



Role of mannoproteins in the activation of wine malolactic fermentation: Influence of the yeast and the extraction method

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ABSTRACT

This study focuses on the characterisation of mannoprotein extracts (MPs) obtained from active dry yeast of *Saccharomyces cerevisiae* and non-*Saccharomyces* strains, including those of *Torulaspora delbrueckii*, *Lachancea thermotolerans*, and *Metschnikowia pulcherrima*, and their effects on wine malolactic fermentation (MLF) carried out by *Oenococcus oeni*. Mannoproteins are essential components of the yeast cell wall, are released during the autolysis process, and vary in size, molecular weight, and protein content depending on the extraction method. In this work, it was observed that the MPs obtained through cellular autolysis induction had a higher molecular weight, a richer sugar composition—including mannose and glucose—and a higher protein content than the MPs obtained through the alkali medium method. All the MPs extracts had beneficial effects on MLF in white and red wines. The consumption of mannoprotein by *O. oeni* was observed in all the fermentations, indicating the relationship between MPs and *O. oeni* metabolism improvement. Finally, the addition of MPs did not significantly affect colour parameters, tannins, or anthocyanins at the end of MLF in red wines, keeping these components stable and, therefore, maintaining the final stability of the wine. In conclusion, this work demonstrates the potential of MPs as effective activators of MLF in wine.

1. Introduction

Yeasts play a crucial role in the conversion of sugars in grape must into ethanol during alcoholic fermentation (AF), the primary transformation in wine production (Bartowsky, 2005; Lerm et al., 2016; Ribéreau-Gayon et al., 2006). Owing to its robust fermentation ability, *Saccharomyces cerevisiae* is the main species performing AF (Ribéreau-Gayon et al., 2006). However, the yeast species present during wine-making extend beyond *S. cerevisiae*, including different non-*Saccharomyces* species with valuable oenological properties. There is a growing interest in the use of certain non-*Saccharomyces* yeasts as starter cultures in the initial stages of AF, which are typically introduced concurrently with a selected *S. cerevisiae* strain to ensure the thorough completion of AF (Padilla et al., 2016). Non-*Saccharomyces* yeasts of oenological interest include *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, and *Lachancea thermotolerans*. They produce secondary metabolites that may

enhance the final flavour and taste of wines (Beltran et al., 2002; Fairbairn et al., 2021; Jolly et al., 2014; du Plessis et al., 2017).

Another significant biochemical transformation in this context is malolactic fermentation (MLF), where lactic acid bacteria (LAB) decarboxylate L-malic acid into L-lactic acid under difficult conditions, typically after AF. In addition to its role in deacidification, MLF ensures microbiological stability and enhances organoleptic characteristics (Bauer and Dicks, 2017; C. R. Davis et al., 1985; Chambers and Pretorius, 2010; Y. Liu et al., 2017; Paramithiotis et al., 2022). *Oenococcus oeni* is the best adapted LAB for wine-demanding environments (Mills et al., 2005). This bacterium is highly adaptable to challenging conditions in wine, including high ethanol, low pH, low temperatures, sulfur dioxide, yeast medium-chain fatty acids, and nutrient depletion (Margalef-Català et al., 2017; Olguín et al., 2009; Yang et al., 2021). Among the possible activators of MLF, the benefits of yeast mannoproteins present in wine lees (Balmaseda et al., 2024) and in yeast mannoprotein extracts

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(Torano et al., 2024; Torano et al., 2025a) have been described.

Mannoproteins constitute a substantial portion—approximately 35–40%—of the cell wall of *S. cerevisiae*. These biomolecules exhibit highly variable sizes ranging from 5 to 800 kDa, although the typical reported range is 50–500 kDa (Díez et al., 2010; Martínez Lapuente et al., 2019). They are distinguished by their significant glycosylation, which is primarily composed of one major glycosidic moiety, which constitutes 50% to 95% of the molecule. This moiety is covalently linked to a minor protein moiety (Klis et al., 2002; Li and Karboune, 2019; Manjón et al., 2020; Snyman et al., 2023; Vidal et al., 2003), which is associated with residues of D-glucose and N-acetylglucosamine (Rodrigues et al., 2012). The degree of glycosylation in mannoproteins is variable, with some instances showing concentrations exceeding 90% sugars, predominantly mannose (Lipke and Ovalle, 1998; Ribéreau-Gayon et al., 2006). Throughout the winemaking process, the liberation of mannoproteins from the yeast cell wall is notable during ageing because of the autolytic process in yeast (Belda et al., 2016; Dupin et al., 2000; Vejarano, 2020). These compounds are also naturally released by yeasts into wine during fermentative and postfermentative processes (Guadalupe et al., 2007). Furthermore, the structural and chemical composition of mannoproteins is directly influenced by the yeast growth phase and environmental conditions, which undergo changes throughout the winemaking process (Ballou, 1976; Klis et al., 2002; Moutounet et al., 2003). These molecules are authorised as additives in wines to improve chemical and sensory stability (Bicca et al., 2023; Caridi, 2006; Lubbers et al., 1994; Oyón-Ardoiz et al., 2022).

