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Influence of grape quality tier, harvest timing, and yeast strain on mannoprotein content, phenolic composition, and color modulation in young red wines

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ABSTRACT

Besides the impact of harvest timing and grape quality, this study investigated the performance of selected yeast strains overproducing mannoproteins versus the conventional strain AGL 804 in terms of mannoprotein release and its consequences on the composition and color modulation of short-aged commercial red wines. The study fills a gap of volume and actual red wine production by comparing 36 winemaking conditions, each of 850 kg of grapes. The results showed that AGL 804 produced the same or more concentration of mannoproteins than the alternatives. Then, an apparent threshold was found for all yeasts when the dissolved solids content of grape musts at harvest exceeded 22 °Bx, beyond which no increase in mannoprotein production was observed. Only below this limit, an independent effect of yeast strains on tannin concentration and tannin-to-anthocyanin ratio (T/A) in wines was observed. These two parameters exhibited a moderate correlation with mannoprotein concentration ($R^2 = 0.534$ and $R^2 = 0.696$, respectively), and a low-moderate correlation for mannoprotein concentration with CIELAB color parameters. Wines produced from grapes > 22 °Bx showed only harvest-related variations in tannin concentration and T/A and no correlation between parameters analyzed and mannoprotein production. The study revealed that, although yeast strains influence the color of red wine after six months of bottling, their effect is secondary to the harvest timing. Moreover, the influence of the yeast strain itself was not consistent across the different harvest dates. Additionally, the study provides winemakers with an improved and practical assay for measuring mannoprotein levels in red wines, especially for small winery laboratories. It also introduces a novel 3D graphical representation of the CIELAB color parameters, which simultaneously integrates the real visible color of the wines and its visual discriminability to the human eye.

1. Introduction

Among the main quality characteristics of red wine, color is one of the most important for commercial evaluation, together with astringency, since both have a significant influence on the marketability of the final product (Guadalupe et al., 2007; Brossard et al., 2016). In fact, in large-scale wine markets, color often plays an even more crucial role, as it largely determines the price of red wine.

The recent shift in consumer preference for fresh, lighter, fruitier red wines has led enologists to adopt shorter vinification and aging methods, reducing the conventional aging times needed to smooth out the astringency. To face these production challenges, it has been proposed to increase the mannoprotein content by using yeast strains overproducing mannoproteins (Peña-Neira, 2019). However, the industry requires a deeper understanding of the factors that govern the release of mannoproteins and their subsequent impact on red wine composition,

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starting from its polyphenolic content and color, which ultimately influence overall quality.

It has been shown in previous studies that yeasts interact with phenolic compounds in red wine, influencing its color through several mechanisms, with Medina et al. (2019) identifying the following as the most relevant to explain these dynamics: (i) cell wall adsorption of anthocyanins (and phenolic compounds in general) resulting in a reduction of their concentration with a consequent loss of color intensity; (ii) release of β-glucosidase enzymes that can cleave anthocyanin glycosides, with consequent loss of color, through the formation of colorless or brown pigments from the released anthocyanins; (iii) production of secondary metabolites, such as pyruvic acid and vinyl-phenols, that can react with anthocyanins and flavanols, forming pigments such as pyroanthocyanins (vitisin A and B), and ethyl- and acetaldehyde-linked anthocyanin-flavanol complexes, which promote the color shift of wine and improve its stability; (iv) release of polysaccharides during fermentation and autolysis, in specific, mannoproteins can interact with phenolic compounds (Mekoue Nguela et al., 2016; Assunção Bicca et al., 2023), potentially influencing red wine color stability by affecting the polymerization of anthocyanins and tannins. This interaction might enhance or reduce polymerization, thus contributing to color preservation or, on the contrary, causing color loss through precipitation of these compounds over time (Guadalupe et al., 2010; del Barrio-Galán et al., 2015; Medina et al., 2019; Oyón-Ardoiz et al., 2022). On the other hand, It has been seen that the particular effect of a specific mannoprotein added to model wine or wine, after alcoholic and malolactic fermentation, would depend on its physicochemical characteristics, such as molecular weight, protein fraction and carbohydrate moiety, and those of the wine, as the ratio of anthocyanins with tannins (Mekoue Nguela et al., 2016; Rinaldi et al., 2021; Wang et al., 2021; Alcalde-Eon et al., 2024; Assunção Bicca et al., 2023). However, the underlying mechanism of phenolic-color stability or its loss in red wines due to mannoproteins released during fermentation and aging on lees is still not fully understood.

Specifically, mannoproteins are highly glycosylated glycoproteins, whose carbohydrate fraction often accounts for more than 90 %, of which mannose is the primary monosaccharide, followed by glucose. Often located in the outermost layer of the cell wall of *Saccharomyces* and *non-Saccharomyces* cells, bounded by covalent bonds to an amorphous β-1,3-glucan matrix, and they may represent more than 50% of the total dry mass of the original cell wall (Lipke & Ovalle, 1998; Klis et al., 2002; Guadalupe et al., 2010). A wide range of molecular weights has been observed, generally between 5 and 400 KDa and reaching up to 800 KDa (Saulnier et al., 1991; Doco et al., 2003). Within it, two types of mannoproteins have been reported, according to the timing of release during vinification: (i) the growing phase by direct excretion, and (ii) the autolysis phase by the action of β-1,3-glucanase. Both have a similar composition, except for the lower protein content of the first type (Guadalupe et al., 2010; Rodrigues et al., 2012). The second type, however, seems to contribute further to the total mannoproteins content of wine (Rosi et al., 2000).

The mannoprotein released in wine will depend on different factors such as: (i) the specific yeast strain or species (*Saccharomyces* or *non-Saccharomyces),* together with the number of cells and physiological conditions (Domizio et al., 2011; Domizio, Liu, Bisson, & Barile, 2014; Rosi & Gheri, 1998; Rosi, Gheri, Domizio, & Fia, 2000); (ii) vinification conditions, including grape variety, initial colloidal content of must, temperature, agitation and extension of fermentation and aging on lees phases (Guilloux-Benatier et al., 1995; Escot et al., 2001; Apolinar-Valiente et al., 2014;Martínez-Lapuente et al., 2018; Ribéreau-Gayon et al., 2021) and (iii) grape maturity, despite contradictory results (Bindon et al., 2013; Gil et al., 2015; Martínez-Lapuente et al., 2016).

Over the years, different methods have been developed for the analysis of mannoproteins in wines based on: (i) precipitation and purification of polysaccharides, hydrolysis, derivatization and gas chromatography (GC) (Ayestarán et al., 2004; Charpentier et al., 2004; Vidal et al., 2004), (ii) gel filtration of the macromolecular fraction, hydrolysis of polysaccharides and high-pressure liquid chromatography with refractive index detection (HPLC-RID) (Quirós et al., 2012), (iii) precipitation of polysaccharide fractions and competitive indirect enzyme-linked lectin sorbent assay (CI-ELLSA) (Marangon et al., 2018), and (iv) tangential ultrafiltration of the macromolecular fraction, hydrolysis of polysaccharides and enzymatic determination of mannose (Buoso et al., 2010; Guaita et al., 2012). Among the described methods, the last one is considered the most suitable assay for the daily routine needs of a winery laboratory. However, it still requires further refinement to improve its effectiveness in red wine.

2. Materials and methods

2.1. Wine production

The Cabernet-Sauvignon red grapes, 850 kg each batch, were hand harvested during the 2021 vintage in three different periods: (i) March 19th-20th, (ii) March 26th-27th, and (iii) April 29th-May 1st. The grapes were collected from two vineyards groups located in the Maule region of Chile (owned by Concha y Toro winery). These vineyards were already classified in the past by internal quality standards, providing grapes suitable for both low-tier and premium-tier commercial red wines. A total of 36 experimental treatments (2 grape quality x 3 harvests x 6 yeast strains) were run in the experimental cellar of the same winery, as described below:

The grapes were harvested by hand in 450 kg plastic bins and, once in the experimental cellar, ca. 850 kg of grapes per treatment were randomly taken, destemmed, crushed and poured together with 5 g/hL of SO_2 into 1,000 L isothermal high-density polyethylene (HDPE) macro T-Bins, in duplicate. A sample of the homogenized must was taken for routine analyses, including the dissolved solids content determined by °Brix (°Bx), total acidity (TA, g/L), pH, and yeast assimilable nitrogen (YAN, mg/L) (**Supplementary Table S1**). Subsequently, the juice pH was corrected to 3.5 (with tartaric acid), and then the bins were inoculated with 20 g/hL of *Saccharomyces cerevisiae* AGL 804 (DSM, Heerlen, Denmark) as control (Y1), and five commercial *S. cerevisiae* yeast strains overproducing mannoproteins (Y2-Y6). Nitrogen content was standardized (1 mg N x 1 °Bx), according to the nitrogen in the sample measured as YAN by the addition of diammonium phosphate (DAP).

During fermentative maceration at 24–26 °C, punch downs twice a day for 5 min each time were performed until the must density reached 1.060 g/cm^3, at which moment oxygen was added by flushing air at saturation level (ca. 8 mg/L) together with an additional 20 g/hL of DAP. Then, punch downs continued twice a day for 2 min until density 1.020 g/cm^3, and finally, below density 1.020 g/cm^3 again twice a day but only to submerge the cap. At the end of each fermentation (< 2 g/L reducing sugars), the free-run wine was racked into stainless steel tanks and to enable malolactic fermentation inoculated with 0.6 g/hL of lactic acid bacteria CH16 Viniflora® (Chr. Hansen, Hoersholm, Denmark). Once the malolactic fermentation was completed (L-malic acid < 0.02 g/L), the wines were racked into stainless steel tanks with N_2 inert gas protection, sulphited with 40 mg/L of SO_2, corrected to pH 3.5 if needed (as detailed above), then homogenized and de-aerated again with N_2. The wines were then corrected to a final value of 35 mg/L of free SO_2, and stored for one month under 1.5 bar pressure of N_2 on their fine less without agitation. Periodically, the wines were checked for free SO_2 content and corrected as above, if necessary. Finally, wines were filtered under N_2 inert gas using membranes of 5.0, 3.0, and 1.0 μm pore size and, then, bottled in 750 cc bottles and screw-capped with a 30x60 mm STELVIN® capsule (Saranex lined).

2.2. Analysis of mannoproteins: Method validation

Mannoprotein content in wines was quantified with the enzymatic method of Buoso et al. (2010) and Guaita et al. (2012), incorporating

modifications during sample preparation and concentration steps. The method was subjected to the following validation procedures: recovery was evaluated using 4 doses of mannan (Mannan from *S. cerevisiae,* Sigma Aldrich): 0, 100, 200, and 300 mg/L. Two improvements were applied to the assay: (1) before the concentration step, 1 g of Polivinilpolipirrolidone (PVPP) was added to 100 mL centrifuged wine, maintained in constant agitation for 5 min, and then filtered through a 0.22 μm Polyvinylidene fluoride (PVDF) membrane filter (Millipore), using a Kitasate flask; (2) the amount of sample used in the enzymatic assay was doubled, from 0.05 mL to 0.1 mL. Following these improvements, another recovery test was performed, this time with 0, 50, and 100 mg/L mannan, analyzing the filtered and retained phase of the wine. Finally, to evaluate the repeatability of the method, two independent samples, with five replicates each, were analyzed at a dose of 50 mg/L of mannan.

