-α-thiol in an acidic medium as described

by Monagas, Gómez-Cordovés, Bartolomé, Laureano, and Ricardo-da-Silva (2003). 100 μl of toluene-α-thiol 5% in methanol 0.2 M HCl were added to 100 μl of proanthocyanidins solution in a hermetically sealed glass tube. The mixture was warmed up to 55 °C on a water bath for 7 min. Ten microlitres of the prepared solution were immediately injected in a HPLC system equipped with a Merck Hitachi Lachrom L-7100 Pump (Merck Hitachi, Tokyo, Japan), followed by a Phenomenex Gemini C18 110 A 150 × 3.0 mm column (Phenomenex, California, USA) and a UV/Vis Waters 2487 Dual Wavelength detector (Waters, Massachusetts, USA), in the following elution conditions: 0.8 ml/min flow with a linear gradient of 15–75% of a solution of acetonitrile/water/formic acid (80:18:2, v/v/v) in a solution of water/formic acid (98:2, v/v), in an 18 min run. The detection was monitored at 280 and 320 nm. The amounts of monomers and of toluene-α-thiol adducts released were calculated from the areas of the chromatographic peaks at 280 nm by comparison with calibration curves (Rigaud, Perez-Ilzarbe, Ricardo da Silva, & Cheynier, 1991).

2.6. Mannoprotein characterisation

A centrifuged solution of commercial mannoproteins at 1 g/l was injected onto a concanavalin-A Sepharose 4B (GE Healthcare Bio-Sciences, Uppsala, Sweden) packed column (100 × 10 mm), and eluted with a sodium acetate-HCl 50 mM pH 5.6, NaCl 150 mM, $CaCl_2$ 1 mM, $MgCl_2$ 1 mM and $MnCl_2$ 1 mM buffer solution at 0.8 ml/min; elution was monitored by a refraction index and a 254 nm wavelength detectors as described by Gonçalves et al. (2002). The bound fraction was eluted with the same buffer solution containing methyl α-D-mannopyranoside, 500 mM. The bound fraction was dialysed against water for 7 days, at 4 °C. After freeze drying of the bound fraction, the sample (1 g/l) was injected onto a FPLC system equipped with a Pharmacia LKB Pump P-500, a 12HR 10/30 FPLC size-exclusion column (GE Healthcare Bio-Sciences, Uppsala, Sweden), eluted with an ammonium acetate 0.3 M buffer solution at 0.3 ml/min and monitored by a Perkin-Elmer LC-30 RI refractive index detector (Perkin-Elmer, Massachusetts, USA) modified with a LED (light-emitting diode) light source (Cromolab, Queijas, Portugal) and a Knauer WellChrom Spectro-Photometer K-2501 wavelength detector (Knauer, Berlin, Germany) at 254 nm. Calibration of the system was performed with Shodex P-82 pullulan standards (Showa Denko K.K., Kanagawa, Japan).

The carbohydrate composition was determined by gas chromatography after derivatization of the samples into their alditol acetates according to Albersheim, Nevis, English, and Karr (1967). Hundred microlitres of a μ-inositol solution (1 mg/ml) and 1 ml of trifluoroacetic acid 2 M were added to 1 ml of polysaccharides solution (1 mg/ml). After hydrolysis at 120 °C, for 75 min, the mixture was washed with 5 ml of water and dried. 500 μl of a saturated sodium borohydride solution in ammonia were added and the mixture reacted for two hours at room temperature. The reaction was stopped by adding some drops of glacial acetic acid and the mixture was washed with 5 ml of a solution of 1% HCl in methanol and dried. One hundred and fifty microlitres of pyridine and 150 μl of acetic anhydride were added to the mixture and left to react for 12 h at room temperature. The reaction was stopped by adding a drop of water in an ice bath. The mixture was washed with 5 ml water, followed by 1 ml of ethanol, and then dried. The alditol acetates were extracted to 200 μl of chloroform and were quantified on a CE Instruments GC 8000 Top gas chromatographer (Thermo Fisher Scientific, Milan, Italy) with a capillary column Zebron ZB-Wax 10 60 × 0.25 mm, 0.25 μm film (Phenomenex, California, US(A) and a FID detector. The column temperature was initially set at 220 °C for 4 min and was raised to 235 °C at 10 °C/min, maintaining this temperature for 5 min. Hydrogen was used as carrier gas at 1 ml/min. μ-Inositol was used as the internal standard, sugar quantification was carried out after determination of each sugar response factor using pure sugars as standards.