Mannoproteins may also play a significant role in MLF by adsorbing medium-chain fatty acids produced by yeasts and phenolic compounds present in grape must, thereby promoting bacterial growth (Alexandre et al., 2004; Caridi, 2006; Díez et al., 2010; Guilloux-Benatier et al., 1995; Liu et al., 2017). Lactic acid bacteria (LAB) can breakdown mannoproteins, which increases the nutritional content of the medium and stimulates their own activity. This process not only enriches the medium but also improves the survival of *O. oeni* in the wine environment. Additionally, previous research has shown that *O. oeni* exhibits glycosidase and peptidase activities, which help release sugars and amino acids from mannoproteins and other macromolecules. (Alexandre et al., 2004; Guilloux-Benatier et al., 2001).

Studies on mannoproteins and their effects on LAB and MLF have focused primarily on those from *S. cerevisiae*, but little is known about the impact of different strains or species. Considering the increasing use of non-*Saccharomyces* yeasts and research showing the positive effects of *T. delbrueckii* and *M. pulcherrima* on MLF (Balmaseda et al., 2018; Ruiz-de-Villa et al., 2023a, 2023b), it is also important to evaluate the influence of mannoproteins from these species on MLF.

The method of mannoprotein extraction can significantly influence the composition of the extract (Oyón-Ardoiz et al., 2022, 2024) and, consequently, its function in wine, including its impact on MLF. Typically, two methods are used for mannoprotein extraction: enzymatic degradation of the yeast cell wall and thermal/chemical autolysis (Breddam and Beenfeldt, 1991). The choice of extraction method can affect the specific mannoproteins released and their interaction with wine components, thereby influencing outcomes such as MLF.

The main objective of this work was to evaluate the influence of the yeast strain/species and the method used to obtain mannoprotein extracts (MPs) from active dry yeast (ADY) on *O. oeni* and MLF.

2. Materials and methods

2.1. Extraction of mannoproteins from *S. cerevisiae* and non-*Saccharomyces* strains

Mannoprotein-rich extracts (MPs) were obtained from 20 g of active dry yeast (ADY) from seven different commercial strains of *S. cerevisiae* and non-*Saccharomyces* species. Four *S. cerevisiae* strains were used in this study: Lalvin QA23 (Lallemand Inc. Montreal, QC, Canada

Lallemand Inc.) (Sc1), CLOS (Lallemand Inc.) (Sc2), Viniferm-3D (Agrovin, Alcázar de San Juan, Spain) (Sc3) and CV K1 Marquée (Lallemand Inc.) (Sc4). Sc1 and Sc2 were specifically chosen for fermenting white and red wines, respectively. Sc3 is described as a high-mannoprotein producer strain, whereas Sc4 contains a killer factor. Three strains belonging to non-*Saccharomyces* species were also included in the study: *T. delbrueckii* Zymaflore Alpha (Laffort, Bordeaux, France) (Tde), *L. thermotolerans* LAKTIA™ (Lallemand Inc.) (Lth) and *M. pulcherrima* Flavia (Lallemand Inc.) (Mpu). These strains were rehydrated at 37 °C for 30 min for the *S. cerevisiae* strains and at 30 °C for 30 min for the non-*Saccharomyces* strains, following the manufacturer's instructions. The cells were subsequently collected through centrifugation (1290 ×g, 10 min) and washed three times with distilled water. The resulting biomass was subjected to two distinct extraction procedures for obtaining the MPs: induced autolysis (MP-A) and alkaline extraction (MP-B), following the procedure described by Oyón-Ardoiz et al. (2024). All the chemical reagents were provided by Panreac (Panreac, Química SLU, Castellar del Vallès, Spain).