The analysis was performed in triplicate, starting from the 100 mL of wine treated with PVPP, which was centrifuged at 3220 g_0 for 20 min at 4 °C (Eppendorf Centrifuge 5804r, A-4–81 rotor, Hamburg, Germany). Then, 20 mL of the supernatant was transferred into 50 mL centrifugal filter tubes (Amicon® Ultra-15, Millipore, Burlington. MA) for retaining all mannoproteins. Then, the sample was centrifuged at 3000 g_0 and 25 °C for 20 min, obtaining two fractions: 1) a permeate with particles smaller than 10 kDa and 2) a retentate concentrated 80–100-fold, containing the mannoproteins of interest for subsequent analysis. The retentate was carefully washed five times by adding 1 mL of deionized water (MilliQ) (5 mL in total), vortexed, and poured into 30 mL glass tubes (screw cap culture tubes PTFE 18x180 mm). After the addition of 415 μm of 96 % H_2SO_4, the tubes were capped and vortexed. Hot acid hydrolysis was then performed by incubating the tubes in an oven (Binder FD 260) at 100 °C for 180 min, followed by cooling the tubes to room temperature. Subsequently, the samples were repeatedly washed with a total of 5 mL of deionized water (MilliQ) and transferred to 50 mL Falcon tubes. The hydrolysate was then carefully neutralized with NaOH to a pH of 7.0 without exceeding a total volume of 20 mL. After the hydrolysis and neutralization, the samples were centrifuged again at 9800 g_0 for 30 min to remove insoluble particles, transferring the supernatant to 20 mL volumetric flasks and top to the graduated line with deionized water. Subsequently, the enzymatic determination of mannose (indicative of mannoprotein concentration) was performed in duplicate by means of the Megazyme®, K-MANGL enzymatic kit (International Ireland Ldt., Bray, Ireland), using 0.1 mL for both sample and deionized water (MilliQ) as blank. Finally, the mannose concentration obtained was used as an indicator of mannoprotein content.

2.3. Physico-chemical analysis of wines

After six months of storage in darkness at 16 °C, the bottled wine was shipped to Italy (Cesena, Emilia-Romagna) by plane in polystyrene boxes. Upon arrival, the wines were analyzed in triplicate for the following basic parameters (**Supplementary Table S2**): total acidity (TA, g/L), volatile acidity (VA, g/L), pH, reducing sugars (RS, g/L), alcohol (% v/v) (FTIR analyzer Bacchus 3, Steroglass, Perugia, Italy), total polyphenol index measuring absorbance at 280 nm (TPI) (mg/L gallic acid) (González-Rodríguez, 2002), POM-test using 420/520 nm for calculations (Celotti et al., 2022), tannin (T) (mg/L) by methylcellulose method (Sarneckis et al., 2006), free anthocyanins (A) (mg/L malvidin-3-glucoside) (Puissant & Léon, 1965), the ratio T/A, and CIELAB color space parameters (C^*, h^*, a^*, b^*, L^*) from 1 mm optical path length glass cuvettes (OIV, 2006). A Cary 60 UV–Vis spectrophotometer (Agilent, Santa Clara, CA) was used for spectrophotometric measurements.

2.4. Statistical analysis

The factorial design of the experiment included two grape qualities (low-tier and premium-tier), six yeast strains (Y1-Y6), and three harvest periods (H1-H3), all in duplicate. Statistical analyses were driven by the following research questions: (1) Does any yeast strain overproducing mannoproteins exhibit a higher mannoprotein yield than the control under the given winemaking conditions, irrespective of grape aptitude or harvest timing? (2) Do the yeast strains, independently of harvest, affect the physico-chemical composition and/or color parameters of red wines six months after bottling and shipping, and does this effect differ depending on the grape quality? (3) Does the initial mannoprotein concentration at bottling correlate with the physico-chemical and/or color parameters of red wines after six months of storage and transport? To address these questions, various statistical methods were applied: Analysis of variance (blocked two-way ANOVA and two-way ANOVA), post-hoc multiple comparations through Tukey's honestly significant differences (between) and Bonferroni (within) corrections, linear (Pearson) and non-linear (Spearman) correlation analysis (r), regression analysis (R^2), and coefficient of variation (CV), as the standard deviation relative to the mean, expressed as a percentage). Data analysis and visualization were conducted using IBM SPSS Statistics® version 25 (2020) and R-studio® software version 4.2.0 (2022). Statistical significances were assessed with a threshold of p-value < 0.05.

3. Results

3.1. Mannoprotein method validation

The initial conditions of the method (Fig. 1**a)** provided unsatisfactory results as of high standard deviation (SD) for the mannose quantification (0.3–27 mg/L), and low recovery (80.2–86 %). The implementation of PVPP pretreatment, together with increasing the sample volume to 0.1 mL (Fig. 1**b**) improved the performance by decreasing the SD (2–15 mg/L) and raising the recovery (89–107 %). With these improvements, the coefficient of variation (CV) of the repeatability test was also considered acceptable (1.4–2.0 %) (Table 1).

In addition, no mannose was found in the filtrate, thus all the mannoprotein was retained in the retentate. Therefore, the findings altogether confirmed an improvement of the present work upon the original enzymatic assay for mannoprotein in red wine (Buoso et al., 2010; Guaita et al., 2012). Since it actually showed satisfactory performance for white wines, but the recovery test was poor on red wines (50.6–54.8 %).

3.2. Mannoprotein concentration in wines derived from different yeast strains

The harvest date (H1-H3) had an inconsistent effect on the mannoprotein content of the wines, as the mannoprotein level was different and lower only in the H2 vintage of low-tier wines, while no differences were observed in the premium-tier wines (Fig. 2).

Therefore, the difference in the mannoprotein levels observed could be more linked to the soluble solids content (°Brix) of grapes than the harvest date itself. Above an apparent threshold of 22 °Bx, the yeast strains do not seem to respond with an increase in mannoprotein production (Fig. 3**).** Low-tier wines (18.6–22.1 °Bx) showed a strong correlation between °Brix and mannoprotein production ($R^2 = 0.734$) with a delta of 27 mg/L of mannose between the highest (Y1 = 186 mg/L) and lowest (Y6 = 159 mg/L) mean of yeast treatment values. In contrast, premium-tier wines (22.2–26.2 °Bx) showed no correlation between °Brix and mannoprotein production ($R^2 = 0.046$), with a delta of only 11 mg/L of mannose between the highest (Y3 = 173 mg/L) and lowest (Y6 = 162 mg/L) mean of yeast treatment values.

Considering the latter results, the subsequent statistical analyses were performed separately for each wine quality (Fig. 4). Within the low-tier wines, only the Y6 showed a different and lower concentration of mannoprotein in comparison with the rest of the yeast strains. While in the premium-tier wines, Y6 was again among the yeast strains that produced less mannoprotein, although with no difference from Y1, Y2,

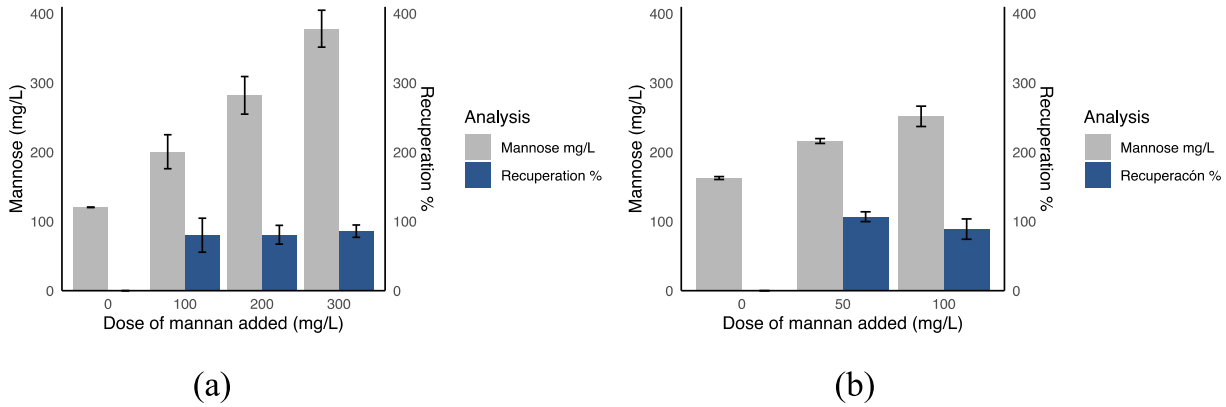

Fig. 1. Validation of mannose assay: a) initial conditions with mannan doses of 0 (control wine), 100, 200, and 300 mg/L; b) Improved assay, with mannan dose of 0 (control wine), 50 and 100 mg/L. Legend of bar colors: gray: mannose concentration (mg/L) and blue: recovery (%).

Table 1
Repeatability test of mannose in red wine.

Mannose (mg/L)	1	2	3	4	5	SD	CV (%)	Recovery (%)
Wine 1 + mannan (50 mg/L)	59.2	51.2	47.2	51.2	50.2	4.4	2.1	100.0
Wine 2 + mannan (50 mg/L)	55.2	51.2	59.2	53.2	54.2	2.3	1.4	100.1

Y4, and Y5 was found.

On the other hand, Y3 and Y6 had consistent mannoprotein production across both low-tier and premium-tier wine qualities. This characteristic may be of special interest in Y3 since its mannoprotein production does not differ from that of control Y1, but the latter does show differences in its production depending on wine quality.

3.3. Physico-chemical composition

Only in low-tier wines, the yeast strain had an effect on tannin concentration and T/A ratio, regardless of the harvest time. In premium-tier wines, however, these same variables were only affected by harvest time (Supplementary Table S3).

Low-tier wines fermented with Y6 showed the lowest values for both mentioned variables, although without differences with respect to Y2, Y3, and Y4 for tannin concentration and Y2 and Y4 for T/A ratio. Conversely, no differences were found between other yeast strains (Y2-Y5) and the control (Y1) for either variable (Fig. 5).

In addition, low-tier wines showed a positive correlation between mannoprotein content at bottling and tannin concentration ($R^2 = 0.534$) and T/A ratio ($R^2 = 0.696$) six months later (Fig. 6), being the highest correlations among all the variables studied (Supplementary Table S4).

On the other hand, the fact that the effect of yeast strain on anthocyanin concentration is not independent of the harvest time (Supplementary Table S3), together with the lack of correlation between mannoproteins and anthocyanin concentration (Supplementary Table S4), is interpreted as the effect of yeast strain on the T/A ratio is mainly due to the effect of yeast strain on tannin concentration. However, it is important to take this ratio into account, as its levels have been related to red wine quality in terms of improved color stability and oxygen consumption during aging. Although, an optimal ratio has not yet been found (Versari et al., 2013; Gambuti et al., 2018).

