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Effect of mannoproteins on red wine colour stability: new insights from collaborative OIV work

Alessandra Rinaldi[1], Alessandra Luciano[1], Stéphanie Carrillo[2], Anne-Laure Gancel[3], Jordi Gombau[4], Silvia Motta[5], Céline Poncet-Legrand[2], Stéphanie Roi[2], Frédéric Véran[2], Antonella Bosso[5], Thierry Doco[2], Pierre-Louis Teissedre[3], Fernando Zamora[4], Luigi Moio[1], and Angelita Gambuti[1,*]

[1] Department of Agricultural Sciences, Grape and Wine Science Division, University of Naples Federico II, Viale Italia, 83100 Avellino, Italy
[2] SPO INRAE, Institut Agro, Univ. Montpellier 2, place Pierre Viala – 34060 Montpellier Cedex 02 – France
[3] UMR Œnologie 1366, Université de Bordeaux, ISVV, 210 Chemin de Leysotte, 33140 Villenave-d'Ornon, France
[4] Departament de Bioquímica i Biotecnologia, Facultat d'Enologia de Tarragona, Universitat Rovira i Virgili, C/Marcel.li Domingo 1, 43007 Tarragona, Spain
[5] CREA – Centro di Ricerca Viticoltura ed Enologia, (CREA-VE), Via Pietro Micca, 35, 14100 Asti, Italia

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*correspondence:
angelita.gambuti@unina.it

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ABSTRACT

Since 2005, mannoproteins (MPs) have been authorised by the European Community (EU, regulation 2165/2005) as an additive for tartaric and protein stabilisation. However, their effect on colour stabilisation in red wine is still a matter for debate in research due to the nature of the product, timing, dose, and wine variety. In recent years, the International Organisation of Vine and Wine (OIV) expert working group has been carrying out collaborative work to elucidate the role of MPs in red wine. The aim of the present work is to show the results of the third year of a study in which five laboratories performed the same analyses on the same wines. Three mannoproteins (A, B, C) at two concentrations (20–40 g/hL) were added to two wines (French and Italian). The equilibration period was one week at cellar temperature (15–18 °C). The wines were then analysed at two aging time points: after one month and five months. The analyses consisted of a cold stability test, CIEL*a*b* coordinates, colour parameters (Abs 420–520–620 nm), and a copigmentation index. An analysis of individual anthocyanins and the characterisation of the MPs was also carried out. Results were expressed as the means of four repetitions carried out in five laboratories. Besides slight variations in colorant intensity and CIEL*a*b* coordinates, none of the wines showed differences visible to the human eye. MPs seem to prevent the loss or degradation of molecular anthocyanins at one month aging. The copigmentation index seemed to increase depending mainly on MP typology, with no difference between the two tested doses, indicating that the 20 g/hL concentration may be appropriate for these wines. The stability of the colouring matter was always observed after the MP treatment. However, the multivariate analyses revealed that after five months of aging the MP-C was the most effective for both wines. Finally, the effect on stability of the colouring matter is MP-dependent, the mannose content (>80 %) and molecular weight (37 < kDa < 79) being the critical factors for the effectiveness of MPs in red wine colour stability.

KEYWORDS: mannoprotein, colour stability, copigmentation, colouring matter, pigments, anthocyanins, red wine

INTRODUCTION

Mannoproteins (MPs) are proteoglycans that are derived from the cell wall of *Saccharomyces cerevisiae*. They are released naturally during alcoholic fermentation and yeast autolysis and can also be introduced exogenously as authorised oenological additives. The European Community authorised their use in winemaking in 2005 (Regulation EC Nº 2165/2005), mainly for tartaric and protein stabilisation. In addition, the efficacy of commercial MPs in modulating phenolic content and influencing the chromatic and sensory properties of wine has also been demonstrated and extensively studied.

Most of the initial studies relate to the role of MPs in interacting with tannins (Poncet-Legrand *et al.*, 2007; Guadalupe *et al.*, 2007), paving the way for possible effects on wine mouthfeel. Subsequently, Rinaldi *et al.* (2012) showed that MPs cause a lower precipitation of salivary proteins when added to wine, and Manjón *et al.* (2021) showed that the formation of salivary protein-mannoprotein complexes, a key factor in modulating astringency perception, is influenced by the hydrophobicity and structural properties of MPs. These findings highlight the importance of considering the specific composition and preparation of commercial MPs when targeting astringency reduction, particularly in tannic red wines. In addition, the duration of contact between MPs and wine has a significant effect on the colloidal equilibrium, thereby influencing sensory results. Rinaldi *et al.* (2021) observed that the tactile effects of MPs varied with the tannin profile of the wine. In wines produced with extended maceration and an anthocyanin/tannin ratio of 0.2, MPs enhanced mouthfeel and contributed to a soft, velvety texture, in addition to improving colour stability. In free-run wines with a higher anthocyanin-to-tannin ratio (0.5), all treatments promoted the formation of polymeric pigments, which were associated with improved tactile sensations (*e.g.*, silky, velvety, mouthcoating) and a concomitant reduction in perceived bitterness.

These results suggest that MPs could serve as effective tools for enhancing both sensory and visual characteristics of tannic red wines. A recent study by Núñez *et al.* (2025) found a strong correlation between perceived wine volume, viscosity, and mannose content in premium wines, a relationship that was less evident in standard quality wines. Mouthfeel attributes such as roundness and smoothness were also positively associated with these parameters. This research highlights the potential of tailor-made yeast strain selection as a practical, sustainable and cost-effective strategy to improve sensory attributes across different wine quality levels.

Despite extensive research, the role of MPs and their molecular structure in red wine colour and stability remains a subject of debate due to the complex mechanisms behind red wine colour. Wine colour is determined by not only the quality and quantity of native pigments (monomeric anthocyanins), but also by the reactions that govern both the degradation of these pigments and the formation of new, more or less stable compounds, as well as the complex mechanisms involved in copigmentation.

MPs interact with wine phenolic compounds through both their glycosidic and protein components, facilitating a variety of non-covalent interactions, including hydrogen bonding and electrostatic forces. These interactions influence the colloidal stability of the wine matrix, inhibit tannin aggregation, and contribute to the stabilisation of anthocyanins, thereby affecting both colorant intensity and longevity. Several studies have demonstrated the ability of MPs to enhance red wine colour stability by protecting anthocyanins from oxidative and chemical degradation (Gonçalves *et al.*, 2018; Oyón-Ardoiz *et al.*, 2022). A recent thermodynamic analysis conducted by Liang *et al.* (2023) on the interaction between cyanidin-3-*O*-glucoside (C3G) and MPs at a pH of 3.2 identified hydrogen bonding and van der Waals forces as the primary binding mechanisms. The authors further hypothesised that MPs may enhance the UV stability of C3G by shielding the C4 position of the molecule, thereby preventing its transformation from the flavylium cation form into a less stable intermediate bearing a hydroxyl group at this site (Furtado *et al.*, 1993; Pina *et al.*, 2012).

Additional studies have reported that MPs can enhance the stability of red wine colour by facilitating the formation of stable pigments, such as pyranoanthocyanins and flavanol–ethyl–anthocyanin adducts (Dong *et al.*, 2024). These findings suggest that the phenolic and chromatic stability of red wines may be influenced by specific MP structural characteristics, including the mannan/glucan ratio of their glycidic moieties, the balance of hydrophobic and hydrophilic amino acids in their protein component, and their molecular weight. These parameters appear to play a critical role in protecting phenolic compounds and promoting the generation of more stable pigment forms.

MPs may also contribute to improving wine colour and stability via their influence on copigmentation, which can lead to bathochromic shifts and enhanced anthocyanin retention, particularly under oxidative or thermal stress conditions. Given that copigmentation between anthocyanins and flavonols is a well-recognised pathway for stabilising red wine colour, Alcalde-Eon *et al.* (2024) examined the effect of various MPs, both individually and in combination with selected flavonols, on the copigmentation process involving malvidin-3-*O*-glucoside. Their results indicate that while MPs did not induce direct copigmentation effects, they modulated the intensity of flavonol–anthocyanin interactions and, in some cases, altered the colloidal behaviour of the resulting coloured aggregates. These findings were further supported by Guo *et al.* (2024), who demonstrated that MPs could protect malvidin-3-*O*-glucoside and quercetin through the formation of binary or ternary complexes. Interestingly, quercetin was more effectively protected than malvidin-3-*O*-glucoside, and the protective activity varied depending on the specific commercial MPs used, highlighting the importance of MPs composition in determining their efficacy as copigmentation modulators and phenolic stabilisers.