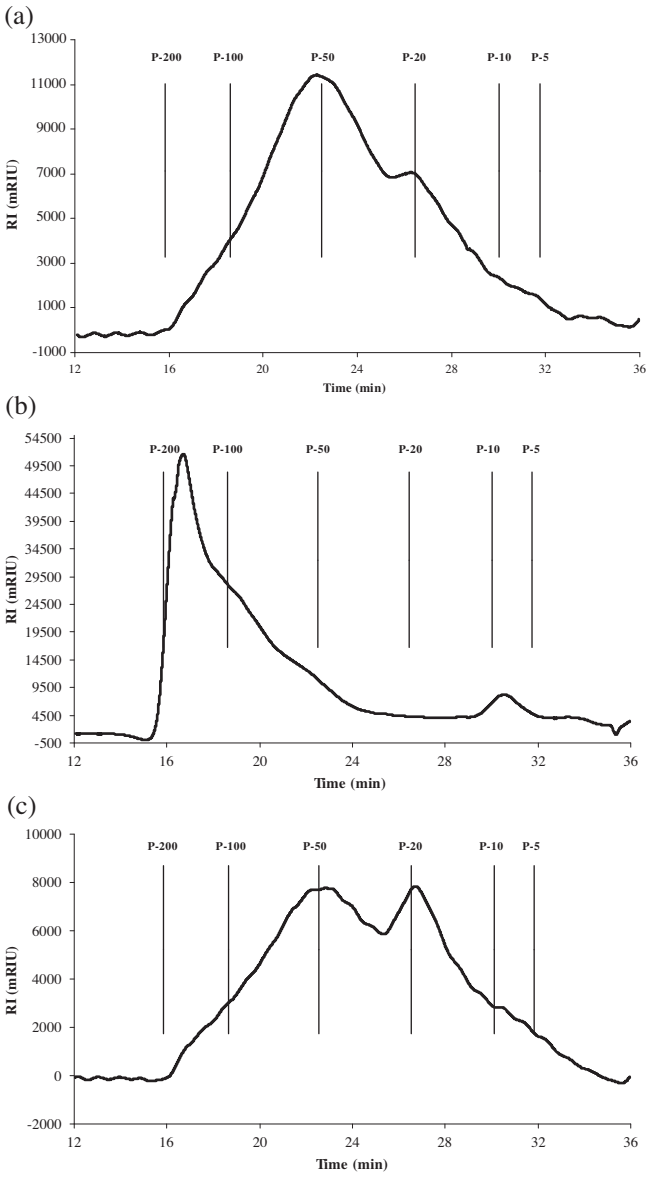

Fig. 1. Molecular weight distribution of the concanavalin-A column retained fraction of the commercial mannoproteins by FPLC on a Superose 12HR column, with the elution of the pullulan standards – (a) MP1, (b) MP2, and (c) MP3.

The total protein content was determined as described by Lowry, Roserbrough, Farr, and Randall (1951) using bovine serum albumin fraction V (Sigma–Aldrich, Missouri, USA) for the calibration curve.

The total polysaccharides content was determined by the phenol–sulphuric method as described by Dubois, Gilles, Hamilton, Rebers, and Smith (1956) using glucose (Panreac, Barcelona, Spain) for the calibration curve.

3. Results and discussion

3.1. Characterisation of the commercial mannoproteins

After purification on concanavalin-A, the retained fractions of the three commercial mannoproteins used were characterised

with regards to their total polysaccharide content, total proteins content and their glycosyl residue composition. MP1 was composed of 11.5% protein and 85.2% of polysaccharides, mannose being the main sugar (99.3%), followed by glucose (0.7%). MP2 was composed of 4.3% protein and 83.1% polysaccharides, and mannose was the only residual sugar detected. MP3 was composed of 0.8% protein and 67.3% polysaccharides, with 99.8% mannose and 0.2% glucose. The molecular weight distribution of the concanavalin-A retained fractions for the three commercial mannoproteins is shown in Fig. 1. MP1 showed two different peaks, corresponding to two fractions with an average molecular weight around 43 and 17 kDa. MP2 also presented two different peaks with an average molecular weight around 164 and 8 kDa, as well as MP3, with two peaks corresponding to average molecular weights around 44 and 19 kDa.