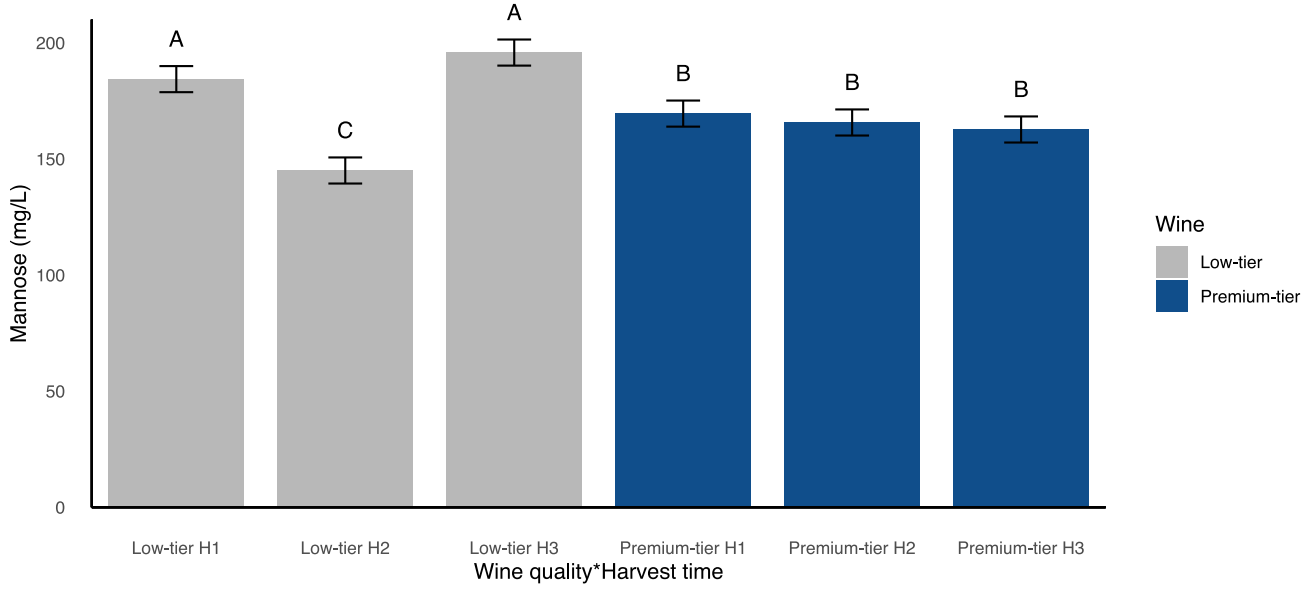

Fig. 2. Post-hoc analysis of the interaction effect of harvest time vs. grape quality. Different letters represent significative differences p-value < 0.05 (Tukey). Bar color: gray: low-tier wines and blue: premium-tier wines.

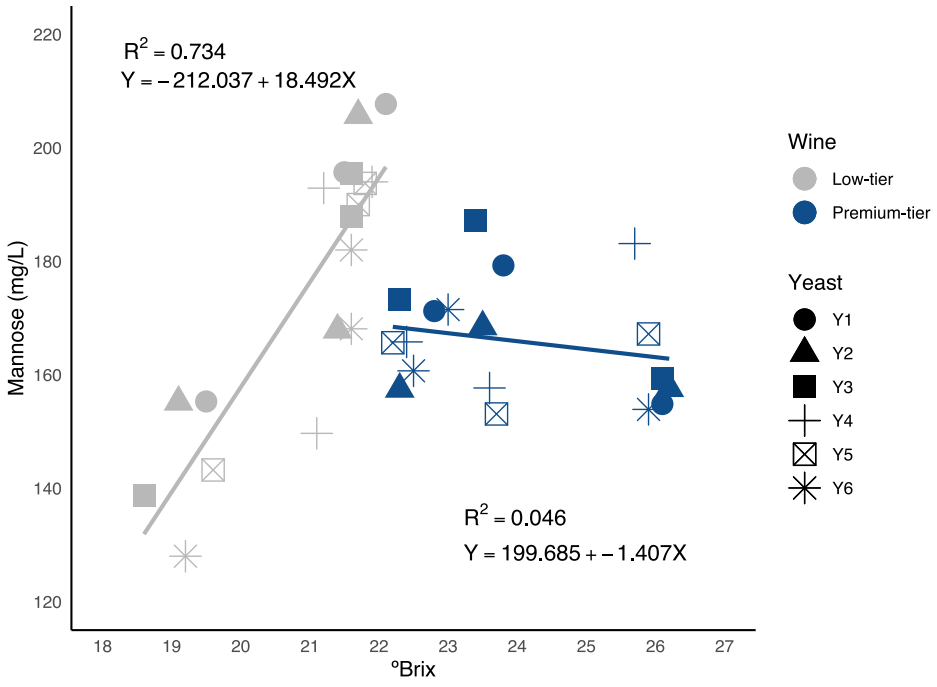

Fig. 3. Linear regression between °Brix of must and the total mannose in low-tier and premium-tier red wines, measured at bottling. Point shapes: ●:Y1,▲: Y2, ■: Y3, +: Y4, □: Y5, *: Y6. Point colors: gray: low-tier wines and blue: premium-tier wines.

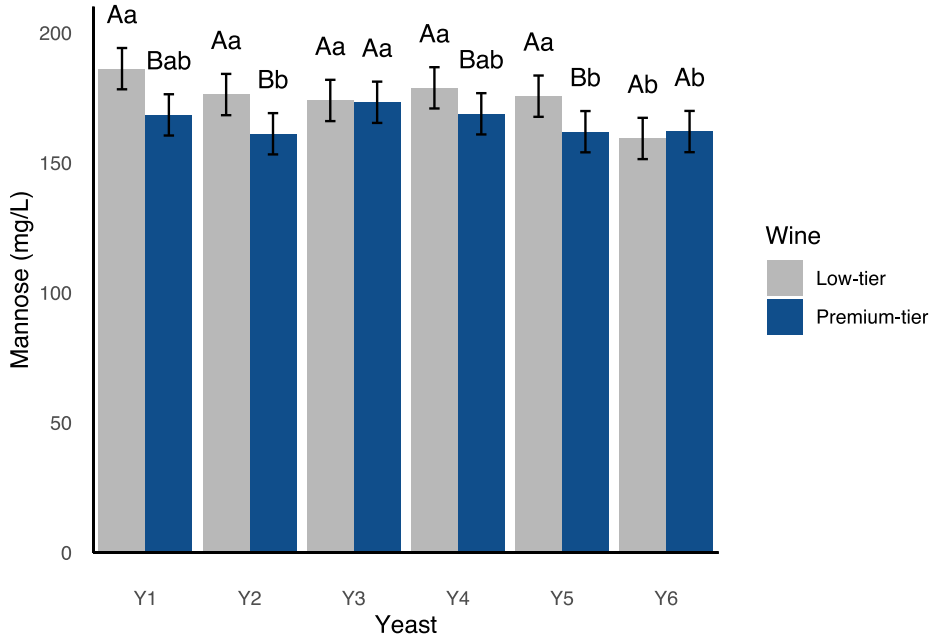

Fig. 4. Post-hoc analysis of the simple effect in each grape quality versus yeast type. Different letters represent significative differences p-value < 0.05 (Bonferroni). Upper case: between wine qualities; lower case: between yeast strain. Bar colors: gray: low-tier wines and blue: premium-tier wines.

3.4. Colorimetric parameters

While yeast strain showed only a harvest-dependent effect on CIELAB color parameters in both wine qualities (**Supplementary Table S3**), significant correlations emerged between mannoprotein concentration at bottling and CIELAB color parameters six months later, but solely in low-tier wines (**Supplementary Table S4**). In this last quality, wines containing higher levels of mannoprotein at bottling exhibited a tendency towards increased yellowness (b^*) ($R^2 = 0.569$), a slight rise in redness (a^*) ($R^2 = 0.249$) and saturation (C^*) ($R^2 = 0.298$), accompanied by a minor reduction in brightness (L^*) ($R^2 = 0.315$). However, no significant correlation was detected for hue (h^*). All this, resulted in a slight increase in color density without increasing the orange hue.

On the other hand, the wine samples of both qualities tended to cluster by harvest time rather than by yeast strain in the CIELAB color space plane (Fig. 7). In particular, low-tier wines exhibited a higher chroma (C^*) and lower brightness (L^*) in their H3 harvest, but without showing great changes in hue (h^*) in the different harvest periods. Suggesting opting for H3 as the optimal harvest date for low-tier wines.

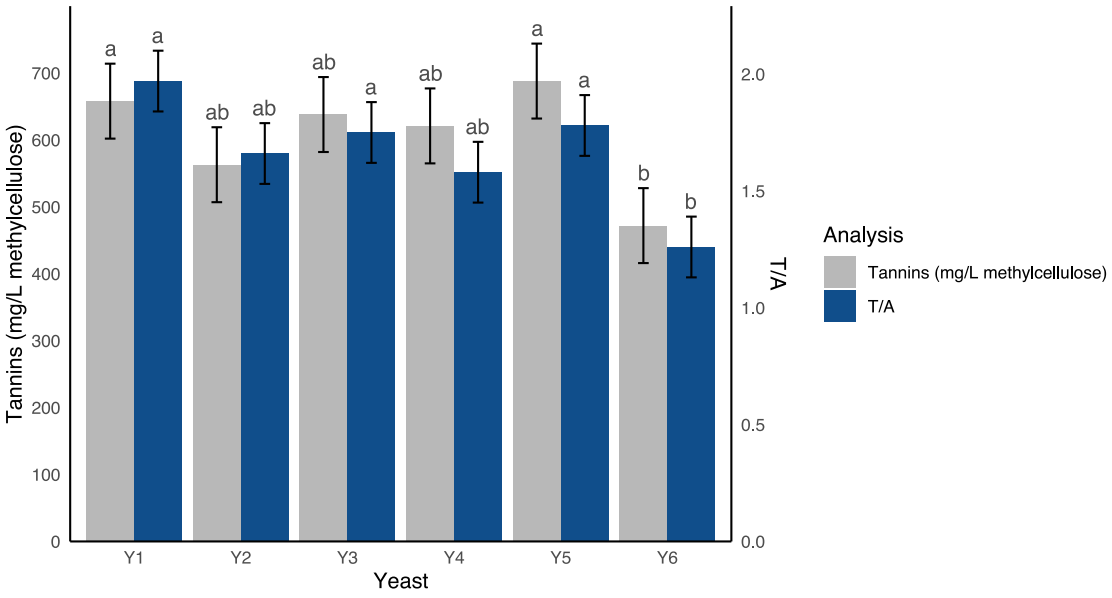

Fig. 5. Post-hoc analysis of the simple effect in low-tier wine for tannin concentration and T/A ratio. Different letters represent significative differences p-value $<$ 0.05 (Bonferroni). Bar colors: gray: Tannins (mg/L methylcellulose) and blue: T/A ratio.