Collectively, these studies confirm that while the role of MPs in red wine colour expression is complex, it is nonetheless significant and highly dependent on both wine matrix and MPs composition. To further elucidate the practical implications of MP application under real winemaking conditions, a collaborative study led by the International Organisation of Vine and Wine (OIV) expert working group was conducted. This study involved five laboratories evaluating the impact of three commercial MPs products (A, B, and C) at dosages of 20 and 40 g/hL on two red wines (French and Italian origin), with an equilibration period of one week at cellar temperature (15–18 °C) prior to pre-bottling filtration. Wines were analysed after one and five months of aging to assess the MPs effects on colour expression and stability. The findings from this multi-year collaboration offer broad insights into the potential of MPs as tools for enhancing chromatic stability across diverse wine matrices.

MATERIALS AND METHODS

1. Mannoproteins analysis

The neutral glycosyl-residues composition of mannoproteins was determined by gas chromatography after polysaccharides hydrolysis with trifluoroacetic acid and neutral sugar conversion in their alditol acetate derivatives. Inositol and allose were added to the samples, after hydrolysis and before reduction and acetylation, as internal standards for quantification (Doco, *et al.*, 1999). The monosaccharides were identified through their retention time. GC-FID (Flame Ionization Detector) was performed by a SHIMADZU GC-2010-Plus gas chromatography system using a fused silica capillary column DB-225 (30 m × 0.25 µm × 0.25 mm ID) (Agilent J&W, Santa Clara, USA) with H_2 as the carrier gas.

The molecular weight distribution and the static and dynamic parameters of the mannoproteins were determined following the protocol described previously by Apolinar-Valiente *et al.* (2013). Briefly, the analyses were performed in an HPSEC (high-performance size-exclusion chromatography) system using two serial Shodex OH-pak KB-803 and KB-805 columns (0.8 × 30 cm^2; Shōwa Denkō, Japan) coupled with an OH-pak KB-800P guard column and equipped with the following set of detectors: a multi-angle (18 angles) laser light (wavelength 666 nm) scattering (MALLS), with one of the angles replaced by a Quasi-Elastic Light Scattering detector (QELS) (DAWN–HELEOS II from Wyatt, CA, USA); an online differential viscometer (ViscoStar II, Wyatt, CA, USA); an UV detector (280 nm) (SPD-20A, Shimadzu, Japan); and a differential refractometer (666 nm, Optilab T-rEX, Wyatt, Santa Barbara, CA, USA). Pools were dissolved in Milli-Q water at 4 g/L and centrifuged at 18,000 g/15 min before being eluted with a filtered (0.1 µm filter, mixed cellulose ester, Millipore) solution (0.1 M $LiNO_3$ + 0.02 % NaN_3) at a flow rate of 1 mL/min and 40 °C. Data were analysed using the ASTRA 6.1.2 software package. An average refractive index increment value (d*n*/d*c*) of 0.150 mg/L was used for the polysaccharide moiety, where *n* was the refractive index of the polysaccharide solution and *c* the polysaccharide concentration in mass. Structural characteristics of the tested MPs (MP-A, MP-B, MP-C) are shown in Table 1.

2. Wine samples

French and Italian wines were used for the experiment: a Grenache–Syrah blend and a Barbera produced during the 2022 vintage in two wineries located in the AOC area (Côtes-du-Rhône, France) and in the Nizza Monferrato DOCG area (Asti, Italy), respectively. Vinification was based on the standard industrial protocol used in each winery. The basic parameters of the wines before the treatment are shown in Table S1.

The two wines were coded as F and W for the French wine aged for one and five months, respectively, and I and H for the Italian wine aged for one and five months, respectively.

TABLE 1. Structural characteristics of MPs.

	MP-A	MP-B	MP-C
Formulation	Powder	Powder	Liquid
Oses	% molar	% molar	% molar
Rhamnose	7.1	0.3	0.0
Arabinose	16.3	4.6	0.8
Apiose	0.0	0.0	0.0
Xylose	0.0	0.0	0.8
Mannose	39.2	51.7	84.5
Galactose	19.7	4.0	0.2
Molar mass moments (g/mol)			
M_n (average molar mass by number)	1.541×10^5 (±0.655 %)	6.723×10^4 (±0.641 %)	3.226×10^4 (±0.626 %)
M_w (average molar mass by mass)	4.229×10^5 (±0.108 %)	2.221×10^5 (±0.191 %)	1.020×10^5 (±0.103 %)
Polydispersity			
M_w/M_n	2.744 (±0.664 %)	3.304 (±0.669 %)	3.162 (±0.634 %)

The French and Italian wines ready for bottling were treated with A, B, and C mannoproteins (provided by Œnoppia, France) at 20 and 40 g/hL in duplicate. The concentrations of 20 and 40 g/hL were the doses normally recommended by the manufacturers. As MP-C was in liquid form (20 % *w/v*) and MP-A and MP-B were in powder, the latter were dissolved at 4 g/20 mL (20 % *w/v*) in Milli-Q water, previously warmed at a temperature of 25–28 °C to aid the dissolution. The concentrations of MPs at 20 and 40 g/hL were prepared as follows:

- 20 g/hL: 1 mL of 20 % MPs solution was added to 1,000 mL of wine (1 + 1,000 mL); the same amount of Milli-Q water was added to 1,000 mL of control wines (20 g/hL); and
- 40 g/hL: 2 mL of 20 % MPs solution was added to 1,000 mL of wine (2 + 1,000 mL); the same amount of Milli-Q water was added to 1,000 mL of control wines (40 g/hL).

The preparation of the wines was carried out at the Department of Agricultural Sciences, Grape and Wine Science Division, University of Naples Federico II (Italy). The procedure was systematised by incorporating MPs into the wine, followed by a one-week equilibration period prior to filtration. The filtration step employed (Merck Millipore glass microfiber filters 2.0 µm (64 g/m^2), Ireland) was consistent with oenological practices typically conducted before the bottling of red wines. Each wine sample was bottled immediately, using narrow-mouth white bottles (120 mL, HPDE, (high-density polyethylene) Thermo Scientific) previously purged with nitrogen, completely filled to eliminate headspace and minimise oxygen ingress, and sealed with a screw cap. Two aging times were set for the analyses: one and five months. F0-20, F0-40 and I0-20, I0-40 represented the French and Italian non-treated wines (the controls), respectively, at one month of aging, and W0-20, W0-40 and H0-20, H0-40 the French and Italian non-treated wines (the controls), respectively, at five months aging.

After preparing the samples, the wines were shipped immediately to the other groups and stored at 16 °C in each research unit. The samples were transported in polystyrene boxes and all shipments dispatched on the same day (22 January 2024). The transport duration was one day, and given the winter conditions, no heat exposure is presumed to have occurred during transit. Each laboratory received 32 experimental wines (controls and treated with MPs A, B and C at 20 and 40 g/hL), 16 (8 × 2 replicates) of which corresponded to samples for the analysis at the one-month aging time (F0-20; F0-40; FA-20; FA-40; FB-20; FB-40; FC-20; FC-40, and I0-20; I0-40; IA-20; IA-40; IB-20; IB-40; IC-20; IC-40, for French and Italian wine, respectively). Sixteen other bottles (W0-20; W0-40; WA-20; WA-40; WB-20; WB-40; WC-20; WC-40, and H0-20; H0-40; HA-20; HA-40; HB-20; HB-40; HC-20; HC-40 for French and Italian wine, respectively), were analysed after an additional four months, representing the five-month aging time.