3.2. Effect of commercial mannoproteins addition on colour compounds evolution

3.2.1. Wines A and B

Table 1 shows the evolution of colour parameters at days 0, 164 and 644. Both studied wines had the same evolution for these parameters. The total polyphenols (TP) represents the absorbance at 280 nm. All wines presented the same evolution of TP, slightly decreasing through time. The colour intensity (CI) represents the sum of absorbances at 420, 520 and 620 nm. In all the experiments of wines A and B CI tended to decrease with time for all samples in the same way, as a result of the polymerisation and precipitation of colour compounds. It seemed that the wines with no commercial mannoproteins addition (T sample) maintained the colour intensity slightly higher than the other samples. This difference ranged from 0.5 to 0.9 in wine A. Guadalupe and Ayestarán (2008) have suggested that mannoproteins can co-precipitate with stable colour but Escot et al. (2001) suggested that mannoproteins could prevent tannin and anthocyanins precipitation. The evolution of colour hue reinforces the fact that there was no stabilisation of colour compounds at 520 nm, evolving to a higher absorbance at 420 nm for both wines. Polymeric pigments (PP) represent longer chain coloured compounds resulting from the condensation reactions between anthocyanins and flavanol units (Somers, 1971). PP tended to increase with time, with no important differences between samples, being slightly higher for the T sample. Coloured anthocyanins are the ones that at wine pH have a red colour, as defined by Somers and Evans (1977). They tended to decrease with time, resulting in wine browning that was in accordance with the results for IC and hue. As with the other parameters no significant differences were seen between samples. For the total anthocyanins (TA), the evolution was also similar for all samples, decreasing with time. At the end of the trail, on day 644, the T sample had less amount of TA in comparison with the other samples, showing a lower stabilisation of these compounds.

3.2.2. Wine C

The evolution of the colour parameters with time for the samples based on wine C are represented in Fig. 2. The CI (Fig. 2a) decreased slightly at the beginning for MP2 and even more for the MP3 sample. Until the end of the experiment, both samples maintained a stable evolution through time. The T sample maintained a stable evolution until the 47th day, increasing at the end of the experiment. For the colour hue (Fig. 2b), the evolution with time was very similar for all samples. The polymeric pigments (Fig. 2c) also increased with time, and at the end of the 60th day, the T sample presented higher values of PP. The total anthocyanins evolution had some differences between samples (Fig. 2d). The MP3 sample had an increase in these compounds at the beginning in contrast to MP2 and T, as well as a slower evolution. At the end of the experiment, the T sample had less anthocyanins than the MP2 and MP3 that behaved in the same way.

3.3. Effect of commercial mannoproteins on proanthocyanidins evolution

3.3.1. Wines A and B

Both studied wines had similar trends for tannin parameters. Concerning wine A (Fig. 3a and b), the T sample had a slower decrease both on mDP and proanthocyanidins content (PA) with time, and MP1C1 had the fastest. The sample MP2C2 seemed to have some protective effect on tannins precipitation as it was the

Table 1
Total polyphenols (TP), colour intensity (CI), hue, coloured anthocyanins (CA), polymerised pigments (PP), and total anthocyanins (TA) for wine A and wine B at day 0, day 164 and day 644.