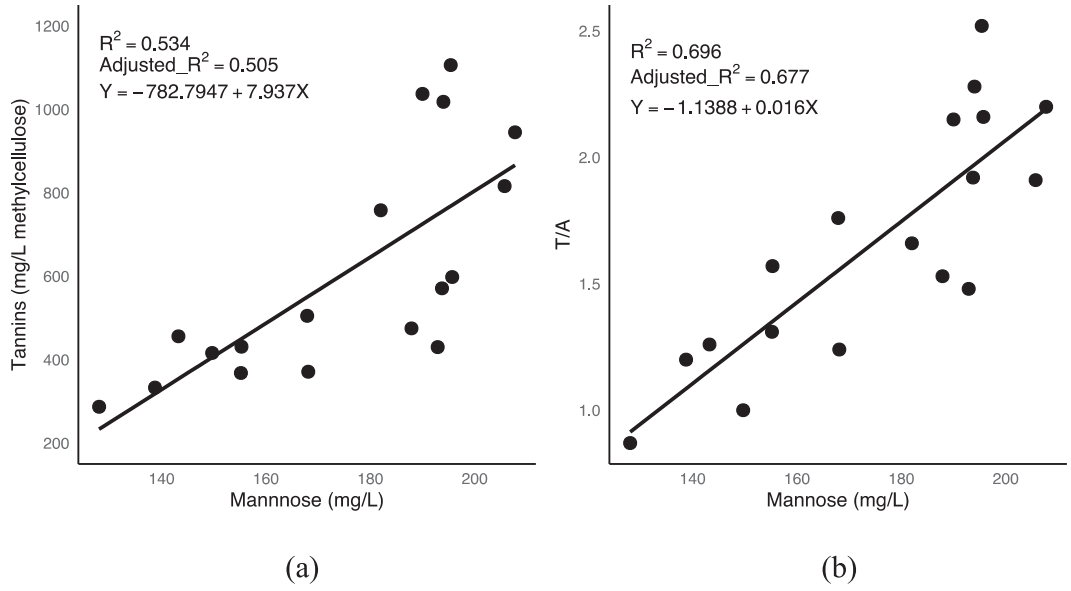

Fig. 6. Linear regression of mannoprotein concentration at bottling vs a) tannin concentration and b) T/A ratio after 6 months of bottling.

In the case of premium-tier wines, H1 and H3 show a higher chroma (C^*) and lower brightness (L^*) than H2, but with a higher hue (h^*) for H3 with respect to the other harvest times. Therefore, H1 seems to be the best option for premium-tier wines.

When analyzing the phenolic composition of the potentially more favorable harvests (Low-tier: H3; Premium-tier: H1 or H3), it was observed that they simultaneously presented the highest levels of TPI, tannins, anthocyanins, and T/A ratio (~2.0), which could explain the better color parameters observed for these harvests.

Among these treatments, only Low-tier H3 and Premium-tier H1 showed high color density (high C^* and low L^*), but no high h^*, as in the case of Premium-tier H3 (**Supplementary Table S5**). Interestingly, these two treatments also exhibited no differences in terms of technological maturity at harvest, measured as the ratio of °Brix to total acidity (5.27–6.02 °Brix/TA). This finding indicates that the incorporation of the °Brix/TA ratio, together with a limit value established for this index, could be crucial in determining the time of harvest for the conditions of the study. In this way, it would be possible to maintain an optimum color of red wine after six months of bottling, independent of the grape quality tier.

On the other hand, the results reveal that a high T/A ratio alone would not be sufficient to explain a superior color. As in the case of the Low-tier H1, whose T/A ratio was not different from that of Low-tier H3, Premium-tier H1, and Premium-tier H3, but had a low color density due to its lower phenolic content.

This was further elaborated by determining the best combination of harvest time treatments and yeast strains for each wine quality in terms of the overall color observed by means of a CIELAB three-dimensional (3D) plot (**Supplementary Fig. SF1a and b**). In the case of low-tier wines, the H3Y4 treatment would be considered the optimal in terms of higher color concentration (high a^*, low L^* *and low* b^*). However, the color of the H3Y4 treatment would not be visibly distinguishable from

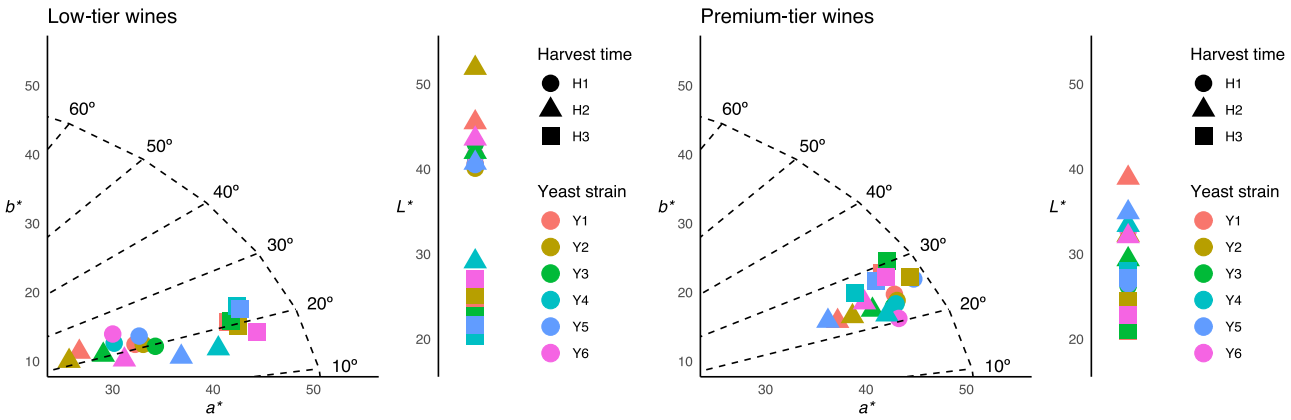

Fig. 7. CIELAB Color Space (a^*, $b^*$and L^*) plane for a) low-tier wines and b) premium-tier wines. Separated by factors Harvest time (●=H1, ▲=H2 and ■=H3) and Yeast strain (red = control (Y1), yellow = Y2, green = Y3, light blue = Y4, blue = Y5, pink = Y6). CIELAB color parameters represent: a^*(green–red), b^*(blue-yellow), and L^* (black: 1 to white: 100).

H3Y5. In the case of premium-tier wines, treatments H3Y1, H3Y3, and H1Y2 exhibited the highest color concentration. Although, the color of H3Y3 and H3Y1 were indistinguishable from each other, and the color of H1Y2 had a lower hue (h^*) compared to H3Y3 and H3Y1, while maintaining a similar chroma (C^*) and brightness (L^*). Therefore, for premium-tier wines, H1Y2 treatment would be preferred.

As expected, the treatments Low-tier H3Y4 and H3Y5 and Premium-tier H3Y2 presented the highest combined levels of TPI, tannins, anthocyanins, and T/A for their respective wine quality, explaining their superior color. Again, a high T/A ratio was not decisive for a superior color (**Supplementary Table S6a and b**).

4. Discussion

4.1. Mannoprotein assay

The analytical modifications proposed were effective for the quantification of mannoprotein in red wine and suitable for common winery laboratories. These improvements derive from two fundamental changes to the original assay (Buoso et al., 2010; Guaita et al., 2012): 1) adding a sample pretreatment step using PVPP, which decreases interferences in colored samples, as the case of this study, for the spectrophotometric determination of mannose at 340 nm, and 2) changing the concentration step of the original method, performed through tangential ultrafiltration, to centrifugal ultrafiltration tubes based on a previous work that successfully used them to concentrate extracellular proteins from yeast culture medium (Wang et al., 2015).

4.2. Mannoprotein released by yeast strains

The minor variations in mannoprotein production by yeast strains, observed across both wine qualities, along with the non-response of the premium-tier wines to grape °Brix, may be attributable to: 1) the length of the autolysis phase that, among other factors, impacts total yeast mannoprotein release (Rosi et al., 2000). In this sense, a brief period of aging on fine lees without stirring, as the case of both qualities in this study, could restrict the potential for mannoprotein release from the yeast strains and the observation of differences. 2) the physiological environment in which the yeast develop during fermentation and aging is mainly determined by grape maturity at the time of harvest, leading to variations in mannoprotein production (Domizio et al., 2011; Domizio, Liu, Bisson, & Barile, 2014; Rosi & Gheri, 1998; Rosi, Gheri, Domizio, & Fia, 2000). In this regard, different works indicate an association between grape maturity and the final concentration of mannoproteins in the wine, linked to the initial soluble solids content of the must or to the phenological stage of the fruit (weeks post-veraison). Bindon et al. (2013) reported an increase in mannoprotein concentration in Cabernet-Sauvignon wines made from grapes harvested at 20 to 26 °Bx (12.0 and 15.5 % v/v alcohol) in 50 kg vinifications. However, they employed a *S. cerevisiae* var. *bayanus* strain (PDM, Maurivin, Sydney, Australia) and standardized the must pH to 3.2 before inoculation, uncommon practices in red wine production. Martínez-Lapuente et al. (2016) obtained similar results with the red grape cv. Tempranillo, confronting prematurely harvested grapes (19.7 °Bx and pH 3.25) with ripe grapes (22.4 °Bx and pH 3.46), in 150 L vinifications (12.3 and 14.0 % v/v alcohol). However, grapes were processed as sparkling wine using *S. cerevisiae* (FERMES 488, Enartis, Italy) and *S. cerevisiae* var. *bayanus* (IOC 18–2007, Institut OEnologique de Champagne, Épernay, France). In contrast, Gil et al. (2015) observed a reduction in mannoprotein concentration in Cabernet-Sauvignon wines fermented in 80 kg batches with *S. cerevisiae* (EC1118; Lallemand Inc., Montreal, Canada) as grape ripening increased in successive harvests (3, 5 and 7 weeks after véraison), and an increase of mannoproteins as the maceration continued (1, 2, 3 and 4 weeks). This reduction in mannoprotein concentration may be due to the fact that the author does not specify grape maturity in terms of sugar content, but only in relation to the number of days from flowering to harvest (véraison). However, a later harvest date does not necessarily correspond to a higher sugar concentration, as factors such as irrigation or rainfall can lower the sugar content of the grapes, making them appear to be at earlier stages of maturity (Intrigliolo et al., 2016). However, it can be seen that the effect of keeping the wine on the lees is still significant despite the above.

Therefore, making a statement in this sense requires a cautious examination, since the evidence available comes from micro-vinifications, from different winemaking protocols and with contradictory results. However, including the present study which fills a gap in volume and real vinification conditions, there is more evidence of an increase in mannoprotein concentration in wine with increasing grape maturity than the opposite. But, their specific influence seems to be largely conditioned by the type of winemaking process. For this reason, this work attempts to make an effort to present winemaking conditions as real as possible and to present the vinification protocol in detail for future comparison.

4.3. Effect of yeast strain and mannoproteins on wine composition

It was observed, only in low-tier wines, that mannoprotein released from yeast strains during fermentation significantly affects tannin concentration and T/A, independent of the effect of the harvest time and without interaction between both factors (**Supplementary Table S3**). This phenomenon can be attributed to the known influence that mannoproteins have over the stability of tannins by affecting their

polymerization and particle size. This effect prevents excessive aggregation and precipitation of tannins, leading to higher tannin concentrations in wine (Poncet-Legrand et al., 2007; Rodrigues et al., 2012; Bindon et al., 2016).