3. Analyses

3.1. Wine main compositional parameters

The chemical and analytical parameters were determined according to the official methods recognised by the OIV. The analytical protocols employed for each parameter are reported below:

- Titratable acidity (Total Acidity): determined according to SECTION 3.1.3 – ACIDS, method OIV-MA-AS313-01 (revision OIV-OENO 551-2015);
- Volatile acidity (Volatile Acidity): determined according to SECTION 3.1.3 – ACIDS, method OIV-MA-AS313-02 (revision 2015);
- Reducing sugars: determined by titrimetry according to SECTION 3.1.1 – SUGARS, Type II method OIV-MA-AS311-01C;
- Alcoholic strength by volume: determined by pycnometry, oscillating densimeter, and hydrostatic balance techniques in accordance with SECTION 3.1.2 – ALCOHOLS, method OIV-MA-AS312-01A (revisions A2/2000, 8/2000, 24/2003, 377/2009, 566/2016);
- Free and total sulfur dioxide: determined by titrimetry according to SECTION 3.2.3 – OTHER NON-ORGANIC COMPOUNDS; *i.e.*, OIV-MA-AS323-04A1 (free SO_2, Type IV) and OIV-MA-AS323-04A2 (total SO_2, Type II), respectively. The most recent updates to these methods are described in RESOLUTION OIV-OENO 661-2021; and
- L-malic acid: determined by enzymatic assay according to SECTION 3.1.6 – ORGANIC ACIDS, method OIV-MA-AS313-11.

3.2. Equipment used for spectrophotometric analyses

Each research group used the following proper spectrophotometers: a UV-1900 Shimadzu UV−visible spectrophotometer; an A7305 spectrophotometer (Jenway Bibby Scientific Limited, Stone, UK); a spectrophotometer UV-Vis Helios Alpha™ (Thermo Fisher Scientific Inc., Waltham, MA, USA); a V-730ST UV/VIS spectrophotometer (Jasco France, Lisses, France); and a UV-Vis JASCO V-630 spectrophotometer (JASCO, USA).

3.3. Colour parameters

Spectrophotometric measurements were performed using a 1 mm quartz cuvette. The Colorant Intensity (CI) was calculated using the method described by Glories (1984) as the sum of the absorbances at 420, 520 and 620 nm (Abs 420 + 520 + 620). This value was multiplied by 10 to correct the absorbance value to the standard optical path of 10 mm.

The CIEL*a*b* coordinates lightness (L*), chroma (C*), hue (h*), red−greenness (a*), and yellow−blueness (b*) were determined according to the OIV method OIV-MA-AS2-11 (2006), using the absorbances measured between 380 and 780 nm with a 5 nm bandwidth. These values were processed using the formula provided by the OIV method using Excel.

The total colour difference (ΔEab*) was calculated as the Euclidian distance between two points in the CIEL*a*b* space using the following formula: $\Delta Eab^* = ((L^*_1 - L^*_2)^2 + (a^*_1 - a^*_2)^2 + (b^*_1 - b^*_2)^2)^{1/2}$.

3.4. Copigmentation index

The copigmentation index (percentage of colour due to copigmentation) was determined following an adaptation of the methods described by Hermosín-Gutiérrez (2003). Briefly, 2 mL of wine are supplemented with 20 mL of a 10 % acetaldehyde solution to prevent the decolorising effect of sulfur dioxide. After allowing the mixture to stand for 45 min, the absorbance at 520 nm was measured using a 1 mm pathlength cuvette (A1). In parallel, an identical sample was diluted 20-fold with a buffer solution adjusted to the same pH as the original wine. Following dilution, the absorbance at 520 nm was measured in a 10 mm pathlength cuvette (A2). The percentage of colour attributable to copigmentation (copigmentation index) was then calculated using the following equation:

$$Copigmentation\ \ index\ \ (\%) = 100x\ \ \frac{A1 - 2xA2}{A1} \quad (1)$$

3.5. Cold test

The cold stability test was conducted following the protocol for colloidal matter stability reported by Bosso *et al.* (2023). The turbidity of the samples was measured in i) the initial wine, ii) the wine after cascade filtering (from 3 to 0.22 mm), and iii) the filtered wine after cold treatment (48 hours in the fridge at 4 °C). Samples were left 15 min on the bench before measurement. They were manually gently shaken three times before reading on turbidimeter. The difference in Nephelometric Turbidity Units (NTU) values before and after cold treatment (ΔNTU) represented the extent of the wine instability.

4. Molecular anthocyanins

Molecular anthocyanins of samples were analysed using HPLC according to González-Centeno *et al.* (2017). Wines were first filtered (0.45 µm) and 20 µL were injected into a Thermo Scientific Accela HPLC (Thermo Fisher Scientific, Waltham, MA, USA) with an Accela 600 pump module and a UV-Visible diode array detector and using the Xcalibur software. Separation was achieved using a reverse-phase C18 Nucleosil (250 × 4.6 mm, 5 µm) and two solvents at a flow rate of 1 mL/min. Solvent A comprised water/formic acid (95:5, *v/v*) and solvent B comprised acetonitrile/formic acid (95:5, *v/v*). The mobile phase gradient was as follows: from 10 % to 35 % B in 25 min, 100 % B at 35 min, 100 % B from 35 to 40 min, 10 % B at 41 min, and then 10 % B for 4 min before the next injection. Eluting peaks were monitored at 520 nm and identified by comparison with injected external standards and according to previous results (Chira, 2009). Malvidin 3-*O*-glucoside was used as external standard to calculate anthocyanins concentrations that were expressed as mg/L malvidin 3-*O*-glucoside equivalents.

5. Statistical analysis

All analyses were performed on two experimental and two analytical replicates, for a total of four replicates. An inter-laboratory proficiency test was carried out to evaluate and compare the measuring results obtained by the five laboratories, and it served as a preliminary step for further statistical analysis. This procedure enables the standardisation of data within each method by providing the *Z*-score, a statistical measure that expresses individual results relative to the group mean and standard deviation. The *Z*-score is important because it allows performance to be evaluated on a comparable scale across laboratories, identifying potential biases or deviations and ensuring data reliability. The *Z*-score, calculated from the analyses at the two aging times, was always <2, indicating that the performance evaluation by the five laboratories can be considered satisfactory. The colorant intensity, CIEL*a*b* coordinates, copigmentation index, and ΔNTU values were expressed as a mean of four replicates across five laboratories. These data were then normalised using the min-max (0–1) normalisation to run ANOVA and carry out multiple comparison. The min-max normalisation was used to adjust the values of variables in the same magnitude, in the range from 0 to 1, to bring data features to the same scale and reduce outliers. This improves the quality and consistency of the data. The min-max normalisation process involves subtracting the minimum value from the data set and then dividing it by the difference between the maximum and minimum values. This is done for each value in the data set. For each attribute, the min-max of an entry is calculated by:

$$x(i)\ \ norm = (x\,(i) - xmin)/(xmax - xmin) \quad (2)$$

where x is the original attribute value, in dataset entry i, and x_{min} and x_{max} are the minimum and the maximum values of the attribute in the data set. It is important to note that min-max normalisation transforms the data to a certain scale, but does not change the shape of the distribution of the original values.

The monomeric anthocyanins were analysed by one laboratory and were expressed as mg/L. The Fisher (LSD) or Tukey (HSD) test was used to compare samples with a significance of 95 %. The proficiency test, ANOVA, two-way ANOVA, Principal Component Analysis (PCA) were carried out by means of XLSTAT 2024 software (Lumivero, France).

RESULTS AND DISCUSSION

1. Colour characterisation

Figure 1 shows the colorant intensity (CI) – which represents the sum of absorbances at 420, 520 and 620 nm (Abs 420 + 520 + 620) – of French (Figure 1A) and Italian (Figure 1B) wines in boxplots and compares different MPs types (A, B, C), dosages (0, 20, 40 g/hL), and aging times (one and five months).