Wine		T	A				T	B			
Sample			MP1		MP2			MP1		MP2	
			C1	C2	C1	C2		C1	C2	C1	C2
TP (AU)	0 days	85.8 ± 0.0	85.8 ± 0.0	85.8 ± 0.0	85.8 ± 0.0	85.8 ± 0.0	79.4 ± 0.0	79.4 ± 0.0	79.4 ± 0.0	79.4 ± 0.0	79.4 ± 0.0
	164 days	73.2 ± 0.0	76.9 ± 0.0	77.4 ± 0.0	75.5 ± 0.0	78.0 ± 0.0	74.4 ± 0.0	72.0 ± 0.0	74.4 ± 0.0	71.2 ± 0.0	75.1 ± 0.0
	644 days	64.0 ± 0.1	67.8 ± 0.0	61.3 ± 0.0	63.2 ± 0.0	62.2 ± 0.0	65.3 ± 0.0	65.5 ± 0.0	69.9 ± 0.0	76.0 ± 0.0	68.7 ± 0.1
CI (AU)	0 days	19.0 ± 0.0	19.0 ± 0.0	19.0 ± 0.0	19.0 ± 0.0	19.0 ± 0.0	17.4 ± 0.0	17.4 ± 0.0	17.4 ± 0.0	17.4 ± 0.0	17.4 ± 0.0
	164 days	15.7 ± 0.2	16.5 ± 0.1	16.2 ± 0.2	15.8 ± 0.0	16.5 ± 0.0	17.0 ± 0.0	16.8 ± 0.0	16.4 ± 0.0	17.8 ± 0.0	16.3 ± 0.1
	644 days	14.7 ± 0.0	13.8 ± 0.1	13.9 ± 0.1	13.9 ± 0.2	14.3 ± 0.2	14.4 ± 0.1	14.4 ± 0.1	14.4 ± 0.0	14.9 ± 0.1	14.2 ± 0.1
Hue (AU)	0 days	0.53 ± 0.00	0.53 ± 0.00	0.53 ± 0.00	0.53 ± 0.00	0.53 ± 0.00	0.55 ± 0.00	0.55 ± 0.00	0.55 ± 0.00	0.55 ± 0.00	0.55 ± 0.00
	164 days	0.68 ± 0.01	0.67 ± 0.00	0.67 ± 0.00	0.69 ± 0.00	0.67 ± 0.00	0.62 ± 0.00	0.65 ± 0.00	0.62 ± 0.00	0.63 ± 0.00	0.66 ± 0.00
	644 days	0.77 ± 0.00	0.79 ± 0.01	0.77 ± 0.00	0.80 ± 0.01	0.76 ± 0.00	0.79 ± 0.00	0.79 ± 0.00	0.77 ± 0.00	0.78 ± 0.00	0.81 ± 0.00
PP (mg/L)	0 days	63 ± 0	63 ± 0	63 ± 0	63 ± 0	63 ± 0	57 ± 0	57 ± 0	57 ± 0	57 ± 0	57 ± 0
	164 days	70 ± 0	72 ± 0	70 ± 0	69 ± 0	72 ± 0	66 ± 0	72 ± 0	63 ± 0	71 ± 0	70 ± 0
	644 days	83 ± 1	84 ± 1	80 ± 1	83 ± 1	80 ± 1	85 ± 0	84 ± 0	81 ± 0	82 ± 0	79 ± 0
CA (mg/L)	0 days	154 ± 0	154 ± 0	154 ± 0	154 ± 0	154 ± 0	141 ± 0	141 ± 0	141 ± 0	141 ± 0	141 ± 0
	164 days	90 ± 2	97 ± 1	96 ± 2	91 ± 0	98 ± 2	116 ± 1	104 ± 0	111 ± 1	117 ± 0	100 ± 1
	644 days	60 ± 1	49 ± 1	55 ± 0	50 ± 2	60 ± 0	53 ± 1	54 ± 1	58 ± 1	61 ± 0	56 ± 1
TA (mg/L)	0 days	715 ± 1	715 ± 1	715 ± 1	715 ± 1	715 ± 1	767 ± 1	767 ± 1	767 ± 1	767 ± 1	767 ± 1
	164 days	436 ± 1	439 ± 1	426 ± 1	474 ± 1	417 ± 0	442 ± 1	424 ± 1	434 ± 1	455 ± 1	464 ± 1
	644 days	104 ± 0	115 ± 1	131 ± 1	113 ± 1	132 ± 1	135 ± 1	141 ± 1	166 ± 0	183 ± 1	179 ± 1

AU is absorbency units (mean ± SD). PP, CA and TA units refer to mg/l of malvidin-3-glucoside. T is the wine with no addition of commercial mannoproteins; C1 is concentration of 0.2 g/L; C2 is concentration of 0.4 g/L; MP1 and MP2 are commercial yeast mannoproteins.