On the other hand, the reason for the lack of significant effect of yeast strain on tannin concentration and T/A ratio for premium-tier wines (**Supplementary Table S3**) might be explained by two key factors: 1) the limited variation of mannoprotein levels induced by yeast strain treatments (mean delta of 11 mg/L in premium-tier wines versus 27 mg/L in low-tier wines). It may not be enough to see differences in aggregation and precipitation of tannins, as described above in low-tier wines, and 2) the major influence of alcohol in polysaccharide-tannin interactions. In this sense, the °Brix threshold found for musts of low-tier and premium-tier wine categories corresponds to an alcoholic strength of approximately 12.0 % v/v alcohol. Studies with model solutions indicate that this alcohol level acts as a tipping point, where, at higher alcohol levels, mannoproteins lose their ability to stabilize tannin aggregation due to increased tannin solubility, while mannoprotein solubility decreases. In contrast, below 12 % v/v alcohol, mannoproteins effectively prevent tannin aggregation (Poncet-Legrand et al., 2007). Therefore, the latter could further explain why low-tier wines ($\leq$ 22 °Bx and $\leq$ 12 % v/v alcohol) tend to have higher tannin concentrations as mannoprotein levels increase, whereas no such effect was observed in premium-tier wines ($\geq$ 22 °Bx and $\geq$ 12 % v/v alcohol).

4.4. Effect yeast on wine color

The present study shows that isolated effect of yeast strain, harvest timing and its interaction significantly influence the CIELAB color parameters of both wine qualities. However, the proportion of variance explained by yeast strain is minor compared to the variance explained by harvest time and the interaction between the two factors (**Supplementary Table S3**). This suggests that, although yeast strain contributes to the variations in CIELAB color parameters, its impact is small and inconsistent across the different harvest periods within each quality. In fact, for low-tier wines only, slight correlations were found between mannoprotein concentration at bottling and CIELAB color parameters six months later. This, as seen before, may be related to the initial ripening conditions of the grapes rather than to differences induced by yeast strain treatments.

Therefore, the color variations observed could be attributed mainly to the differences in the maturity of each grape quality within the harvest dates, which influences differences in physico-chemical factors that, in turn, impact the total compounds extracted from the skin and seed grape matrices. These factors include: 1) the concentration of anthocyanins in the grape skin and their degree of extractability, and 2) the

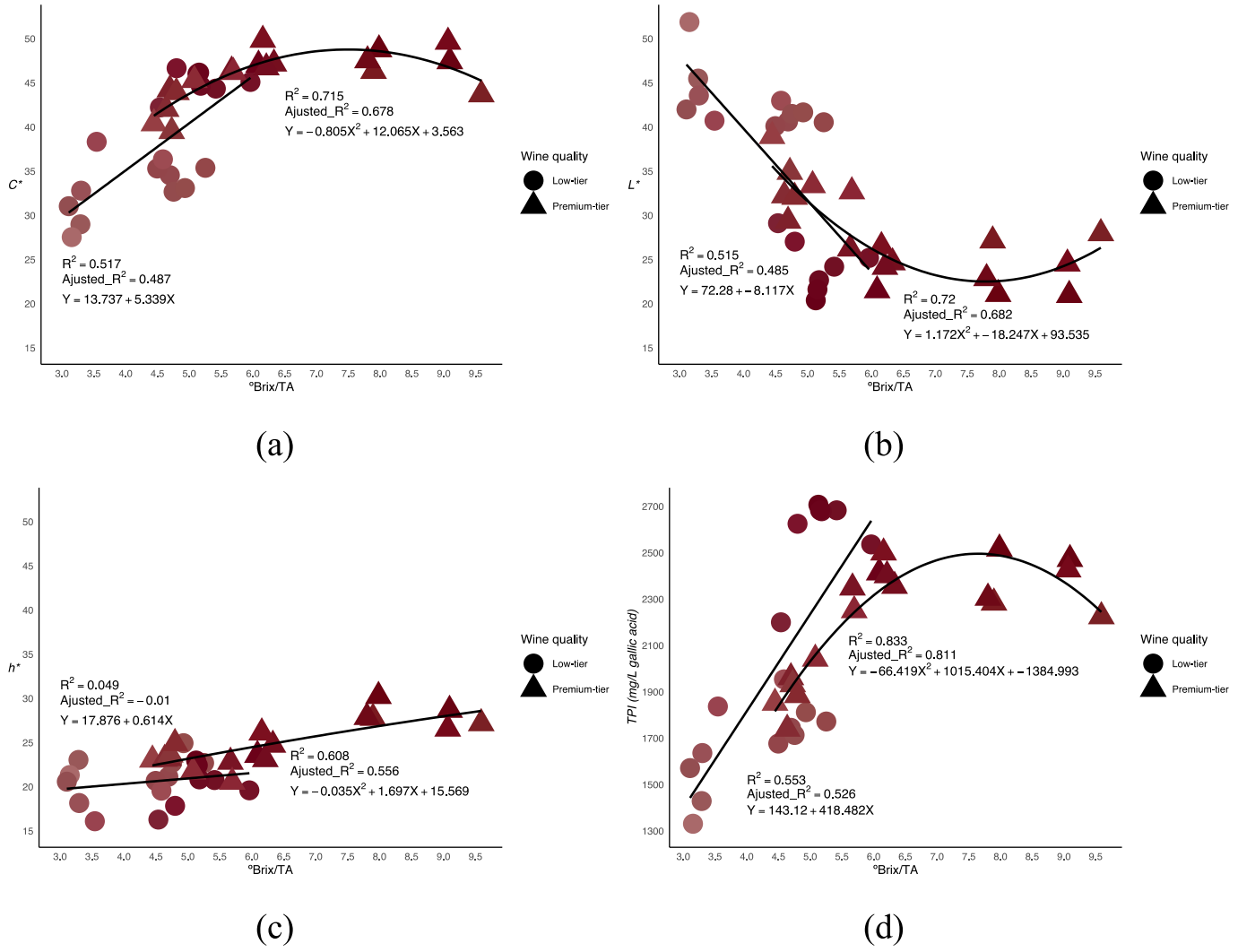

Fig. 8. Regression analysis on the technological maturity (°Brix/TA) of grapes at harvest versus: a) total phenolic index (TPI), b) chroma (C^*), c) brightness (L^*) and d) Hue (h^*), CIELAB color parameters of wine, six months after bottling. Legend: low-tier wines: ●, premium-tier wines: ▲. The color of points is the actual visible color of wine calculated by CIELAB color parameters.

flavanol concentration in skins and seeds and their degree of polymerization (Río Segade et al., 2019). That, subsequently, can affect the color of red wine, its stability, and the global phenolic composition (De Freitas et al., 2017; Escribano-Bailón et al., 2019; Río Segade et al., 2019).

In this regard, different responses were observed for CIELAB wine color parameters six months after bottling according to the technological maturity (°Brix/TA) of the grape at harvest. A linear fitting for °Brix/TA to C^* ($R^2 = 0.517$) was observed for the low-tier wines, and a convex quadratic response of °Brix/TA to C^* ($R^2 = 0.715$) for the premium-tier wines (Fig. 8**a**). A linear fitting of °Brix/TA to L^* ($R^2 = 0.515$) was detected for low-tier wines, and the concave quadratic response of °Brix/TA to L^* ($R^2 = 0.720$) for the premium wines (Fig. 8**b**). Finally, a linear fitting for °Brix/TA to h^* was only found for premium-tier wines ($R^2 = 0.608$) (Fig. 8**c**).

The variable most closely related to these CIELAB color parameters was TPI for both wine qualities (**Supplementary Table S7a and b**). This variable was also affected by technological maturity, as expected, with a linear fitting for °Brix/TA to TPI ($R^2 = 0.553$) in low-tier wines, and a convex quadratic response for °Brix/TA to TPI ($R^2 = 0.833$) in the premium-tier wines (Fig. 8**d**). Thus, the correlations of TPI with CIELAB were strong and positive for C^* (low-tier: $R^2 = 0.950$, and premium-tier: $R^2 = 0.771$) and negative for L^* (low-tier: $R^2 = 0.945$, and premium-tier: $R^2 = 0.716$). In contrast, for TPI and h^*, there was no correlation for low-tier wines, and only a slight positive correlation was found for premium-tier wines ($R^2 = 0.247$) (**Supplementary Table S7 a and b**).

The correlation between h^* and technological maturity found only for premium-tier wines and its lack of a strong correlation with TPI, implies that factors other than total phenol content may influence the hue (h^*) of red wines, especially during higher ripening stages. The main factor that could explain these observations is the formation of pyranoanthocyanins (reaction products of anthocyanins) during fermentation and aging, which contribute with orange hues of the wine (higher h^*). Since it is known that the concentration in the wine, as well as its orange hue, is higher in wines produced with more mature vintages (Pérez-Magariño and González-San José, 2004; Rentzsch et al., 2007; Escribano-Bailón et al., 2019).

All the above suggests the occurrence of an optimal °Brix/TA ratio, where the observed TPI and wine color values begin to stabilize (°Brix/TA ~ 6.5, with TPI = 2409 mg/L gallic acid, C^*= 47.98, and L^*= 24.45). Beyond this optimal point, additional increases in technological maturity resulted in marginal improvements in terms of C^* and L^*, reaching value beyond which a declining trend is observed (°Brix/TA ~ 7.5, with TPI = 2494 mg/L gallic acid, and C^*= 48.78, L^*= 22.61), and increase of L^* and h^* is observed.

In addition to the above, higher maturities would need adding increased amounts of DAP, which can be detrimental to the color stability of red wines (Medina et al., 2019), and could further diminish the overall color of the wine.

Consequently, the above could explain the differences observed in color between wine qualities and harvest and, on the other hand, would suggest that it does not seem advisable to harvest with °Brix/TA values higher than 6.5–7.5 for Cabernet-Sauvignon red wines, as this could lead to a loss of color C^* and an increase in L^* and h^* after six months of bottling under the winemaking conditions of the present study. On the other hand, it helps to understand why Low-tier H1 and Premium-tier H3 had optimal color parameters at similar technological maturity (5.3–6.0 °Brix/TA). Finally, it suggests that a higher mannoprotein concentration at bottling could lead to slight increases in C^* and decreases in L^* without increases in h^* six months later, for < 22 °Bx musts, as in the case of low-tier wines, under the winemaking conditions presented in this work and the ranges of mannoprotein concentrations found.

5. Conclusions

This study sheds light on the crucial and controversial role of mannoproteins in shaping red wine quality by addressing a key gap in volume and real winemaking conditions. Specifically, its reveals how harvest timing, grape quality (low-tier and premium-tier) and yeast strain choice, influence mannoprotein release and red wine characteristics, particularly in the production of commercial, ready-to-drink, red wines.

The modified assay employed in this research offers winemakers a practical and improved tool for assessing mannoprotein levels in red wines, particularly suited for small winery laboratories. Additionally, this work introduces a novel graphical representation that integrates CIELAB color parameters, visible wine color, and its visual discriminability (ΔE^*_{ab}) (**Supplementary Fig. SF1a and b**). This new approach simplifies the identification of treatments that enhance color quality, providing a clearer alternative to traditional 2D CIELAB color space charts and multiple ΔE^*_{ab} comparison tables (Fan et al., 2023; Wu et al., 2024), especially when dealing with numerous treatments.