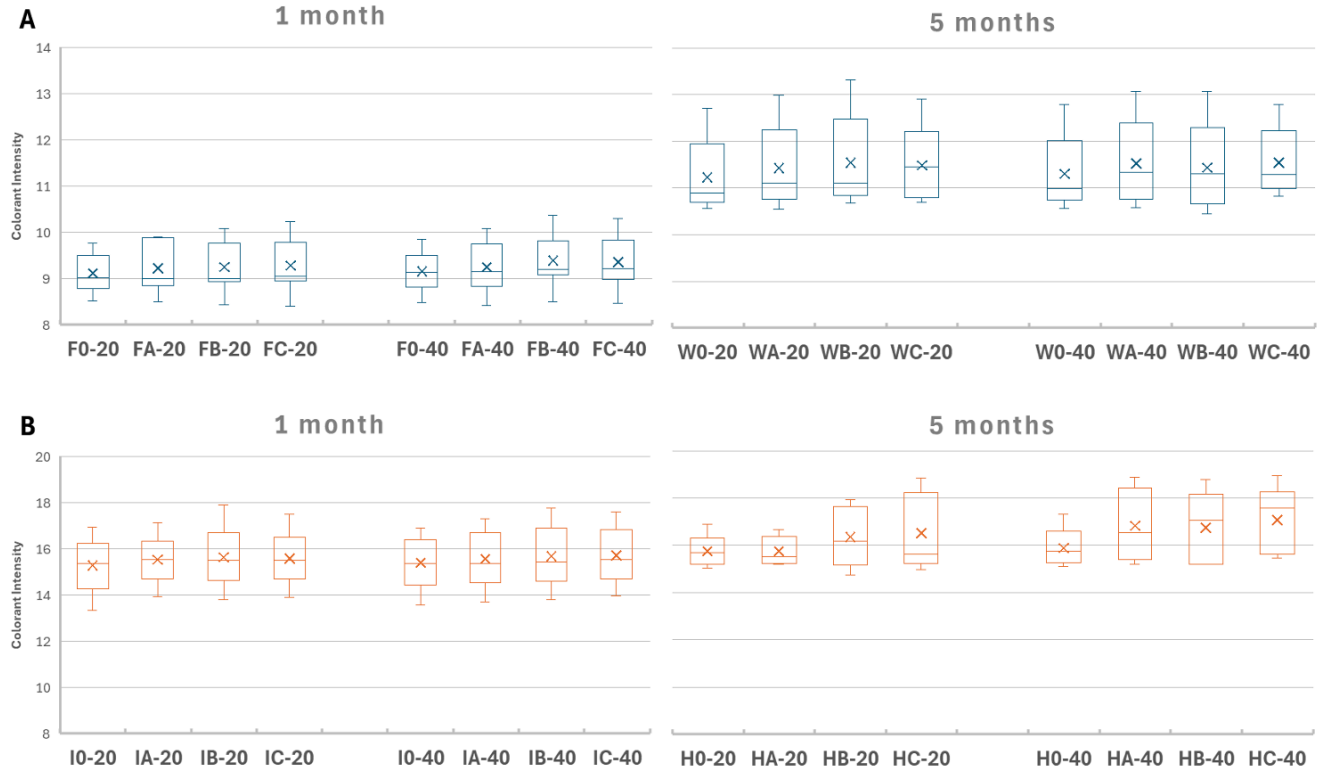


FIGURE 1. Colorant intensity (Abs 420 + 520 + 620) at one and five months of aging of French (F and W series, respectively; 1A) and Italian (I and H series, respectively; 1B) wines treated with MPs (A, B, C) at 20 and 40 g/hL. Control wines are: F0-20, F0-40, and I0-20, I0-40 for the French and Italian wines at one month of aging, and W0-20, W0-40, and H0-20, H0-40 for the French and Italian wines at five months of aging, respectively.

Each boxplot represents the range of the values provided by the five laboratories for each sample along with four repetitions. At one month of aging, the colorant intensity (CI) of the French wines (F series) exhibited comparable values across treatments. After five months (W series), a general increase in CI was observed, aligning with patterns reported in wines characterised by low initial colorant intensity (Rinaldi *et al.*, 2019), but in contrast to the decline observed in Portuguese wines under similar aging conditions (Rodrigues *et al.*, 2012). The Italian wine (Figure 1B) displayed consistently higher baseline colorant intensities suggesting a higher anthocyanin content, potentially linked to grape variety, phenolic extractability, or vinification style. No differences were denoted between Italian wines (I series) at one month of aging as a result of the addition of MPs at either concentration. However, at five months (H wines), the differences between treatments seemed to become more pronounced. MPs-treated wines, mainly at 40 g/hL, exhibited increases in CI indicating a dependence from the dose. Since the colorant intensity as well as the other colour parameters showed variations between laboratories, data were normalised to allow multiple comparisons. ANOVAs were performed on the means of normalised data for spectrophotometric analyses (CI, Abs 420 nm, 520 nm, 620 nm), CIEL*a*b* coordinates (L*, a*, b*) for the French (F) and Italian (I) wines at one month (Table 2), and for the French (W) and Italian (H) wines at five months, and significancy of multiple comparison was shown in Table 3.

Significant differences were observed for CI and absorbances (especially at 620 nm), depending on MPs type, wine and dose. In French wines, the addition of B and C mannoproteins resulted in higher values of CI and absorbance across all wavelengths at 40 g/hL. Lightness (L*) and redness (a*) were significantly lower than in the control wine (F0-40), indicating a darker and less red hues (bluer). In Italian wines, all MP-treated wines showed higher CI and absorbance values. By contrast, no differences were observed for CIEL*a*b* values. Differences in the effect of MPs on CI and CIEL*a*b* have been observed in wines with different anthocyanin/tannin (A/T) ratios, showing wines with higher A/T ratios (*e.g.*, free-run wines), enhanced colour intensity and a better preservation of anthocyanins (Rinaldi *et al.*, 2021).

After five months of aging, while no significant differences in these parameters were observed for the French and Italian wine after the treatment at 20 g/hL, significant differences were observed for the concentration at 40 g/hL, as shown in Table 3. In the French wine, the MP-A and MP-C (WA-40 and WC-40) showed higher absorbance values than in the control at all three wavelengths indicating a high yellow intensity, as well as red and blue intensity, which is consistent with previous research that showed the effect of MPs in enhancing colorant intensity (Rinaldi *et al.*, 2019; Guadalupe *et al.*, 2010). This enhancement has been attributed to mannoproteins' ability to interact with polyphenols, thereby preventing precipitation of pigments and maintaining a vivid wine colour over time. For the

TABLE 2. Spectrophotometric (CI, Abs 420 nm, 520 nm, 620 nm) and CIEL*a*b* values of French (F) and Italian (I) wines treated with mannoproteins (MPs) A, B, and C at 20 and 40 g/hL after one month of aging. Values represent the mean of absolute data across four repetitions over five laboratories. Different letters denote significant differences within each group according to the Fisher (LSD) test applied on normalised data ($p < 0.05$). Bolded *p*-values indicate statistical significance ($p < 0.05$).

Wines	CI	420 nm	520 nm	620 nm	L*	a*	b*
F0-20	9.12	3.54	4.42	1.17 b	21.44	40.83	18.10
FA-20	9.23	3.61	4.48	1.20 b	20.85	40.39	17.33
FB-20	9.25	3.62	4.47	1.22 ab	20.80	40.28	17.52
FC-20	9.29	3.63	4.49	1.23 a	20.65	40.19	17.45
Pr > F	0.411	0.119	0.057	**0.012**	0.286	0.956	0.704
F0-40	9.16 b	3.56 b	4.43 b	1.18 b	21.46 a	41.03 a	18.14
FA-40	9.25 b	3.61 b	4.45 b	1.21 b	20.69 b	40.09 b	17.32
FB-40	9.39 a	3.67 a	4.53 a	1.24 a	20.38 c	39.93 c	17.19
FC-40	9.36 a	3.66 a	4.50 a	1.24 a	20.39 bc	39.94 bc	17.10
Pr > F	**0.001**	**0.001**	**0.000**	**<0.0001**	**0.000**	**<0.0001**	0.130
I0-20	12.48 b	11.56 b	20.41 b	4.44 b	11.56	37.37	8.53
IA-20	12.73 a	11.65 a	20.48 a	4.52 a	11.38	37.06	8.17
IB-20	12.84 a	11.68 a	20.59 a	4.52 a	11.43	37.10	8.15
IC-20	12.79 a	11.69 a	20.51 a	4.53 a	11.50	37.41	8.43
Pr > F	**0.015**	**0.007**	**0.048**	**0.001**	0.333	0.070	0.055
I0-40	12.65 c	11.58	20.45 b	4.44 c	11.64	37.11	8.67
IA-40	12.80 b	11.63	20.46 b	4.50 b	11.05	36.00	7.49
IB-40	12.92 ab	11.68	20.53 ab	4.54 ab	11.11	36.35	7.74
IC-40	12.94 a	11.68	20.57 a	4.53 a	11.19	36.57	7.76
Pr > F	**0.000**	0.112	**0.004**	**<0.0001**	0.364	0.094	0.071

Italian wines, HC-40 (MP-C) had the highest absorbance at 620 nm, the only significant parameter, suggesting it has a role in preserving blue/purple pigments, which are often linked to derived anthocyanins. The different effects of the MPs on the French and Italian wine may also have depended on the differences in the composition of the native anthocyanins remaining in the wine after five months, as well as on the formation of new pigments resulting from condensation reactions over time.