The extraction of mannoproteins through induced autolysis involves several steps (Oyón-Ardoiz et al., 2024). Initially, the biomass underwent induced autolysis, following a modification of the procedure proposed by Liu et al. (2008). The wet biomass was suspended in 3% NaCl (w/v) at a concentration of 15% (w/v), and yeast cell autolysis was carried out for 24 h at 55 °C with agitation (120 rpm). The suspension was subsequently heated at 80 °C for 15 min to deactivate the cellular enzymes. The autolysed cells were collected by centrifugation (5170 ×g, 15 min) and washed twice with 3% NaCl (w/v). The supernatants, containing mannoproteins liberated during the autolysis process, were combined and precipitated in 70% (v/v) ethanol. The resulting precipitates were washed three times with 70% (v/v) ethanol and then freeze-dried. The autolysed cells were further subjected to chemical hydrolysis.

The extraction of mannoproteins (Oyón-Ardoiz et al., 2024) through chemical hydrolysis (MP-B) was conducted with a modification of the method outlined by Kim and Yun (2006). Initially, the autolysed cells were suspended in 2% NaOH (w/v) at a concentration of 15% (w/v), and the suspension was heated for 1 h at 90 °C. Next, the suspension was cooled to room temperature and subjected to centrifugation (5170 ×g, 10 min). Upon adjustment of the pH of the supernatant to neutrality with 3 M HCl, the mannoproteins were precipitated in 70% (v/v) ethanol. The resulting precipitates were washed three times with 70% (v/v) ethanol and subsequently freeze-dried. Finally, the three MPs obtained were dissolved in water and subjected to dialysis using membranes with a 6–8 kDa molecular weight cut-off for 72 h at 10 °C against 5 L of distilled water with periodic water changes.

2.2. Characterisation of the mannoprotein extracts (MPs)

The protein and glycoprotein profiles of the extracts rich in mannoproteins (MPs) were visualised through SDS–PAGE employing 4–15% linear gradient Mini-PROTEAN® TGX™ precast gels from Bio-Rad Laboratories, Inc. (Hercules, CA, USA), following the protocol reported by Oyón-Ardoiz et al. (2024): the extracts, dissolved in water at a concentration of 37.5 mg/mL, were diluted 1:1 with 2× sample loading buffer (100 mM Tris–HCl pH 6.8, 20% (v/v) glycerol, 4% (v/v) SDS, 25 mM DTT, and traces of bromophenol blue), and heated to 100 °C for 5 min. Subsequently, 16 µL of each sample was loaded into the gel, and electrophoresis was conducted at a constant voltage of 180 V for 50 min at room temperature using Tris–Glycine SDS as the running buffer (24.8 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3, Bio-Rad Laboratories, Inc.). Protein bands were stained with Coomassie Brilliant Blue G-250 (Alfa Aesar, Thermo Fisher Scientific, Germany), and glycoproteins were detected using the Periodic Acid-Schiff (PAS) staining method. The results of both staining processes were scanned using a Lexmark XC4140 scanner (Lexington, KY, USA). Molecular weights (MWs) were estimated with a protein ladder (PageRuler™ Plus Prestained Protein Ladder,

10–250 kDa, Thermo Fisher Scientific, Germany) (Oyón-Ardoiz et al., 2024).

The total protein concentration of the extracts was assessed using the Lowry method (Lowry et al., 1951) with a DC protein assay kit (Bio-Rad Laboratories, Inc.) and a calibration curve based on bovine serum albumin. The total protein content (w/w) was derived from the concentration data.

The molecular weight (MW) distribution of the MP extracts was determined through HRSEC-RID following the method outlined by Manjón et al. (2020). This involved an HPLC system (an Agilent 1260 Infinity system (Agilent Technologies, Palo Alto, CA, USA)) equipped with two serial Shodex OHpak SB-803 HQ and SB-804 HQ columns (8 mm × 300 mm) (Showa Denko Europe GmbH, Germany) and a refractive index detector (RID). The MW calibration curve was established using various pullulan standards with MWs ranging from 342 Da to 805,000 Da.