The findings suggest that a careful yeast selection and appropriate harvest timing can effectively enhance mannoprotein content, tannin concentration, and T/A ratio, as well as global wine color, six months after bottling. These results are of particular interest for fresh, low-alcohol red wines made from early ripening vintages (≤ 22.0–23.0 °Bx and maximum 6.0–6.5 °Brix/TA ratio).

However, the complex production of mannoproteins by yeast strains and their impact on wine composition and color, will require additional research to clarify their underlying mechanisms and thus improve the quality of fresh red wines, through the use of model wines with controlled initial sugar levels and different levels of anthocyanins/tannins.

furthermore, there is a need to improve mannoprotein analysis proposed by making it even more practical and specific, potentially through the use of combined ultrafiltration steps to target specific molecular weight ranges, and able to obtain more accurate conclusions from model wine research and industrial trials.

6. Metadata

Metadata will be made available and searchable as FAIR on AMS Acta repository (https://amsacta.unibo.it/) or FigShare repository (https://figshare.com/) by recording the DOI of the article soon after publishing.

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CRediT authorship contribution statement

Cristian Galaz Torres: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Josefina Vidal:** Formal analysis, Data curation, Conceptualization. **Sebastian Vargas:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Jorge Zincker:** Validation, Funding acquisition, Conceptualization. **Natalia Brossard:** Writing – review & editing, Methodology, Conceptualization. **Edmundo Bordeu:** Writing – review & editing, Methodology, Conceptualization. **Arianna Ricci:** Writing – review & editing, Conceptualization. **Giuseppina P. Parpinello:** Writing – review & editing, Supervision, Conceptualization. **Andrea Versari:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2025.115956.

Data availability

Data will be made available on request.

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Supplementary table S1: Base enological parameters of low-tier and premium-tier wines from different harvest timing.

Harvest	ºBx	TA g/L	pH	YAN mg/L
Low-tier H1	21.52 ± 0.20	4.51 ± 0.23	3.40 ± 0.02	288 ± 6
Low-tier H2	19.52 ± 0.85	5.66 ± 0.53	3.24 ± 0.08	306 ± 46
Low-tier H3	21.77 ± 0.20	4.15 + 0.28	3.59 ± 0.03	179 ± 25
Premium-tier H1	23.50 ± 0.28	3.91 ± 0.15	3.37 ± 0.03	285 ± 16
Premium-tier H2	22.42 ± 0.21	4.75 ± 0.23	3.44 ± 0.04	351 ± 18
Premium-tier H3	25.98 ± 0.18	3.05 ± 0.27	3.96 ± 0.02	253 ± 12

Supplementary table S2: Base enological parameters of low-tier and premium-tier wines six month after bottling

Treatment	Alcohol (% v/v)	pH	Total acidity (TA) (g/L)	Volatil acidity (VA) (g/L)	Reducing sugars (RS) (g/L)
Low-tier H1 Y1	12.52 ± 0.20	3.55 ± 0.02	5.46 ± 0.11	0.46 ± 0.04	1.22 ± 0.33
Low-tier H1 Y2	12.25 ± 0.11	3.54 ± 0.01	5.81 ± 0.15	0.41 ± 0.05	0.66 ± 0.02
Low-tier H1 Y3	12.29 ± 0.34	3.46 ± 0.01	5.56 ± 0.16	0.50 ± 0.01	0.64 ± 0.12
Low-tier H1 Y4	12.13 ± 0.33	3.46 ± 0.01	5.58 ± 0.07	0.47 ± 0.02	0.44 ± 0.01
Low-tier H1 Y5	12.59 ± 0.13	3.46 ± 0.01	5.55 ± 0.10	0.52 ± 0.02	0.70 ± 0.27
Low-tier H1 Y6	12.38 ± 0.31	3.46 ± 0.01	5.41 ± 0.11	0.49 ± 0.02	0.75 ± 0.09
Low-tier H2 Y1	10.97 ± 0.37	3.57 ± 0.01	5.60 ± 0.16	0.40 ± 0.03	0.22 ± 0.14
Low-tier H2 Y2	10.77 ± 0.33	3.50 ± 0.01	5.63 ± 0.09	0.43 ± 0.02	0.28 ± 0.17
Low-tier H2 Y3	10.32 ± 0.11	3.46 ± 0.01	5.61 ± 0.14	0.49 ± 0.05	0.37 ± 0.09
Low-tier H2 Y4	12.31 ± 0.27	3.47 ± 0.01	5.58 ± 0.15	0.42 ± 0.03	0.44 ± 0.03
Low-tier H2 Y5	11.64 ± 0.20	3.46 ± 0.01	5.46 ± 0.08	0.43 ± 0.04	0.57 ± 0.11
Low-tier H2 Y6	10.95 ± 0.14	3.45 ± 0.03	5.36 ± 0.08	0.43 ± 0.01	0.16 ± 0.22
Low-tier H3 Y1	12.26 ± 0.27	3.52 ± 0.02	5.81 ± 0.17	0.46 ± 0.03	1.13 ± 0.04
Low-tier H3 Y2	12.17 ± 0.23	3.52 ± 0.02	5.70 ± 0.20	0.44 ± 0.01	0.90 ± 0.36
Low-tier H3 Y3	12.16 ± 0.30	3.50 ± 0.01	5.42 ± 0.10	0.50 ± 0.04	0.71 ± 0.06
Low-tier H3 Y4	12.26 ± 0.11	3.46 ± 0.01	5.58 ± 0.14	0.48 ± 0.04	0.86 ± 0.09
Low-tier H3 Y5	12.3 ± 0.20	3.46 ± 0.02	5.46 ± 0.13	0.45 ± 0.04	0.87 ± 0.11
Low-tier H3 Y6	12.23 ± 0.16	3.45 ± 0.01	5.61 ± 0.08	0.47 ± 0.01	0.94 ± 0.21
Premium-tier H1 Y1	14.21 ± 0.11	3.47 ± 0.01	5.48 ± 0.11	0.50 ± 0.01	1.65 ± 0.36
Premium-tier H1 Y2	13.94 ± 0.21	3.54 ± 0.01	5.76 ± 0.07	0.39 ± 0.03	1.62 ± 0.05
Premium-tier H1 Y3	13.42 ± 0.37	3.42 ± 0.01	5.41 ± 0.08	0.48 ± 0.04	1.42 ± 0.11
Premium-tier H1 Y4	13.87 ± 0.23	3.48 ± 0.02	5.70 ± 0.03	0.46 ± 0.06	1.62 ± 0.10
Premium-tier H1 Y5	13.65 ± 0.31	3.48 ± 0.01	5.28 ± 0.16	0.46 ± 0.05	1.57 ± 0.26
Premium-tier H1 Y6	13.74 ± 0.33	3.54 ± 0.02	5.59 ± 0.17	0.42 ± 0.02	1.47 ± 0.68
Premium-tier H2 Y1	12.71 ± 0.23	3.45 ± 0.02	5.63 ± 0.15	0.46 ± 0.05	1.00 ± 0.07
Premium-tier H2 Y2	12.73 ± 0.10	3.55 ± 0.01	5.82 ± 0.10	0.48 ± 0.02	0.75 ± 0.01
Premium-tier H2 Y3	12.86 ± 0.34	3.46 ± 0.02	5.38 ± 0.07	0.52 ± 0.05	1.37 ± 0.01
Premium-tier H2 Y4	12.96 ± 0.25	3.45 ± 0.01	5.48 ± 0.08	0.48 ± 0.01	0.89 ± 0.12
Premium-tier H2 Y5	12.79 ± 0.11	3.46 ± 0.01	5.46 ± 0.16	0.50 ± 0.01	1.05 ± 0.08
Premium-tier H2 Y6	13.05 ± 0.23	3.51 ± 0.02	5.66 ± 0.18	0.44 ± 0.06	1.57 ± 0.27
Premium-tier H3 Y1	14.08 ± 0.23	3.49 ± 0.01	5.92 ± 0.08	0.49 ± 0.03	1.44 ± 0.06
Premium-tier H3 Y2	14.25 ± 0.21	3.52 ± 0.02	5.98 ± 0.01	0.41 ± 0.05	2.02 ± 0.50
Premium-tier H3 Y3	14.34 ± 0.23	3.47 ± 0.01	5.68 ± 0.08	0.50 ± 0.03	2.20 ± 0.08
Premium-tier H3 Y4	14.03 ± 0.18	3.49 ± 0.01	5.78 ± 0.12	0.49 ± 0.04	1.27 ± 0.24
Premium-tier H3 Y5	14.52 ± 0.16	3.47 ± 0.01	5.76 ± 0.16	0.56 ± 0.04	2.06 ± 0.23
Premium-tier H3 Y6	14.13 ± 0.33	3.52 ± 0.01	5.66 ± 0.14	0.41 ± 0.05	1.79 ± 0.21

Supplementary table S3: ANOVA summary for physicochemical parameters after 6 months of bottling for both wine qualities

Variables	Low-tier			Premium-tier		
	Harvest	Yeast	Harvest xYeast	Harvest	Yeast	Harvest xYeast
POM test 420/520 nm	38% ***	15% ***	46% ***	27% ***	31% ***	40% ***
TPI (mg/L gallic acid)	86% ***	6% ***	8% ***	84% ***	3% **	12% ***
Tannins (T) (mg/L Methyl cellulose)	82% ***	7% *	4% ns	60% ***	8% ns	14% ns
Anthocyanins (A) (mg/L malvidin-3-glu)	80% ***	9% ***	10% ***	65% ***	11% ***	24% ***
T/A ratio	59% ***	20% **	10% ns	46% ***	14% ns	18% ns
Alcohol (% v/v)	62% ***	11% ***	20% ***	84% ***	0.4% ns	8% ns
*L**	80% ***	8% ***	11% ***	63% ***	9% ***	27% ***
*a**	70% ***	7% ***	22% ***	53% ***	6% ***	37% ***
*b**	83% ***	6% ***	9% ***	70% ***	4% ***	23% ***
*C**	75% ***	8% ***	17% ***	58% ***	4% ***	35% ***
*h**	22% ***	4% **	71% ***	71% ***	7% ***	19% ***

Significant codes: ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

% is the percentage of variability explained by the factor or interaction

Supplementary table S4: Correlation (Pearson and Spearman) and determination coefficient between mannoprotein at bottling and variables measured 6 months after bottling for both wine qualities

Variables	Mannoprotein					
	Low-tier wines			Premium-tier wines		
	Pearson's correlation	Spearmann correlation	Regression coefficient	Pearson correlation	Spearmann correlation	Regression coefficient
POM test 420/520 nm	r = -0.56*	r = -0.43	R2=0.319	r = 0.16 ns	r = 0.22 ns	R2=0.027
TPI (mg/L gallic acid)	r = 0.62**	r = 0.59*	R2=0.379	r = -0.12 ns	r = -0.30 ns	R2=0.014
Tannins (T) (mg/L Methyl cellulose)	r = 0.73***	r = 0.80***	R2=0.534	r = -0.10 ns	r = -0.20 ns	R2=0.01
Anthocyanins (A) (mg/L malvidin-3-glu)	r = 0.38 ns	r = 0.32 ns	R2=0.147	r = -0.14 ns	r = -0.18 ns	R2=0.02
T/A ratio	r = 0.83***	r = 0.86***	R2=0.696	r = -0.06 ns	r = -0.17 ns	R2=0.003
Alcohol (% v/v)	r = 0.74***	r = 0.49*	R2= 0.541	r = -0.09 ns	r = -0.12 ns	R2=0.009
*L**	r = -0.56*	r = -0.63**	R2=0.315	r = 0.22 ns	r = 0.32 ns	R2=0.048
*a**	r = 0.50*	r = 0.48*	R2=0.249	r = -0.18 ns	r = -0.14 ns	R2=0.031
*b**	r = 0.75***	r = 0.78***	R2=0.569	r = -0.40 ns	r = -0.43 ns	R2=0.162
*C**	r = 0.55*	r = 0.49*	R2=0.298	r = -0.29 ns	r = -0.44 ns	R2=0.085
*h**	r = 0.38 ns	r = 0.34 ns	R2=0.143	r = -0.36 ns	r = -0.44 ns	R2=0.131
Significant codes: ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$						

*Red treatments are potentially more favorable vintages for superior red wine color.