No significant differences were detected for CIEL*a*b* parameters within each wine, thus indicating visual hue differences were less pronounced, despite some spectral variation in colour absorbance. The total colour difference (ΔEab*) of treated samples for both French and Italian wines after one and five months was always lower than 2 units. Because differences below 3 ΔEab*units were not detectable by the human eye (Martínez *et al.*, 2001), the observed differences in colour were not visible.

2. Effect of MPs on molecular anthocyanins

Table 4 summarises the monomeric anthocyanin concentrations (mg/L) for French and Italian control wines at one (F0-20, F0-40, and I0-20, I0-40, respectively) and five (W0-20, W0-40, and H0-20, H0-40, respectively) months of aging, and wines treated with mannoproteins A, B, and C at 20 and 40 g/hL, along with *p*-values from one-way ANOVA assessing treatment effects. The initial concentration of anthocyanins and the total sum were similar in both wines (F and I), despite the differences observed in the colorant intensity. At one of month aging, total anthocyanin concentrations were markedly higher in the treated samples, particularly in the wines treated with B and C mannoproteins, suggesting an early protective or stabilising effect. This was especially evident in malvidin derivatives (mal-Glc, mal-acetyl-Glc, mal-coumaroyl-Glc), which consistently displayed the highest concentrations and statistical significance across treatments ($p < 0.0001$), indicating a key role in wine colour stability within one month of aging. Malvidin-3-*O*-glucoside (mal-Glc), recognised as the most stable and predominant anthocyanin in red wine, was found in higher concentrations in the treated wines, indicating that the addition of these MPs contributes to the preservation of wine pigments during aging. As shown in Table 4, the concentration of mal-acetyl-Glc differed significantly between the two wines. MP-C exhibited a protective effect in the French wine at both concentrations tested and in the Italian wine at 40 g/hL. At 20 g/hL in the Italian wine, a similar protective effect was observed for both MP-B and MP-C ($p < 0.0001$).

TABLE 3. Spectrophotometric (CI, Abs 420 nm, 520 nm, 620 nm) and CIEL*a*b* values of French (W) and Italian (H) wines treated with mannoproteins (MPs) A, B, and C at 40 g/hL after five months of aging. Values represented the mean of absolute data across four repetitions over five laboratories. Different letters denote significant differences within each group according to the Fisher (LSD) test applied on normalised data ($p < 0.05$). Bolded *p*-values indicate statistical significance ($p < 0.05$).

Wine	CI	420 nm	520 nm	620 nm	L*	a*	b*
W0-40	11.30	3.97 c	5.37 c	1.48 c	16.38	38.40	13.51
WA-40	11.53	4.07 ab	5.45 ab	1.51 ab	16.13	38.17	12.92
WB-40	11.44	4.03 bc	5.41 bc	1.49 bc	16.13	38.45	13.26
WC-40	11.54	4.11 a	5.54 a	1.55 a	16.17	38.28	13.01
Pr > F	0.130	**0.005**	**0.010**	**0.019**	0.532	0.591	0.128
H0-40	15.89	5.08	8.40	2.18 b	10.90	34.30	8.30
HA-40	16.83	5.37	8.74	2.42 a	9.35	29.79	6.80
HB-40	16.75	5.27	8.65	2.36 ab	9.45	28.62	6.77
HC-40	17.08	5.37	8.78	2.40 a	9.44	29.98	6.61
Pr > F	0.109	0.090	0.093	**0.034**	0.195	0.392	0.154

Delphinidin-3-*O*-glucoside (del-Glc), petunidin-3-*O*-glucoside (pet-Glc), and peonidin-3-*O*-glucoside (peo-Glc) were less reduced with MPs in a significant way in F and I wines ($p < 0.0001$). The MP-C showed the best protective effects of all the MPs.

These findings support the hypothesis that mannoproteins contribute to anthocyanin stabilisation during early aging stages and may delay pigment degradation (Guadalupe & Ayestarán, 2008). Cyanidin-3-*O*-glucoside (cya-Glc) did not show significant changes in F, but in I wines the trend was similar to the other anthocyanins. It seems that MPs better protected the stable forms of anthocyanins from degradation possibly by forming complexes or stabilising them in colloidal structures (Alcalde-Eon *et al.*, 2024). MPs had already shown a protective effect on anthocyanins such as cya-Glc from thermal degradation (Liu *et al.*, 2023) and ultraviolet radiation (Liang *et al.*, 2023) in model solutions. However, the formation of stable complexes that preserve the stability and colour properties of anthocyanins appeared to be more difficult in red wine.

Total monomeric anthocyanins dropped significantly from one to five months across all samples and treatments with a general decrease of about 86 % for both wines (Table 4). Only about 50 % of total anthocyanins were detected in wines, specifically del-Glc, pet-Glc, peo-Glc, mal-Glc, and the more stable acylated derivatives, namely the acetylated and coumaroylated forms. In Italian wines (H series), the total concentration of molecular anthocyanins was higher than in the French ones (W series), and the controls showed higher content of total anthocyanins than the treated wines. In the W wines, no significant differences were found after mannoprotein treatment except for mal-Glc at 40 g/hL; meanwhile, differences found in the H wines depended on the concentration and type of MP, the decrease of monomeric anthocyanins possibly being associated with the formation of polymeric pigments. The mal-Glc and its acetylated and coumaroylated forms showed different trends across the MP treatments and dosage in H wines. The MP-C was found to be the most efficient at protecting this compound. However, a future study of polymeric pigments may provide valuable insights into the fate of monomeric anthocyanins in forming these compounds during aging.

The benefit of MPs was clearer at one month of aging than at five months, when many of the differences disappeared. Aging reduced the effect of MPs on maintaining molecular anthocyanins in solution, probably due to polymerisation, copigmentation, or precipitation effects. As regards the concentration effect, the increase of the MP dose from 20 to 40 g/hL did not show significant improvements, suggesting that 20 g/hL may be appropriate for these wines. Alcalde-Eon *et al.* (2019) have shown MPs to be non-linear and dose-dependent, observing that at lower doses MPs enhance copigmentation by stabilising specific pigment families, while high doses may limit anthocyanin availability for copigmentation through adsorption or interference with pigment interactions, leading to diminished effects. This indicates that higher doses may disrupt the balance of pigments necessary for effective copigmentation.

3. Effect of MPs on the copigmentation index

MPs can contribute to colour stabilisation through the enhancement of copigmentation by improving phenolic solubility and supporting molecular stacking interactions (Guadalupe & Ayestarán, 2008; Alcalde-Eon *et al.*, 2024). Figure 2 shows the means of the copigmentation index for the two wines (French and Italian) across the four conditions: MP-A, MP-C, MP-B, and the control (no MPs). While the copigmentation index was higher in the Italian wine treated with MP-C than in the control and other treatments, in French wine it was higher in the MP-A- and MP-C-treated wine than in the control, but was not different from MP-B.

A two-way ANOVA was performed to understand the effect of MPs and aging time, and their interaction on the copigmentation index. In French wines, the efficacy of mannoproteins in promoting copigmentation was

TABLE 4. Monomeric anthocyanins (mg/L) in French and Italian wines treated with mannoproteins (MPs) A, B, and C at 20 and 40 g/hL after one and five months of aging. Different letters denote significant differences within each wine series in row according to the Tukey (HSD) test ($p < 0.05$). Bolded *p*-values indicate statistical significance ($p < 0.05$).