To analyse the monosaccharide composition of the MPs, the method described by (Oyón-Ardoiz et al., 2022) was employed. The MPs underwent acid hydrolysis, followed by derivatization with 1-phenyl-3-methyl-5-pyrazolone (PMP). The resulting monosaccharide derivatives were analysed via HPLC-DAD-MS using an Agilent 1200 Series HPLC instrument equipped with an Agilent Poroshell 120 EC-18 column (2.7 µm, 4.6 mm × 150 mm) (Agilent Technologies) coupled to a mass spectrometer API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany). This system featured an ESI source and a triple quadrupole-ion trap mass analyser controlled by Analyst 5.1 software. Chromatograms were recorded at 250 nm as the preferred wavelength. A calibration curve was established using commercial glucose, mannose, and xylose standards from Sigma-Aldrich (St. Louis, MO, USA) and AlfaAesar (Kandel, Germany).

2.3. Bacterial strain and culture conditions

O. oeni PSU-1 (ATCC BAA-33), the first strain with a fully annotated genome, was used in this study. The cells were maintained on MRSmf plates (Torano et al., 2024) and stored at 4 °C. To prepare the inoculum, a single colony was selected from the plates and cultivated in liquid media at 27 °C within a 10% CO₂ environment. Then, 500 µL of this culture was introduced into 50 mL of the same newly prepared liquid media.

2.4. Malolactic fermentation

MLF was carried out in a wine like medium (WLM) as described by Torano et al. (2024). The WLM composition included 2 g/L L-malic acid, 0.5 g/L citric acid, 1.25 g/L Bacto TM casamino acids (BD, Le Pont de Claix Cedex, France), and 1.25 g/L peptone (Panreac), with a pH of 3.4 and 14% ethanol (v/v). The *O. oeni* PSU-1 strain was collected at the end of the exponential growth phase (OD_{600 nm} ≈ 1) and inoculated at a population of 10⁶ cells/mL, according to its growth curve, into WLM in 12 mL tubes under two fermentation conditions; the first condition was the addition of 2 g/L of MP-A and MP-B extracts from the different yeast strains (Sc1, Sc2, Sc3, Sc4, Tde, Lth, and Mpu) and the second was a control condition without MP addition. All fermentations were conducted under 10% CO₂ and static conditions at 20 °C in triplicate.

The progress of fermentation was monitored daily by measuring L-malic acid consumption using an enzymatic kit with a Y15 analyser (Biosystems S.A., Barcelona, Spain), and bacterial population growth was monitored as previously described. MLF was deemed complete when the concentration of L-malic acid fell below 0.1 g/L. The samples from each replicate were centrifuged and stored at −20 °C before and after MLF.

Fermentation in wine was carried out using Muscat of Alexandria and Tempranillo grapes from cultivars of the cellar Mas dels Frares (Universitat Rovira i Virgili, Constantí, Spain) during the 2023 harvest. AF was conducted in filled stainless steel tanks with a capacity of 50 L.

Both red and white wines were inoculated with *S. cerevisiae* Lalvin QA23. The resulting wines were stabilised for 4 days at 4 °C and used for the MLF assays. To verify the absence of indigenous LAB in wines, viability was assessed on MRSmf plates before *O. oeni* PSU-1 inoculation. No viable LAB populations were detected prior to inoculation. In addition, L-malic acid determination in must and wines after AF confirmed that there had not been consumption of this acid before starting MLF assays.

The oenological parameters at the end of AF for Muscat wine were as follows: 11.8% ethanol (v/v), pH 3.13, titratable acidity, 4.65 g tartaric acid/L, 1.68 g/L L-malic acid, 3.59 g/L glucose–fructose, 12 mg N/L α-amino nitrogen (NOPA) and 12 mg N/L NH₄⁺. The final parameters for Tempranillo were as follows: 13.8% ethanol (v/v), pH 3.51, titratable acidity, 5.6 g tartaric acid/L, 1.78 g/L L-malic acid, 0.69 g/L glucose–fructose, 40 mg N/L α-amino nitrogen (NOPA) and 16 mg N/L NH₄⁺.

In this assay, the MLF volume was maintained at 12 mL, but the added concentration of mannoproteins was 0.5 g/L, the concentration used in other studies (Poncet-LeGrand et al., 2007; Rinaldi et al., 2021; Sun et al., 2019) and reported by commercial manufacturers.