Supplementary table S5: Pc c analysis of technological maturity, phenolic composition after bottling by quality and l st

Treatment	Brix/TA	TPI (mg/L gallic acid)	Tannins (T) (mg/L Methyl cellulose)	Anthocyanins (A) (mg/L malvidin-3-glu)	A	*a**	*b**	*L**	*C**	*h**
Low-tier H1	4.79 ± 0.27c	1779 ± 98c	492 ± 85b	294 ± 12c	).33ab	32.05 ± 1.67b	12.88 ± 0.73d	41.23 ± 1.(	5 ± 1.42b	21.95 ± 1
Low-tier H2	3.49 ± 0.54d	1667 ± 314c	382 ± 64b	325 ± 57c	25c	31.65 ± 5.88b	10.83 ± 0.69d	42.13 ± 7.4	9 ± 5.69b	19.25 ± 2
Low-tier H3	5.27 ± 0.39bc	2652 ± 63a	947 ± 135a	447 ± 20a	).3a	42.53 ± 1.04a	16.1 ± 1.43c	23.52 ± 2.3	± 0.92a	20.74 ± 1
Premium-tier H1	6.02 ± 0.28b	2379 ± 83b	788 ± 129a	458 ± 17a	).26a	43.28 ± 0.75a	18.83 ± 1.91b	25.95 ± 3.7	2 ± 1.34a	23.48 ± 1
Premium-tier H2	4.73 ± 0.21c	1902 ± 103c	470 ± 50b	383 ± 21b	).16bc	39.1 ± 2.19a	16.82 ± 1.03bc	33.49 ± 3.2	7 ± 2.3a	23.28 ± 1
Premium-tier H3	8.57 ± 0.77a	2372 ± 117b	791 ± 107a	437 ± 34ab	).27a	41.67 ± 1.79a	22.22 ± 1.51a	24.09 ± 2.(	3 ± 2.08a	28.05 ± 1

Different epresent significative differences p-value < 0.05 (Tuk

Low-tier wines

Tannins (T) mg/L Methyl cellulose)	Anthocyanins (A) (mg/L malvidin-3-glu)	T/A ratio	*a**	*b**	*L**	*C**	*h**	Mean colo	Mean sible color
98 ± 78bcde	277 ± 8hi	: 0.22abcd	32.22 ± 0.57 def	12.45 ± 0.53 efg	40.63 ± 0.16 c	34.55 ± 0.72 de	21.13 ± 0.49 bcde	#934	
95 ± 52cde	287 ± 7hi	: 0.23abcdef	33.04 ± 0.51 de	12.46 ± 0.3 efg	40.1 ± 1.25 c	35.31 ± 0.37 cde	20.66 ± 0.74 cde	#934	
75 ± 3cde	311 ± 5fg	: 0.03bcdef	34.25 ± 0.56 cd	12.17 ± 0.61 efgh	42.98 ± 0.03 bc	36.35 ± 0.74 cd	19.56 ± 0.61 ef	#9D4	
50 ± 23cde	290 ± 6ghi	: 0.11bcdef	30.15 ± 0.91 fg	12.62 ± 0.26 efg	41.49 ± 0.8 bc	32.68 ± 0.94 ef	22.72 ± 0.2 abcd	#934	
71 ± 50bcde	298 ± 1ghi	: 0.17abcde	32.63 ± 0.47 def	13.65 ± 0.52 def	40.54 ± 1.15 c	35.38 ± 0.63 cde	22.7 ± 0.48 abcd	#944	
71 ± 21de	300 ± 5gh	: 0.05ef	30.00 ± 0.37 fg	13.95 ± 0.63 cdef	41.66 ± 0.13 bc	33.09 ± 0.6 ef	24.94 ± 0.71 a	#944	
51 ± 21cde	276 ± 1i	: 0.08bcdef	29.66 ± 0.76 g	10.02 ± 0.55 i	31.89 ± 0.94 a	27.54 ± 0.91 h	21.33 ± 0.48 bcde	#A96	
58 ± 42e	281 ± 7hi	: 0.18cdef	25.66 ± 0.761	10.02 ± 0.551	31.89 ± 0.94 a	27.54 ± 0.91 h	21.33 ± 0.48 bcde	#A96	
53 ± 3e	279 ± 9hi	0.03ef	29.06 ± 1.02 gh	10.91 ± 0.58 ghi	41.99 ± 0.59 bc	31.04 ± 1.16 fg	20.58 ± 0.35 de	#934	
6 ± 2de	417 ± 7d	02f	40.52 ± 0.68 b	11.84 ± 0.71 fghi	29.12 ± 1.13 d	42.22 ± 0.45 b	16.29 ± 1.18 g	#7D2	
56 ± 81cde	364 ± 4e	: 0.23def	36.83 ± 0.68 c	10.63 ± 0.27 ghi	40.72 ± 1.07 c	38.33 ± 0.57 c	16.1 ± 0.67 g	#994	
37 ± 58e	331 ± 5f	: 0.18f	31.17 ± 0.52 efg	10.22 ± 0.39 hi	43.57 ± 1.55 bc	32.8 ± 0.62 ef	18.16 ± 0.36 fg	#995	
45 ± 166ab	429 ± 4cd	2.2 ± 0.37abc	41.47 ± 0.87 b	15.72 ± 0.62 bcd	24.19 ± 1.05 efg	44.35 ± 1.04 ab	20.76 ± 0.35 bcde	#711	
6 ± 62abc	427 ± 1cd	1.91 ± 0.14abcde	42.49 ± 0.71 ab	15.13 ± 0.4 cd	25.19 ± 0.33 def	45.1 ± 0.81 ab	19.6 ± 0.17 ef	#751	
06 ± 252a	440 ± 5bcd	2.52 ± 0.54a	41.74 ± 0.74 b	15.89 ± 0.71 abc	22.67 ± 0.58 fg	44.67 ± 0.94 ab	20.84 ± 0.51 bcde	#6D0	
918 ± 103a	448 ± 5bc	2.28 ± 0.25ab	42.37 ± 0.43 ab	17.96 ± 0.59 a	20.39 ± 2.47 g	46.03 ± 0.62 a	22.97 ± 0.46 abc	#680	
037 ± 194a	481 ± 8a	2.15 ± 0.37abcd	42.67 ± 0.42 ab	17.62 ± 0.62 ab	21.62 ± 0.03 fg	46.16 ± 0.62 a	22.43 ± 0.52 bcd	#6B0	
58 ± 31abcd	457 ± 1b	1.66 ± 0.07abcdef	44.42 ± 0.8 a	14.29 ± 0.54 cde	27.03 ± 0.81 de	46.66 ± 0.93 a	17.84 ± 0.33 fg	#7C1	

Different letters represent significative differences p-value < 0.05 (Tukey)

tentially more favorable vintages for superior red wine color.

Supplementary table S6: Post-hoc analysis of phenolic composition and CIELab and C and yeast strain for low-tier wines

tentially more favorable vintages for superior red wine color.

Supplementary table S6b: Post hoc analysis of phenolic composition and CIELab and CI and yeast strain for premium-tier wines

				Premium-tier wines						
	TPI (mg/L gallic acid)	Tan (mg/L cat	l	Anthocyanins (A) (mg/L malvidin-3-glu)	T/A	*a**	*b**	*L**	*C**		Me color
1 Y1	648 ±			451 ± 6cd	1.44 ± 0.03abc	42.83 ± 0.94 abc	19.72 ± 0.48 cd	24.66 ± 0.76 efg	47.16 ± 0.65 abcd	2	cde	#750E
1 Y2	832 ±			482 ± 4a	1.73 ± 0.32abc	43.1 ± 0.85 abc	18.81 ± 0.92 de	21.5 ± 1.18 g	47.03 ± 1.15 abcde	2	def	#6C01
1 Y3	939 ±			453 ± 3bcd	2.07 ± 0.18ab	42.74 ± 0.48 abc	17.98 ± 0.75 def	26.22 ± 1.24 def	46.36 ± 0.74 bcdef	2	efg	#7914
1 Y4	918 ±			462 ± 4abc	1.99 ± 0.1abc	42.99 ± 0.72 abc	18.34 ± 0.68 def	24.15 ± 0.67 efg	46.73 ± 0.93 abcdef	2	g	#730C
1 Y5	746 ±			470 ± 9abc	1.59 ± 0.49abc	44.76 ± 0.78 a	21.94 ± 0.54 abc	26.49 ± 1.11 def	49.85 ± 0.47 a	2	bcd	#7C10
1 Y6	645 ±			432 ± 8de	1.49 ± 0.16abc	43.27 ± 0.49 abc	16.21 ± 0.5 ef	32.7 ± 1.15 bc	46.21 ± 0.63 bcdef	2	g	#8B26
2 Y1	456 ±			375 ± 7gh	1.22 ± 0.01bc	37.17 ± 0.81 fg	15.82 ± 0.61 f	38.96 ± 0.89 a	40.4 ± 0.98 h	2	efg	#963E
2 Y2	533 ±			362 ± 6h	1.47 ± 0.11abc	38.65 ± 0.62 efg	16.51 ± 0.55 ef	32.24 ± 0.45 bc	42.03 ± 0.79 gh	2	efg	#853E
2 Y3	433 ±			395 ± 7fg	1.11 ± 0.06bc	40.64 ± 0.5 cde	17.45 ± 0.77 def	29.37 ± 1.23 cd	44.23 ± 0.85 defg	2	ef	#7F21
2 Y4	534 ±			394 ± 8fg	1.36 ± 0.09abc	42.08 ± 0.64 abcd	16.76 ± 0.59 ef	33.4 ± 0.92 b	45.3 ± 0.81 cdef	2		#8C2A
2 Y5	431 ±			412 ± 1ef	1.05 ± 0.04c	36.21 ± 0.67 g	15.84 ± 0.62 f	34.9 ± 1.1 b	39.52 ± 0.37 h	2	def	#8935
2 Y6	433 ±			359 ± 8h	1.21 ± 0.06bc	39.84 ± 0.6 def	18.51 ± 0.31 de	32.08 ± 0.8 bc	43.93 ± 0.41 efg	2	cde	#8629
3 Y1	826 ±			475 ± 0ab	1.74 ± 0.3abc	41.61 ± 0.73 cd	22.76 ± 0.96 ab	20.97 ± 1.16 g	47.43 ± 1.1 abc	2		#6903
3 Y2	729 ± 23abc			477 ± 1a	1.53 ± 0.06abc	44.39 ± 0.4 ab	22.2 ± 0.52 abc	24.52 ± 1.06 efg	49.63 ± 0.59 a	2	bc	#7609
3 Y3	959 ± 206a			432 ± 4de	2.22 ± 0.45a	42.13 ± 0.87 abcd	24.57 ± 0.84 a	21.08 ± 0.46 g	48.77 ± 1.17 ab	3	a	#6A02
3 Y4	720 ± 97abc			387 ± 5g	1.86 ± 0.23abc	38.86 ± 0.66 efg	19.94 ± 0.5 cd	27.96 ± 0.31 de	43.68 ± 0.36 fg	2	bc	#7A20
3 Y5	666 ± 111abc			427 ± 1e	1.56 ± 0.25abc	40.99 ± 0.37 cde	21.6 ± 0.69 bc	27.14 ± 1.1 de	46.33 ± 0.65 bcdef	2	ab	#7A1A
3 Y6	846 ± 230abc			421 ± 6e	2.01 ± 0.52abc	42.02 ± 0.42 bcd	22.22 ± 0.69 abc	22.87 ± 1.08 fg	47.53 ± 0.69 abc	2		#6F09