	French wine										Italian wine									
Monomeric anthocyanin (mg/L)	**One month aging**																			
	F0-20	**FA-20**	**FB-20**	**FC-20**	***p*-value**	**F0-40**	**FA-40**	**FB-40**	**FC-40**	***p*-value**	**I0-20**	**IA-20**	**IB-20**	**IC-20**	***p*-value**	**I0-40**	**IA-40**	**IB-40**	**IC-40**	***p*-value**
del-Glc	10.08 c	11.30 b	11.02 b	11.93 a	**<0.0001**	9.62 c	11.44 a	10.81 b	11.82 a	**<0.0001**	10.01 c	10.95 b	11.59 a	11.38 a	**<0.0001**	10.13 c	10.96 b	11.55 a	11.21 b	**<0.0001**
cya-Glc	2.14	2.27	2.14	2.43	0.289	2.03	2.15	2.23	2.32	0.166	2.87 c	3.02 b	3.18 a	3.19 a	**<0.0001**	2.93 b	3.13 a	3.22 a	3.15 a	**0.005**
pet-Glc	8.34 d	9.52 b	9.11 c	9.98 a	**<0.0001**	8.27 c	9.66 a	8.91 b	9.75 a	**<0.0001**	12.66 c	13.80 b	14.86 a	14.53 a	**<0.0001**	12.95 d	14.13 c	14.96 a	14.52 b	**<0.0001**
peo-Glc	6.56 d	7.79 b	7.42 c	8.32 a	**<0.0001**	6.30 c	7.58 b	7.58 b	8.09 a	**<0.0001**	5.51 c	6.13 b	6.67 a	6.54 a	**0.000**	5.67 c	6.12 bc	6.77 a	6.48 ab	**0.001**
mal-Glc	46.25 d	54.46 c	52.40 b	56.29 a	**<0.0001**	45.45 c	54.82 a	51.06 b	55.91 a	**<0.0001**	50.76 c	56.09 b	59.83 a	58.74 a	**<0.0001**	51.72 d	56.44 c	60.48 a	58.79 b	**<0.0001**
del-acetyl-Glc	5.29	5.42	5.48	5.29	0.384	5.31	5.09	5.25	5.30	0.572	3.82	3.77	3.75	3.84	0.740	3.88	3.82	3.92	3.87	0.818
cya-acetyl-Glc	1.34	1.37	1.38	1.41	0.952	1.35	1.53	1.52	1.40	0.633	1.42 a	1.19 b	1.26 b	1.14 b	**0.009**	1.46 a	1.04 c	1.21 b	1.26 b	**0.001**
pet-acetyl-Glc	1.87	2.30	2.03	2.24	0.096	1.94	2.07	2.17	2.31	0.199	1.90	1.97	1.95	1.92	0.780	1.94 a	1.77 b	1.96 a	1.90 a	**0.001**
peo-acetyl-Glc	2.02	3.66	2.03	2.35	0.310	1.95	2.24	2.13	2.34	0.060	0.96	0.90	1.12	1.02	0.110	0.89	0.92	0.95	0.92	0.921
mal-acetyl-Glc	14.22 d	16.84 b	15.98 c	17.51 a	**<0.0001**	14.19 d	16.683 b	15.80 c	17.39 a	**<0.0001**	10.09 b	10.92 b	11.64 a	11.54 ca	**<0.0001**	10.04 d	11.04 c	11.94 a	11.56 b	**<0.0001**
peo-coumaroyl-Glc	1.10	1.17	1.19	1.15	0.117	0.93 b	1.12 a	1.05 ab	1.18 ba	**0.014**	0.63	0.65	0.65	0.66	0.954	0.65	0.63	0.61	0.63	0.848
mal-coumaroyl-Glc	2.94 c	3.67 b	3.56 b	3.91 a	**<0.0001**	3.02 b	3.32 b	3.36 b	3.87 a	**0.001**	3.57 c	4.10 b	4.45 a	4.33 a	**<0.0001**	3.74 d	4.18 c	4.56 a	4.35 b	**<0.0001**
Total	102.06 c	119.8 a	113.75 b	122.83 a	**<0.0001**	100.35 ad	117.69 b	111.88 c	121.67 a	**<0.0001**	104.18 c	113.51 b	120.96 a	118.84 a	**<0.0001**	106.00 d	114.18 c	122.13 a	118.64 b	**<0.0001**
	Five months aging																			
	W0-20	**WA-20**	**WB-20**	**WC-20**	***p*-value**	**W0-40**	**WA-40**	**WB-40**	**WC-40**	***p*-value**	**H0-20**	**HA-20**	**HB-20**	**HC-20**	***p*-value**	**H0-40**	**HA-40**	**HB-40**	**HC-40**	***p*-value**
del-Glc	0.66	0.73	0.68	0.68	0.801	0.61	0.71	0.68	0.63	0.418	1.45	1.26	1.3726	1.48	0.406	1.73 a	1.20 b	1.23 b	1.29 b	**0.030**
pet-Glc	0.69	0.79	0.72	0.77	0.533	0.65	0.70	0.67	0.73	0.722	2.11 a	1.44 c	1.56 bc	1.82 b	**0.001**	2.17 a	1.54 b	1.50 b	1.62 b	**0.021**
peo-Glc	0.47	0.49	0.49	0.47	0.959	0.48	0.44	0.51	0.41	0.244	0.86	0.63	0.68	0.73	0.060	0.89	0.66	0.68	0.69	0.064
mal-Glc	4.60	5.21	4.67	4.81	0.282	4.12 c	4.69 ab	4.36 bc	4.73 a	**0.008**	7.64 a	5.59 c	6.19 bc	7.01 ab	**0.001**	8.36 c	5.45 b	5.53 b	6.25 b	**0.026**
mal-acetyl-Glc	1.42	1.70	1.49	1.58	0.065	1.45	1.65	1.41	1.60	0.409	1.42 ab	1.24 b	1.49 ab	1.74 a	**0.032**	1.74 a	1.23 c	1.40 bc	1.63 ab	**0.009**
mal-coumaroyl-Glc	1.48	1.55	1.46	1.65	0.498	1.50	1.52	1.59	1.66	0.509	0.79 b	0.87 b	1.04 a	0.92 ab	**0.031**	0.94	0.92	0.87	0.98	0.426
Total	9.32	10.47	9.50	9.97	0.205	8.80	9.72	9.22	9.76	0.093	14.27 a	11.02 c	12.34 bc	13.70 ab	**0.005**	15.84 a	10.99 b	11.21 b	12.46 b	**0.020**

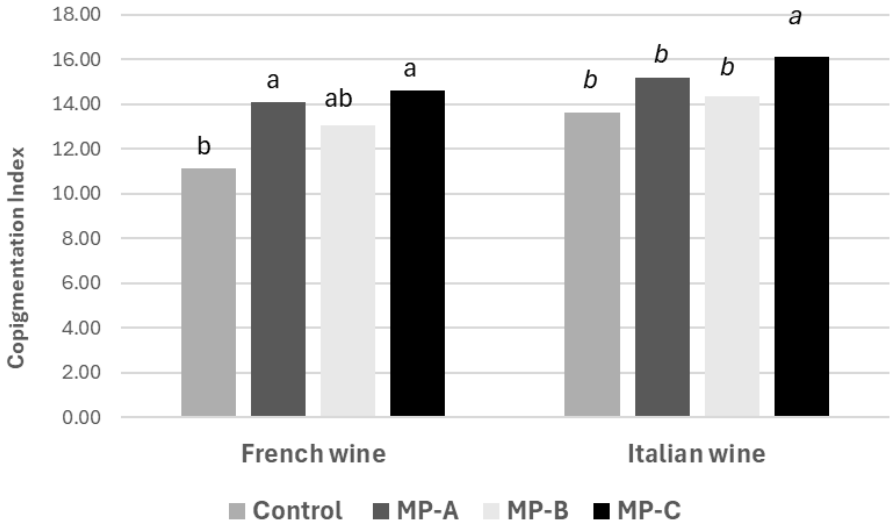


FIGURE 2. Copigmentation index (mean) measured across the MPs treatments (A, B, C) in French and Italian wine. Control represents the wines with no MPs addition. Histograms with different letters within each wine typology show statistical difference according to the Fisher (LSD) test applied on normalised data ($p < 0.05$).

significantly influenced by MP type ($p = 0.013$) and its interaction with aging time ($p = 0.044$), with MP-C and MP-A demonstrating the highest effectiveness. In the case of Italian wines, copigmentation was influenced solely by type of MP added ($p = 0.000$), and not by aging time, with MP-C proving to be the most effective in enhancing the copigmentation index; this may be due to synergistic interactions between the structural features of the mannoproteins and the phenolic composition of the wine matrix (Alcalde-Eon *et al.*, 2024). Overall wine composition, including the presence of other polysaccharides and polyphenols, can also modulate the effect of mannoproteins on the copigmentation index. Guadalupe *et al.* (2007) showed that mannoproteins could significantly enhance colour and colloidal stability in wines, the extent to which depending on interaction with other wine components. Regarding the mannoprotein MP dose, no significant differences in the copigmentation index were observed between the 20 and 40 g/hL concentrations, suggesting that 20 g/hL may represent the appropriate dose for both wine typologies with respect to this parameter as well. According to Snyman *et al.* (2023), the application of high doses of MPs (0.5 mg/mL) did not result in a proportional improvement in colour intensity or polymeric pigment formation, indicating that lower concentrations can be more effective depending on the specific typology of the MPs. The dose-dependent effects also highlighted the importance of optimising MPs concentrations for specific wine properties.