2.5. Mannoprotein consumption

The consumption of mannoproteins was analysed at the beginning and end of the MLF, primarily relying on the methodology outlined by Quirós et al. (2011), with certain adaptations (Torano et al., 2024). In this process, 5 mL samples were collected after overnight cold precipitation at −20 °C, followed by centrifugation (4600 ×g for 15 min). To these samples, five volumes of absolute ethanol were added, and the samples were incubated overnight at 4 °C. Afterwards, the samples were centrifuged at 2800 ×g for 10 min, and the resulting pellet was washed with two volumes of 96% ethanol. The pellet was subsequently resuspended in 1 mL of ethanol, transferred to a 2 mL tube, and subjected to further centrifugation at 6600 ×g for 5 min. The resulting pellet was then dried using a speed vacuum (Univap 148100ECH, Progen Scientific, London, UK) for 60 min.

Following the drying process, the samples were hydrolysed by adding 1 mL of 5 M H₂SO₄ (Panreac) and incubated for one hour at 95 °C in a thermoblock (Labnet, Madrid, Spain). Once the sample reached room temperature, neutralisation was performed with 1 M NaOH (for HPLC analysis) or 10 M NaOH (Panreac) for enzymatic analysis according to the method of Torano et al. (2024). The samples for Agilent 1100 HPLC (Agilent Technologies) were filtered with 0.22 µm pore filters before injection (Agilent Technologies). The HPLC instrument was equipped with a Hi-Plex H (300 mm × 7.7 mm) column inside a 1260 MCT (Infinity II Multicolumn Thermostat). The column conditions were 25 °C for 30 min, and the mobile phase was 10 mM H₂SO₄ (Panreac) and 10% CH₃CN (Panreac) at a flow rate of 0.6 mL/min. Finally, the chromatograph was equipped with an RID detector at 35 °C (1260 Infinity II refractive index detector) (Agilent Technologies).

2.6. Analysis of wine parameters

The concentrations of sugars (glucose and fructose), L-malic acid, acetic acid, D- and L-lactic acid, NH₄⁺, α-amino nitrogen (NOPA), and citric acid were determined with a Y15 autoanalyser (Biosystems SA). pH was determined using a Crison micro pH 2002 pH meter (Crison, Barcelona, Spain), and alcohol content was determined using ebulliometry (electronic ebulliometer uEBU6576, GAB Sistemática Analítica SL, Barcelona, Spain) as in Balmaseda et al. (2021).

2.7. Colour parameters, anthocyanins, and pyroanthocyanins

CIELab coordinates were determined following the methodology outlined by (Ayala et al., 1997). The colorimetric parameters, including hue (H*), lightness (L*), and chroma (C*), were computed using MSCV software (<http://www.unizar.es/>). The colour intensity (CI) was

calculated by summing the absorbances at 420, 520, and 620 nm, which were measured with a spectrophotometer via the method described by Glories (1984). According to the chromatic characteristics defined by CIELab (OIV, 2006), the limit for lightness (L_{ab}^*) ranges from 0 to 100, hue (h_{ab}) ranges from 0 to 360°, and Chroma (C_{ab}^*) has no specified interval.

Oenological tannin extracts were analysed via the methylcellulose precipitation method as described by Sarneckis et al. (2006). A fixed volume of 150 µL from each sample was placed in two centrifuge tubes. To tube A, 1 mL of methylcellulose solution (0.4% w/v) and 1 mL of saturated ammonium sulfate solution were added, and the volume was adjusted to 5 mL. Tube B was similarly prepared, excluding the addition of 1 mL of methyl cellulose solution, which was replaced with 1 mL of distilled water. After standing at room temperature for 20 min, the samples were centrifuged (4600 ×g for 15 min), and the absorbance of the supernatant was measured at 280 nm using a Helios alpha UV – visible spectrophotometer (Thermo Fisher Scientific). The difference in absorbance at 280 nm (ΔA_{280}) between tube B and tube A represented the tannin content of the sample. All the samples were analysed in triplicate.