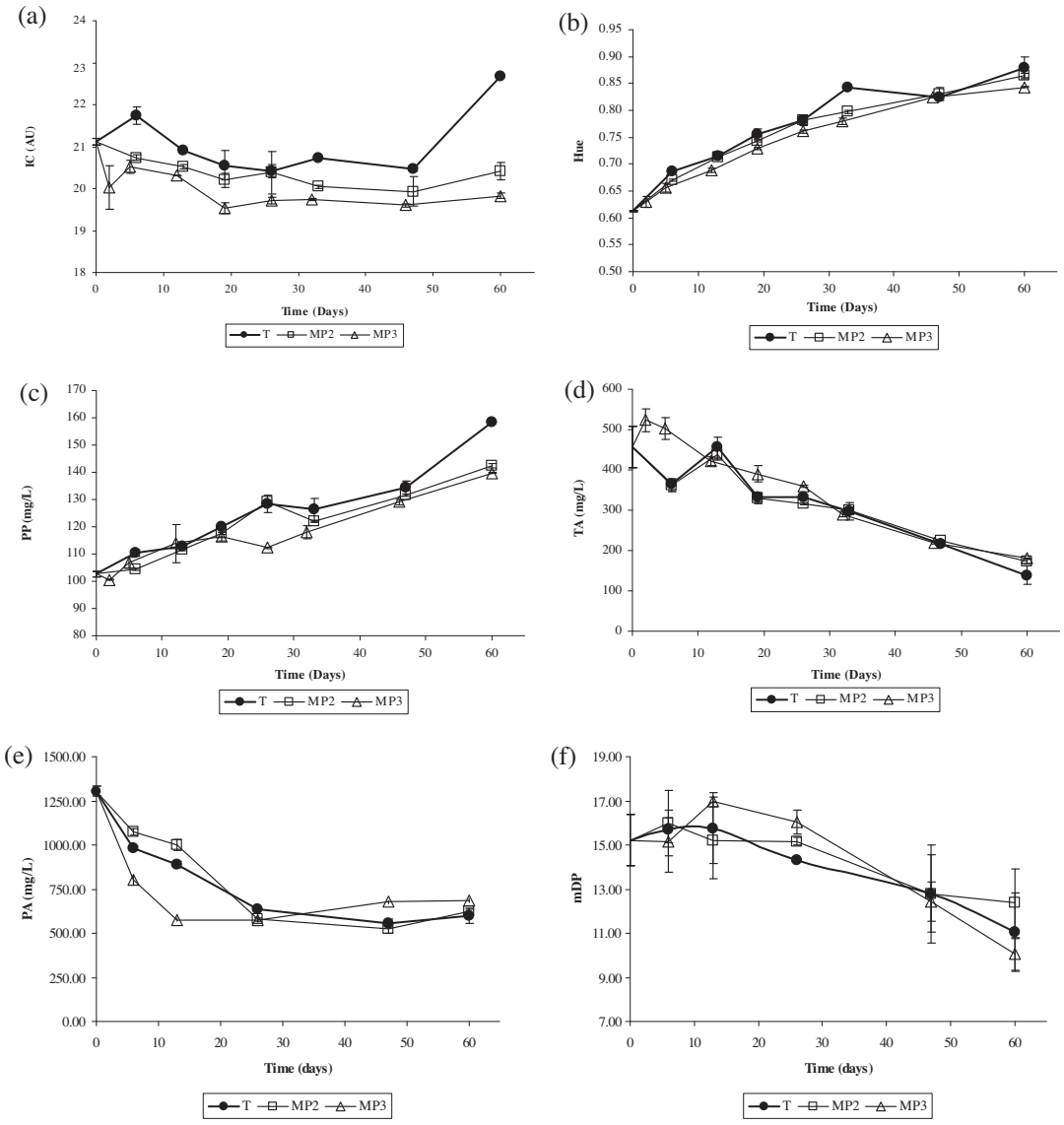

Fig. 2. (a) Colour intensity (CI), (b) hue, (c) polymeric pigments (PP), (d) total anthocyanins (TA), (e) total proanthocyanidins (PA), and (f) mDP evolutions of wine C.

one that showed the slowest rate of decrease until the end of the experiment. Referring to wine B, the situation was similar, with a decrease of mDP and PA for all samples (Fig. 3c and d). The MP1C2 sample seemed to have a slower decrease of mDP. The quantity of proanthocyanidins was slightly higher for the T sample after 164 days, than for the mannoprotein added samples. It is important to notice that the method used to achieve the concentration of proanthocyanidins on wine refers only to native tannins, resulting in a big decrease of these forms as they conjugate with anthocyanins directly, or with an ethanol bridge during wine ageing.

3.3.2. Wine C

In order to understand what was happening among the proanthocyanidins in the samples with added commercial mannoproteins, and as other authors already have reported the lack of a tannin stabilisation character of commercial mannoproteins on the wine polyphenolic and colour composition (Guadalupe & Ayestarán, 2008; Guadalupe et al., 2007, 2010), it was important to verify if there was any influence by the commercial mannoproteins in the different proanthocyanidins polymer chains, according to their degree of polymerisation. For this, wine C was added with two different commercial mannoproteins, at the same concentration, and incubated at 35 °C permanently for sixty days, in order to accelerate any polymerisation reactions that could occur between proanthocyanidins themselves and other wine compounds, namely with anthocyanins. Each sample was then divided into eight polymer fractions and submitted to thiolysis for galloylation and prodelphinidins percentage and estimation of their mDP and PA content.

Table 2 shows the evolution of galloylation and prodelphinidins percentage for global proanthocyanidins and for each mDP interval. Both galloylation and prodelphinidins percentage with time was constant, with no significant differences between samples. By evaluating the evolution of the total proanthocyanidins present in the wine (Fig. 2e), it was possible to conclude that at day 61 the quantity of tannins was slightly higher on MP3 sample than on T and MP2. It was also possible to see a constant evolution on the quantity of proanthocyanidins with time, with a slower decrease on MP3 when compared with the evolution of T and MP2 experiments. In Fig. 2f it was possible to observe the overall mDP of the three experiments. The evolution of T and MP2 samples was more constant, decreasing with time, though the T wine had at the end a smaller mDP than MP2. Concerning the MP3 wines, there