4. Effect of MPs on the stability of the colouring matter

The colloidal stability of the colouring matter in red wine refers to the ability of the wine to maintain its colour and clarity over time, preventing the formation of hazes or precipitates. A cold test was used to measure the colloidal stability of colouring matter, because cold treatment can exacerbate instability by causing precipitation of pigments, either directly due to their own instability or indirectly through adsorption onto tartrate crystals (Alcalde-Eon *et al.*, 2014). An increase in turbidity values after a cold treatment (ΔNTU) indicates an increase in instability of the colouring matter. A two-way ANOVA was carried out on French and Italian wines to assess the effect of the MP typology (A, B, C) and concentration (20 and 40 g/hL) variables on ΔNTU. In both wines, the main effect may be associated with the typology of MPs, which differed in their mannose content and molecular weight (Table 1). Figure 3 showed the ΔNTU values measured in French (F and W, after one and five months of aging) and Italian (I and H, after one and five months of aging) wines, both for untreated controls (no MPs) and for wines treated with the three MPs.

The controls always showed the highest turbidity change in both wines and for both aging times, indicating a higher instability of the colouring matter without treatment. The addition of MPs (MP-A, MP-B, MP-C) significantly reduced turbidity compared to control after cold treatment. After one month of aging in F wines, MP-B and MP-C were more effective than the control, but not significantly different from MP-A. MP-C was the most effective in both wines after five months of aging. The MPs can interact with colouring matter in wine by acting as protective colloids, helping to stabilise pigments and prevent their aggregation and precipitation (Escot *et al.*, 2001). The composition of MPs such as polysaccharide content, purity, and structural features directly affected their ability to colloidally stabilise the colouring matter (Alcalde-Eon *et al.*, 2019), and the interaction with pigments (Palomero *et al.*, 2007). Characterised by a lower molecular weight (37 to 79 kDa), MP-C had the most pronounced effect, except in the case of Italian wine after one month of aging. Similar to our results, an MP with low molecular weight was found to be more effective in stabilising Barbera wine, without reducing the total anthocyanins or flavonoids content (Bosso *et al.*, 2023).

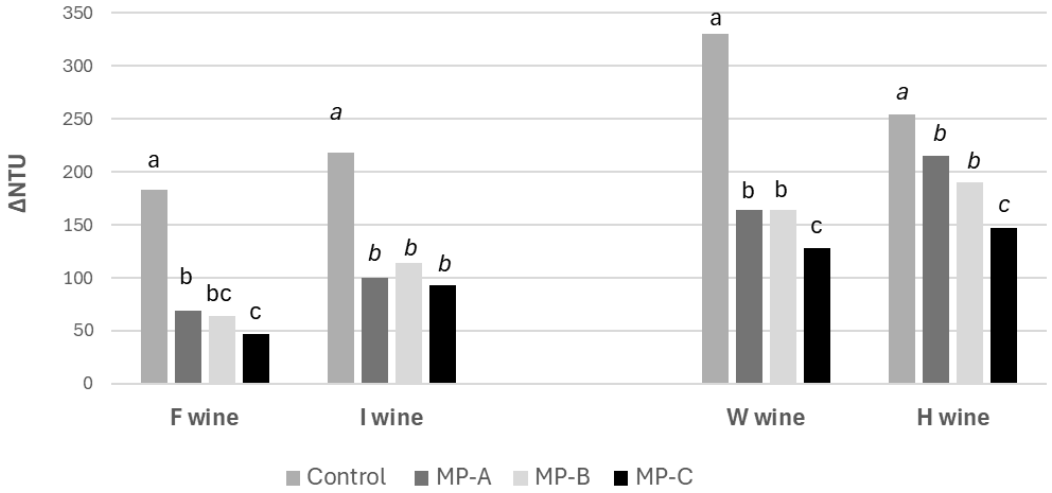


FIGURE 3. ΔNTU measured in French and Italian wine after one month (F and I wine, respectively) and five months of aging (W and H wine, respectively) across the MP treatments (A, B, C). Control represents wine with no MP addition. Histograms with different letters within each wine typology show statistical differences according to the Fisher (LSD) test applied on normalised data ($p < 0.05$).

5. Overall effect of MPs on red wines

A multivariate analysis was performed on the overall data (CI, Abs 420, 520, 620 nm, L*, a*, b* coordinates, copigmentation index, molecular anthocyanins, ΔNTU) in which the MP treatment was considered a categorical variable. Figure 4 represents the principal component analysis (PCA) for French (F series) and Italian (I series) analysed after one month of aging. The first two axes explain the 80.47 % of total variance, with a clear separation between the control wines (no MPs) and the treated ones. The controls were characterised by higher CIEL*a*b* coordinates, as well as a high instability of the colouring matter. On the other hand, the wines treated with MP-B and MP-C were stable and characterised by higher values of colorant intensity, copigmentation index, and the main molecular anthocyanins. This indicates that these MPs are better suited for stabilising pigments without altering the wine colour parameters, while contributing to the colloidal stability of the colouring matter at both concentrations after one month of aging. However, some differences between the two wines seem to be related more to the varietal composition of the molecular anthocyanins than to the MP treatment.

Figure 5 represents the PCA for French (W series) and Italian (H series) analysed after five months aging. The first two axes explained the 71 % of total variance, with a clear separation between the control wines (no MPs) and the treated ones. After additional four months of aging, the untreated wines were still characterised by a high instability of the colouring matter as well as high values in CIEL*a*b* coordinates and content in molecular anthocyanins. The more stable wines from a colloidal point of view were associated with the treatment with MP-C. They also showed high colorant intensity. The other MPs had no significant impact on the studied variables at five months of aging. The efficiency of the MP-C in reducing the instability of the colouring matter over time seems to have depended on its composition; *i.e.*, high mannose content (>80 %) and medium-low molecular weight (37 < kDa < 79). Mannoproteins with high mannose content seem to have been more prone to adsorbing pigmented molecules, thus reducing hydrophobic and π–π interactions that lead to aggregation and precipitation (Alcalde-Eon *et al.*, 2024). Additionally, the medium to low molecular weight of MPs (*e.g.*, ~62 kDa and ~51 kDa) plays an important role in stabilising tannin and pigment colloids – better than high molecular weight (~337 kDa) (Poncet-Legrand *et al.*, 2007) – and in preventing aggregation and precipitation of pigments like anthocyanin-derived compounds (Alcalde-Eon *et al.*, 2024).

CONCLUSION

The use of mannoproteins as an alternative to traditional aging techniques, such as lees aging, has gained popularity in the wine industry, with various commercial preparations available. However, their effectiveness on the stabilisation of red wine colour may depend on many factors. In this collaborative study, we found that the effectiveness of commercial MPs in stabilising wine colour in terms of colloidal matter is closely related to their chemical composition, particularly mannose content and molecular weight. Despite measurable variations in colour parameters, such as colorant intensity (CI) and CIEL*a*b* coordinates, after one and five months of bottle aging, these changes were not associated with any perceptible visual differences (ΔEab*), suggesting limited visual relevance under the tested conditions. However, the impact of MPs on molecular anthocyanin preservation differed depending on MP type, dose, and aging time. The increase in the copigmentation index was also influenced by the typology of mannoproteins applied. Furthermore, the main effect of MP addition was an enhancement in the stability of colouring matter, which was evident even at a lower dose of 20 g/hL for both wine typologies.