This ethanol index establishes the percentage of tannins bound to polysaccharides (nonastringent tannins) as described by Ribéreau-Gayon et al. (2006). It was obtained by measuring the absorbance at 280 nm. One millilitre of the sample was mixed with 9 mL of absolute ethanol, homogenised, and allowed to settle overnight at 4 °C. After this period, the mixture was centrifuged (6600 ×g for 5 min). The supernatant was diluted 1:100 with distilled water, and the absorbance at 280 nm was measured. Additionally, the absorbance at 280 nm of the original sample diluted to 1:100 was determined. The ethanol index was determined by the difference in absorbance measurements in a 1 cm quartz cuvette at time zero and at the end of the treatment.

The analysis of free and acylated anthocyanins, as well as pyranoanthocyanins, was performed using an Agilent 1200 series liquid chromatograph (HPLC–diode array detection) with an Agilent Zorbax Eclipse XDB-C18 column (4.6, 250 mm × 5 mm, Agilent Technologies). The analytical procedure followed the method outlined by Gil et al. (2012). An external malvidin-3-monoglucoside calibration curve was employed for the quantification of phenolic compounds.

2.8. Statistical analysis

Three independent biological samples were analysed for each combination of strains per condition. ANOVA with Tukey's honestly significant difference (HSD) test was performed with the XLSTAT software 2023.4 package (Addinsoft, Paris, France), with a statistical level of significance of $p < 0.05$.

3. Results and discussion

3.1. Characterisation of mannoprotein extracts from *S. cerevisiae* and non-*Saccharomyces* strains

MPs from ADY were successfully obtained from all the yeast strains by the two extraction procedures—induced autolysis and alkaline extraction. The analysis of the MPs of different yeast strains via SDS-PAGE (Fig. S1) indicated that the two applied extraction methods resulted in the solubilisation of mannoproteins from the cell wall of the different yeast strains. This was evidenced by the presence of pink bands stained with the Schiff reagent in the electrophoresis gel, as well as the extraction of some non-glycosylated proteins (blue bands stained with Coomassie Brilliant Blue). The glycoprotein bands were located above 250 kDa, indicating the presence of high-molecular-weight (MW) molecules in all of the samples. Possible differences in the profiles of the mannoproteins were observed since MP-A extracts (obtained from induced autolysis) resulted in a higher concentration of the band at the beginning of the lane than did the MP-B extracts (obtained from alkaline

extraction). However, this analysis was intended only as a qualitative test to confirm the extraction of mannoproteins. The HRSEC-RID analysis provided quantitative data supporting the differences in the MW distributions of the obtained MPs, confirming the existence of significant structural differences between them (Fig. S2). Table 1 displays the distribution and average MW of the different MPs, as well as the total protein content.

In terms of the average MW of the extracts, those obtained from autolysis (MP-A) were larger than those obtained from alkaline extraction (MP-B) (Table 1). A greater average MW of mannoproteins was observed in the strain of *Lachancea thermotolerance* (Lth) with ca. 415 kDa, and the strain of *Metschnikowia pulcherrima* (Mpu) with 210 kDa. In contrast, the strain of *Torulaspora delbrueckii* (Tde) presented the smallest mannoprotein size, with an average MW of ca. 56 kDa. Among the strains of the species *Saccharomyces cerevisiae* (Sc), some differences were detected; however, these differences were much smaller than those among the non-*Saccharomyces* strains. In this case, the average MW for the four Sc strains ranged between ~106 and ~136 kDa. The average MW observed in MP-B also significantly differed but to a lesser extent; the average MW varied between ~69 kDa for Lth and ~49 kDa for Sc2.

The induced autolysis of the *S. cerevisiae* strains resulted in the

Table 1

Molecular weight (MW) distribution and average MW (kDa) of the mannoproteins determined by HRSEC-RID and total protein (w/w) in the extracts from Sc1, Sc2, Sc3, Sc4 (*S. cerevisiae*) Tde (*T. delbrueckii*), Lth (*L. thermotolerans*) and Mpu (*M. pulcherrima*) obtained by induced autolysis (MP-A) and alkaline extraction (MP-B). Different lowercase letters indicate significant differences among strains ($p < 0.05$). All data are expressed as the arithmetic average of three biological replicates ± standard deviation.