Different letters represent significative differences p-value < 0.05 (Tukey)

Supplementary table S7a: Summary of correlation (Pearson) and linear regression coefficients of the variables measured 6 months after bottling for low-tier wines

Low-tier wines

Variables	POM test 420/520 nm	TPI (mg/L gallic acid)	Tannins (T) (mg/L Methyl cellulose)	Anthocyanins (A) (mg/L malvidin-3-glu)	T/A ratio	Alcohol (% v/v)	*L**	*a**	*b**	*C**
TPI (mg/L gallic acid)	r=-0.58* R2= 0.337									
Tannins (T) (mg/L Methyl cellulose)	r=-0.55* R2= 0.308	r= 0.89*** R2= 0.795								
Anthocyanins (A) (mg/L malvidin-3-glu)	r=-0.50* R2= 0.247	r= 0.94*** R2= 0.892	r= 0.80*** R2= 0.64							
T/A ratio	r=-0.48* R2= 0.235	r= 0.63** R2= 0.395	r= 0.89*** R2= 0.784	r= 0.45 ns R2= 0.198						
Alcohol (% v/v)	r=-0.76*** R2= 0.584	r= 0.55* R2= 0.307	r= 0.50* R2= 0.246	r= 0.39 ns R2= 0.149	r= 0.530* R2= 0.284					
*L**	r= 0.50* R2= 0.25	r=-0.97*** R2= 0.945	r=-0.89*** R2= 0.784	r=-0.930*** R2= 0.866	r=-0.630** R2= 0.400	r=-0.51* R2= 0.263				
*a**	r=-0.57* R2= 0.329	r= 0.96*** R2= 0.917	r= 0.80*** R2= 0.639	r= 0.950*** R2= 0.900	r= 0.520* R2= 0.266	r= 0.56* R2= 0.315	r=-0.94*** R2= 0.881			
*b**	r=-0.55* R2= 0.306	r= 0.86*** R2= 0.748	r= 0.91*** R2= 0.827	r= 0.74*** R2= 0.545	r= 0.79*** R2= 0.624	r= 0.61** R2= 0.378	r=-0.87*** R2= 0.754	r= 0.74*** R2= 0.544		
*C**	r=-0.59* R2= 0.347	r= 0.97*** R2= 0.950	r= 0.84*** R2= 0.700	r= 0.95*** R2= 0.903	r= 0.56* R2= 0.319	r= 0.59* R2= 0.344	r=-0.96*** R2= 0.917	r= 1.00*** R2= 0.992	r= 0.79*** R2= 0.63	
*h**	r= 0.01 ns R2= 0,053	r=-0.11 ns R2= 0.012	r= 0.15 ns R2= 0.023	r=-0.28 ns R2= 0.077	r= 0.370 ns R2= 0.140	r= 0.11 ns R2= 0.013	r= 0.08 ns R2= 0.007	r=-0.35 ns R2= 0.119	r= 0.37 ns R2= 0.139	r=-0.26 ns R2= 0.069

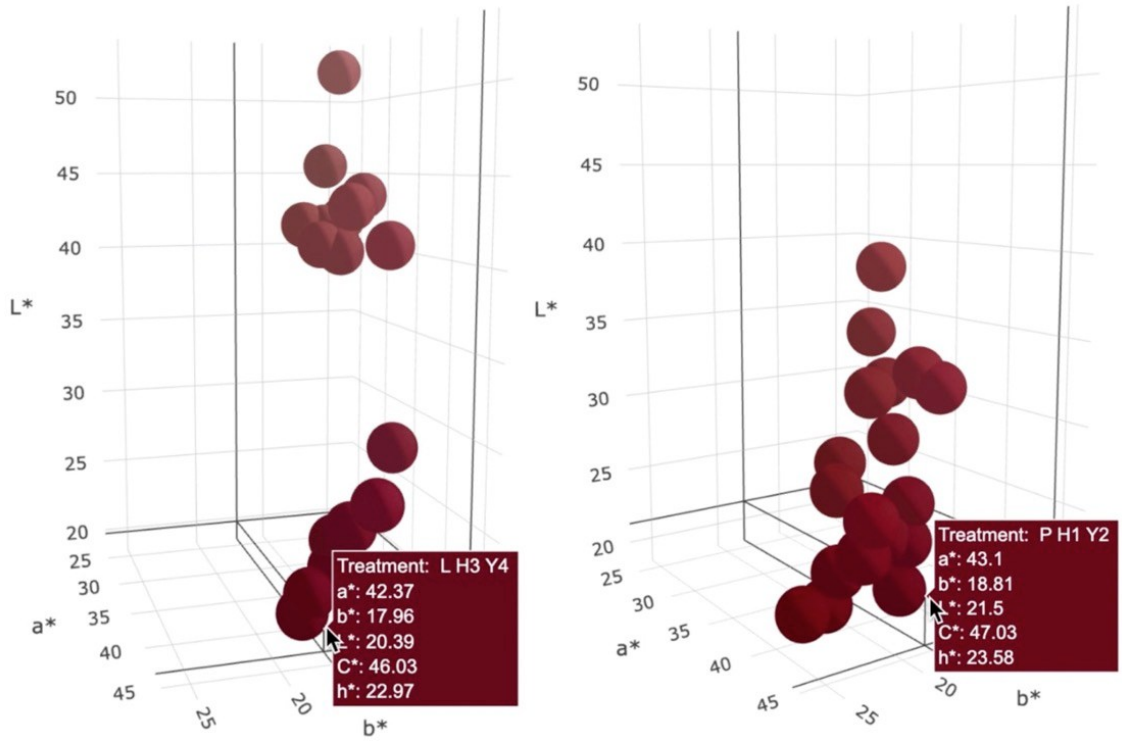
Significant codes: ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Supplementary table S7b: Summary of correlation (Pearson) and linear regression coefficients of the variables measured 6 months after bottling for premium-tier wines

Premium-tier wines										
Variables	POM test 420/520 nm	TPI (mg/L gallic acid)	Tannins (T) (mg/L Methyl cellulose)	Anthocyanins (A) (mg/L malvidin-3-glu)	T/A ratio	Alcohol (% v/v)	*L**	*a**	*b**	*C**
TPI (mg/L gallic acid)	r=-0.11 ns R2= 0.012									
Tannins (T) (mg/L Methyl cellulose)	r=-0.03 ns R2= 0.001	r= 0.86*** R2= 0.738								
Anthocyanins (A) (mg/L malvidin-3-glu)	r=-0.07 ns R2= 0.005	r= 0.88*** R2= 0.779	r= 0.71*** R2= 0.499							
T/A ratio	r=-0.03 ns R2= 0.001	r= 0.71** R2= 0.506	r= 0.95*** R2= 0.907	r= 0.46 ns R2= 0.212						
Alcohol (% v/v)	r=-0.22 ns R2= 0.05	r= 0.85*** R2= 0.728	r= 0.72*** R2= 0.522	r= 0.65** R2= 0.422	r= 0.65** R2= 0.425					
*L**	r=-0.04 ns R2= 0.002	r=-0.85*** R2= 0.716	r=-0.83*** R2= 0.689	r=-0.73*** R2= 0.535	r=-0.730*** R2= 0.539	r=-0.80*** R2= 0.642				
*a**	r= 0.31 ns R2= 0.098	r= 0.78*** R2= 0.609	r= 0.63** R2= 0.392	r= 0.74*** R2= 0.554	r= 0.47* R2= 0.221	r= 0.59** R2= 0.349	r=-0.65** R2= 0.425			
*b**	r=-0.27 ns R2= 0.072	r= 0.74*** R2= 0.554	r= 0.63** R2= 0.402	r= 0.49* R2= 0.245	r= 0.60** R2= 0.359	r= 0.80*** R2= 0.642	r=-0.80*** R2= 0.637	r= 0.49* R2= 0.237		
*C**	r= 0.13 ns R2= 0.016	r= 0.88*** R2= 0.771	r= 0.72*** R2= 0.519	r= 0.75*** R2= 0.560	r= 0.59** R2= 0.351	r= 0.76*** R2= 0.577	r=-0.80*** R2= 0.642	r= 0.94*** R2= 0.878	r= 0.76*** R2= 0.579	
*h**	r=-0.44 ns R2= 0.196	r= 0.50* R2= 0.247	r= 0.440 ns R2= 0.194	r= 0.22 ns R2= 0.050	r= 0.470* R2= 0.224	r= 0.65** R2= 0.428	r=-0.62** R2= 0.383	r= 0.10 ns R2= 0.01	r= 0.92*** R2= 0.841	r= 0.44 ns R2= 0.194

Significant codes: ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Supplementary figure SF1: Color contrast by spheres in three-dimensional CIELab color space



Captures of interactive spatially representations of the CIELab color parameters in three-dimensional (3D) plots (*L**, *a**, and *b**) Each treatment combination was depicted as a sphere with a radius of 1.5 units. Consequently, if any two spheres intersect it means that they have a ΔE*ab value of less than 3.0 units and the human eye would not be able to perceive a color difference between the corresponding wines. At the same time, if they do not intersect, it means that their color difference is perceptible to the human eye and this difference is noticeable the farther apart they are. (Fairchild, M. D. (2018). The colors of wine. International Journal of Wine Research, 13-31). The above, together with the information of *h*, C** and the actual observed color visually represented on the surface of each sphere in the graph help easily determine the best combination in each wine quality. a) low-tier wines (**Video1a)**, b) premium-tier wines (**Video1b).**