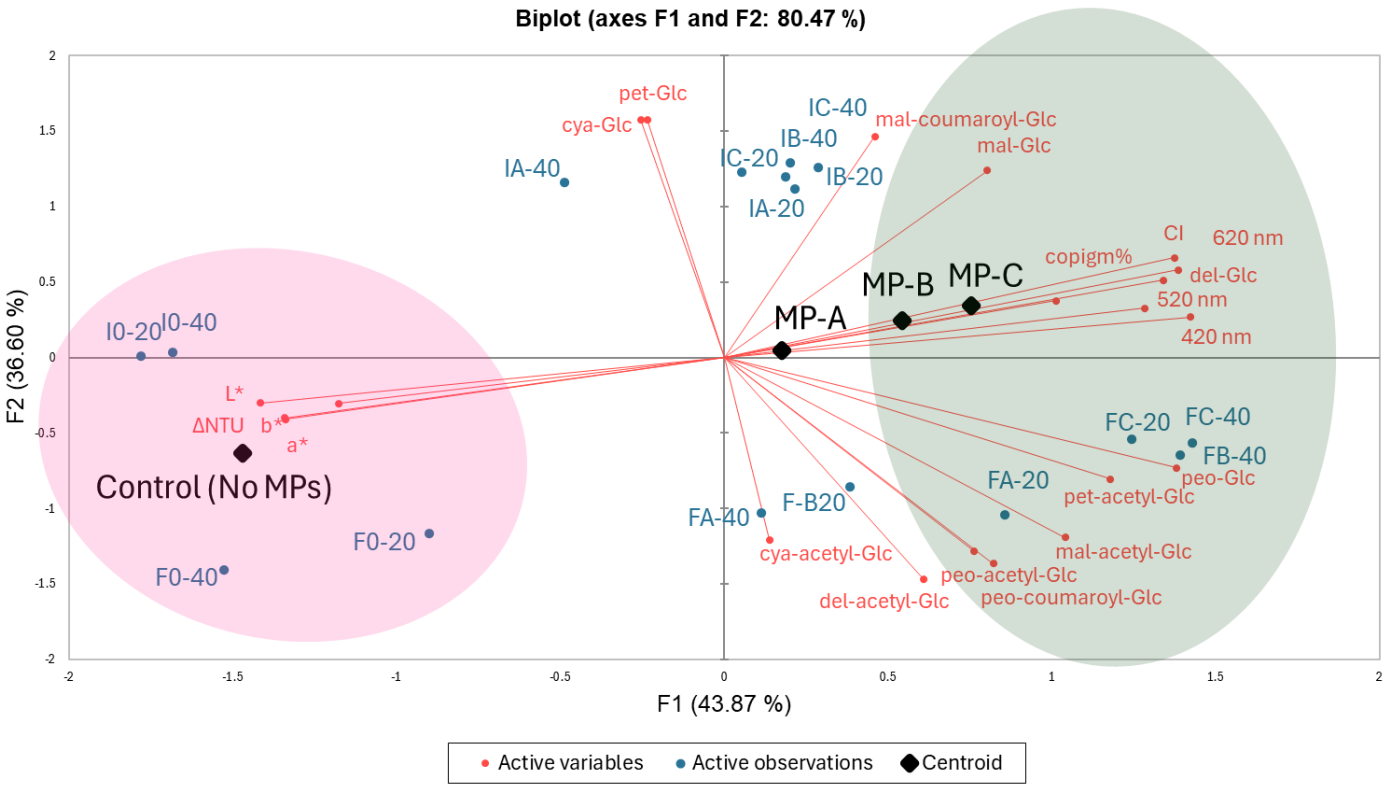


FIGURE 4. Principal component analysis of French (F series) and Italian (I series) wines analysed after one month of aging.

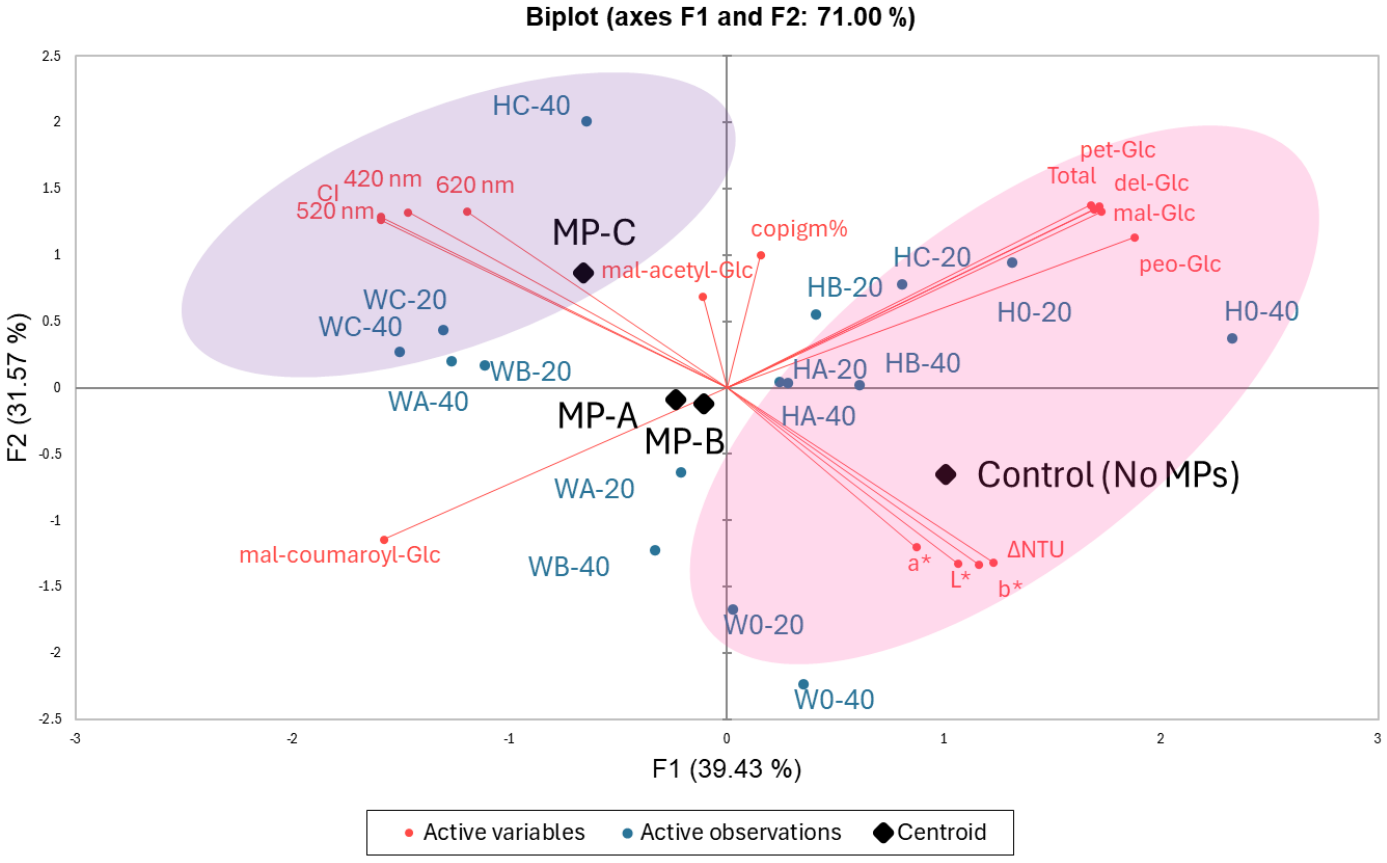


FIGURE 5. Principal component analysis of French (W series) and Italian (H series) wines analysed after five months of aging.

Among the tested MPs, MP-C was the most effective in preserving colouring matter after five months of aging in both red wines. This enhanced efficacy is likely due to structural features, such as high mannose content (>80 %) and a molecular weight range of between 37 and 79 kDa. Different techniques, such as cold stabilisation and filtration, are commonly employed in winemaking to enhance the colloidal stability of colouring matter; however, the addition of mannoproteins may offer a low-energy alternative for achieving colloidal stabilisation in red wines.

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