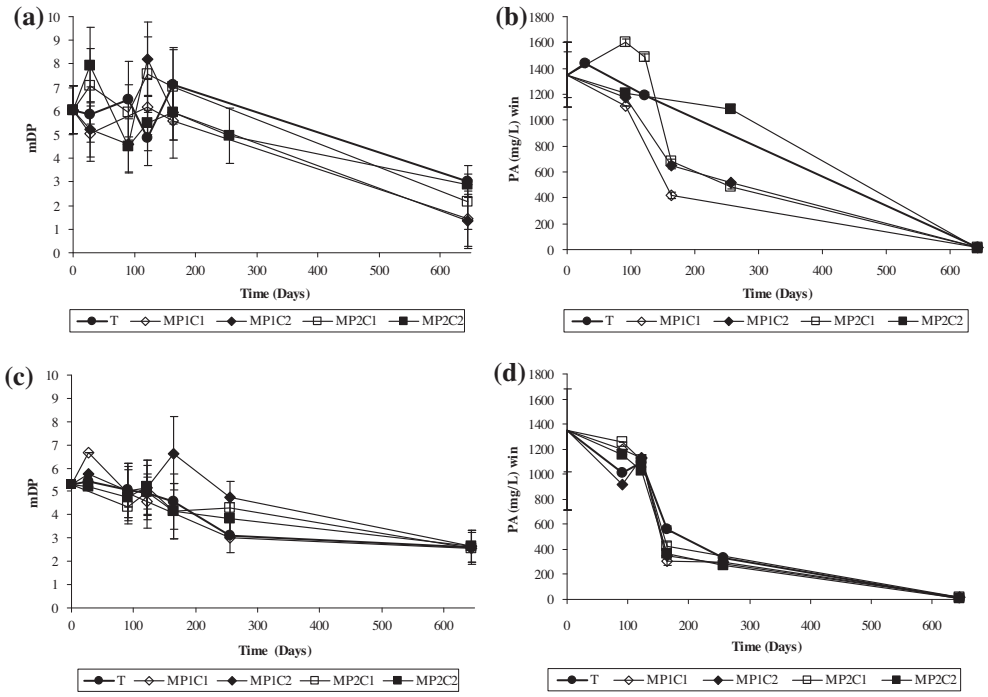

Fig. 3. Wine A (a) mDP for and (b) proanthocyanidins content (PA); wine B (c) mDP for, and (d) proanthocyanidins content (PA).

Table 2
Structural characteristics of the proanthocyanidins of wine C.

Sample	Time (days)	Global		[3–8]		[8–14]		[14–20]		[20–30]	
		% Gal	% Prodelph	% Gal	% Prodelph	% Gal	% Prodelph	% Gal	% Prodelph	% Gal	% Prodelph
T	0	2.5 ± 0.3	24.1 ± 1.0	2.4 ± 0.2	18.7 ± 0.6	2.9 ± 0.3	24.1 ± 0.1	2.5 ± 0.2	25.7 ± 0.4	2.6 ± 0.3	26.3 ± 1.8
	26	2.3 ± 0.4	25.5 ± 3.6	2.2 ± 0.8	21.0 ± 5.0	2.4 ± 0.2	24.9 ± 3.3	2.3 ± 0.3	27.9 ± 2.2	2.0 ± 0.4	27.7 ± 4.4
	60	2.8 ± 0.3	25.6 ± 2.3	2.5 ± 0.7	21.6 ± 4.7	2.8 ± 0.2	25.3 ± 1.5	2.9 ± 0.1	28.5 ± 1.0	–	–
MP2	0	2.5 ± 0.3	24.1 ± 1.0	2.4 ± 0.2	18.7 ± 0.6	2.9 ± 0.3	24.1 ± 0.1	2.5 ± 0.2	25.7 ± 0.4	2.6 ± 0.3	26.3 ± 1.8
	26	2.3 ± 0.3	27.5 ± 3.2	2.3 ± 0.1	23.5 ± 1.4	2.1 ± 0.7	22.9 ± 0.6	2.5 ± 0.3	29.8 ± 5.0	2.1 ± 0.2	27.3 ± 1.9
	60	2.6 ± 0.3	25.0 ± 2.0	2.7 ± 0.5	21.8 ± 3.7	2.6 ± 0.2	26.4 ± 1.3	2.5 ± 0.3	25.4 ± 1.6	–	–
MP3	0	2.5 ± 0.3	24.1 ± 1.0	2.4 ± 0.2	18.7 ± 0.6	2.9 ± 0.3	24.1 ± 0.1	2.5 ± 0.2	25.7 ± 0.4	2.6 ± 0.3	26.3 ± 1.8
	26	2.5 ± 0.3	24.9 ± 0.9	2.3 ± 0.1	21.4 ± 0.5	2.6 ± 0.1	23.8 ± 0.7	2.4 ± 0.2	26.2 ± 0.7	2.9 ± 0.7	26.7 ± 1.3
	60	2.6 ± 0.4	25.2 ± 1.9	2.5 ± 0.8	21.3 ± 2.1	2.6 ± 0.2	26.1 ± 1.9	2.7 ± 0.4	29.3 ± 0.8	–	–

% Gal – percentage of galloylation, % prodelph – percentage of prodelphinidins. T is the wine with no addition of commercial mannoproteins; MP2 and MP3 are commercial yeast mannoproteins. Global, [3–8], [8–14], [14–20] and [20–30] are ranges of mean polymerisation degrees.

was a slight increase of the mDP at the beginning of the experiment, and subsequently a more evident decrease to lower values when compared with the other two wines.

Fig. 4 shows the quantity of proanthocyanidins per mDP interval and by day of sampling for each experiment. It is important to notice that the mDP values obtained for each of the eight purified fractions varied between 3.61 and 32.06 (data not shown), resulting in a very large range of values for this parameter. The highest weighted average mDP found in the three wine experiments was 16.97. Sun et al. (1998) and Maury, Sarni-Manchado, Lefebvre, Cheynier, and Moutounet (2001) have found values of mDP on wines of 22.1 and 27, respectively. The fact that these long chain tannins remain in solution is probably due to: (a) stabilisation resulting from the complexation between polymeric tannins and anthocyanins, maintaining tannins soluble even with high mDP values as suggested by Maury et al. (2001); (b) eventually this stabilisation can result from the interaction between the condensed tannins and other wine macromolecules. Because of this variation of values among the eight tannin fractions obtained on the glass powder separation column we decided to show the results in intervals of mDP values, from 3 to 8, 8 to 14, 14 to 20 and 20 to 30 in order to facilitate the results evaluation. For the T sample (Fig. 4a), on day 0, the quantity of PA was similar to all groups of mDP. On day 26, there were proanthocyanidins of all mDP intervals, but in higher amounts on [3–8] and [8–14] mDP, decreasing on the other two intervals. On day 61, there were no proanthocyanidins with mDP [20–30], existing mainly on [3–8] and [14–20]. Concerning the MP2 added samples (Fig. 4b), the evolution of proanthocyanidins on day 26 was similar to day 0, except for the fact that the quantity of proanthocyanidins decreases for the [8–14] mDP interval. On day 61, there was a prevalence of proanthocyanidins with mDP between 14 and 20, followed by [8–14] and finally [3–8], with no proanthocyanidins on the [20–30] interval. Regarding samples T and MP2, it seemed that there was no stabilisation effect but a continuous evolution of the polymerisation reactions, and precipitation of the polymers with the biggest chains and highest molecular weight. The MP3 added samples presented a smaller quantity of proanthocyanidins with mDP between 8 and 14 when comparing it with the other samples on day 26 (Fig. 4c). On day 61, most of the proanthocyanidins had an mDP on the interval [8–14], followed by the intervals [3–8] and [14–20]. At the end of the experiment it seemed that there was a stabilisation of the proanthocyanidins chains with mDP between

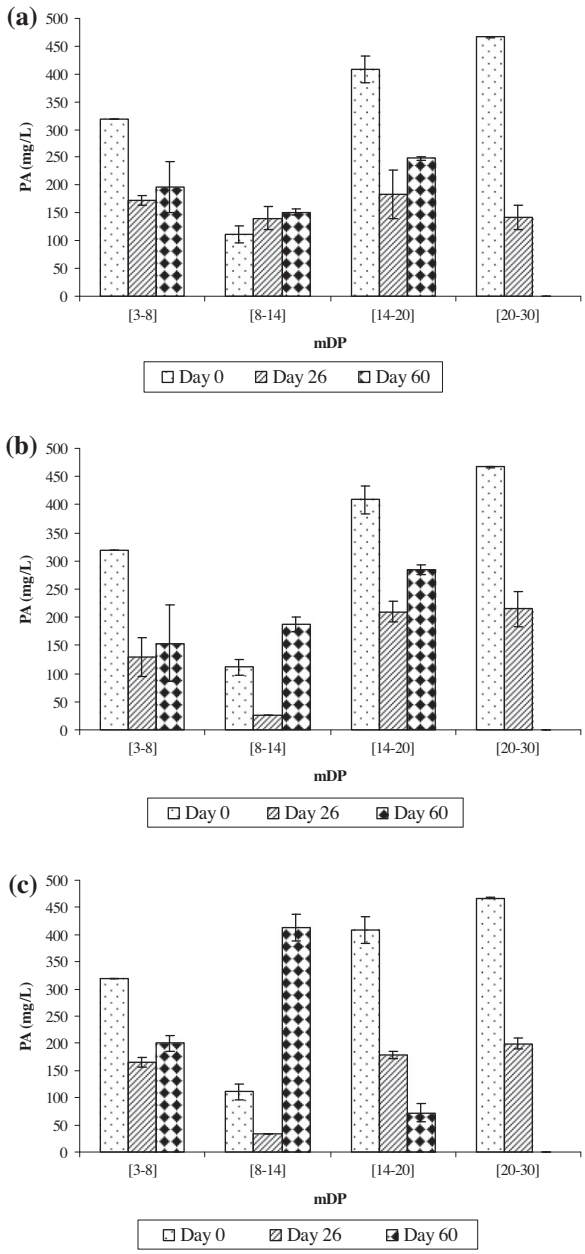

Fig. 4. Evolution of the quantity of proanthocyanidins per mDP interval and by day of sampling for wine C, (a) with no commercial mannoproteins addition, (b) with MP2 commercial mannoprotein addition, and (c) with MP3 commercial mannoprotein addition.

8 and 14, as most of the proanthocyanidins appeared with mDP in this interval, contrary to T and MP2. In fact, on T and MP2 samples, there was an evidence of a continuous evolution of the chain length until precipitation, with no stabilizing effect at all. Riou et al. (2002) verified that in a wine model solution, polysaccharides, namely mannoproteins, interfered with the particle size of proanthocyanidins, although they did not protect the tannins initial aggregation. Escot et al. (2001) and Guadalupe et al. (2007) demonstrated that mannoproteins addition alter significantly the mouth and structural properties of red wines, leading to a decrease in red wine astringency. If mannoproteins prevent tannin particle growth after the particle size reaches a certain number of monomers, then it is possible to explain why mannoproteins can contribute to tannin stabilisation and the achievement of a more rounded wine with less astringent tannins. The explanation for these properties can be explained not only by a precipitation of long chain tannins at the beginning of ageing, but also by this interaction between mannoproteins and tannins leading to the maintenance of the polymers in solution, preventing their growth and possibly their interaction with saliva proteins.

Though there was no evidence of a stabilizing effect of MP2 on tannins, it could be that there was a smaller interaction due to the characteristics of the commercial product itself that could act at a smaller scale than MP3. The molecular weight profile of MP3 commercial product, after purification on concanavalin-A, presented two polysaccharides with average molecular weights of 44 and 19 kDa. Comparing with the MP2 product, there were also two polysaccharides with average molecular weight of 164 and 8 kDa. This difference in polysaccharide composition could be the reason why the MP3 commercial product showed a stabilisation effect on tannin growth and MP2 had the same behaviour with the T samples (with no commercial mannoprotein addition). Poncet-Legrand et al. (2007) have shown that low molecular weight mannoproteins have stabilising effects on the grape seed tannin aggregation, and that high molecular weight molecules do not have any impact, suggesting that the stabilisation mechanism can be a steric stabilisation, explaining why the medium and low molecular weight molecules were more efficient.

The fact that for all modalities at day 61 there were no proanthocyanidins with a mDP higher than 20 was probably due to the conjugation of three hypotheses: (a) the polymerisation of flavan-3-ol units results in the formation of high molecular weight polymers that precipitate; (b) the association between tannins and anthocyanins as $T–A^+$ complexes promotes the stability of the molecule without any further growth (Vidal Cartalade, Souquet, Fulcrand & Cheynier, 2002); (c) as tannin tends to aggregate through time and further to precipitate, this leads to a reduction of the number of flavan-3-ol units existing in the solution, limiting the reaction between monomers and monomers with oligomers in order to form higher chain polymers.

4. Conclusions

The enriched mannoprotein commercial preparations did not have an effect on colour stabilisation of the studied wines. The evolution of colour parameters with time was similar for all experiments, showing no efficient effect of the three commercial products on the colour stability parameters of the wines. The global tannin profile evolution did not show significant differences. When it came to analysing the intervals of the several tannin polymerisation degrees it was possible to see differences between one of the commercial mannoproteins studied and the non-added mannoproteins experiment. MP3 seemed to stabilise the growth of tannins with a polymerisation degree between 8 and 14, suggesting that the interaction between mannoproteins and tannins leads to the maintenance of the tannins with an mDP in this interval in wine. The fact that the two concanavalin-A purified polysaccharides presented low molecular weights (44 and 19 kDa) supports the hypothesis of an interaction occurring between low-medium MW mannoproteins and proanthocyanidins. Although there was no influence on the colour parameters, it is possible that specifically the commercial mannoprotein MP3 used in this work has some influence on the wine tannin aggregation evolution, contributing to the delay of tannin polymerisation in red wines. This study demonstrated the importance of studying not only the wine global mDP evolution but also the several existing polymerisation degrees, as there were significant differences between the modalities when it comes to a more detailed study. Further studies should be made with this commercial product, namely its interaction in wine model solutions in order to better understand the mechanisms of this interaction. The results of this work can be

applied directly to the winery process with the aim of applying commercial mannoprotein enriched products to red wines for tannin stabilisation.

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