	MPs	MW (kDa)	%	Average MW (kDa)	Protein content (%)
MP-A	Sc1	958–30	41.78	106 ± 2.26 ^b	24 ± 1.47 ^c
		30 al 1	49.45		
		< 1	8.77		
	Sc2	876–136	46.9	136 ± 6.38 ^d	6 ± 0.33 ^b
		136–20	37.71		
		20 al 3	8.81		
		< 3	6.58		
	Sc3	936–140	39.38	121 ± 2.51 ^c	19 ± 0.87 ^d
		140–24	18.52		
		24 al 7	9.5		
		< 7	32.6		
	Sc4	856–140	39.38	118 ± 7.45 ^{bc}	22 ± 1.03 ^c
		140–24	18.52		
		24 al 7	9.5		
		< 7	32.6		
	Tde	836–188	14.15	56 ± 3.5 ^a	12 ± 0.22 ^c
		188–35	28.18		
		35–7	9.69		
MP-B		< 7	47.98		
	Lth	1070–48	91.78	415 ± 2.44 ^f	11 ± 2.17 ^c
		48–14	4.47		
		< 14	3.75		
	Mpu	96–26	92.42	210 ± 4.07 ^e	4 ± 1.45 ^a
		26 al 2	3.01		
		< 2	4.57		
	Sc1	303–4	95.48	52 ± 0.45 ^{ab}	2 ± 0.32 ^a
		< 4	4.52		
	Sc2	508–3	96.61	49 ± 0.14 ^a	4 ± 1.36 ^{ab}
		< 3	3.39		
	Sc3	327–6	95.97	58 ± 0.73 ^c	6 ± 0.15 ^b
		< 6	4.03		
	Sc4	395–5	95.28	52 ± 1.78 ^{ab}	23 ± 2.32 ^d
		< 5	4.72		
	Tde	386–4	97.34	53 ± 0.87 ^b	5 ± 1.84 ^b
		< 4	2.66		
	Lth	934–287	2.6	70 ± 0.22 ^d	6 ± 0.38 ^b
		287–4	93		
		< 4			
	Mpu	557–254	1.16	54 ± 0.81 ^b	3 ± 0.35 ^d
		254–4	94.56		
		< 4	4.27		

solubilisation of a significant fraction of MW mannoproteins, between 136 and 902 kDa (Table 1), which resulted in different fractional distributions among these yeasts. The *S. cerevisiae* strains are distributed into four fractions, except for Sc1, which is distributed into three fractions. The percentage of the first fraction, which ranges between 136 and 936 kDa, is the highest percentage: 46.90% for Sc2, 39.38% for Sc3, and 39.38% for Sc4. The fraction corresponding to a percentage of 41.78%, with a wider range between 30 and 958 kDa, is associated with Sc1 (Table 1).

The distributions of non-*Saccharomyces* species also differ. Tde presents four fractions, with the highest percentage of 47.98% in the fraction below 7 kDa. Lth and Mpu have three fractions with well-defined ranges. In the case of Lth, there is a fraction with an MW between 48 and 1070 kDa that represents 91.78%, whereas Mpu has a fraction with an MW between 26 and 96 kDa, representing 92.42% (Table 1).

With respect to alkaline extraction (MP-B), all the mannoproteins eluted in a main peak, which accounted for more than 93% of the total extract content, depending on the yeast strain. This peak represented more than 94% of the total mannoproteins detected and was divided into two fractions for all *S. cerevisiae* strains and Tde, with an MW between 3 and 395 kDa, except for Sc2, which had a wider range of up to 508 kDa. In non-*Saccharomyces* species, Lth and Mpu presented a fraction whose MW was between 4 and 287 kDa among the three fractions observed.

Several studies have reported varying ranges of MWs associated with mannoprotein extracts. Oyón-Ardoiz et al. (2024) observed structural differences in the mannoproteins of *T. delbrueckii* by employing enzymatic and chemical extraction methods. Snyman et al. (2023) also reported differences in species such as *Saccharomyces boulardii*, *S. cerevisiae*, *M. pulcherrima*, and *T. delbrueckii* when enzymatic and ultrasound treatments were applied for purification.

The quantification of the protein fraction revealed significant differences between the MP-A and MP-B extracts, with a greater protein yield observed with induced autolysis. Sc2, Sc4, and Mpu presented similar protein contents for both types of extraction methods. However, the remaining strains showed notable differences depending on the extraction method, highlighting the influence of the purification treatment on the characteristics of the obtained MPs. Sc1 was the strain with the greatest difference in protein content, with the highest protein content among all strains in MP-A (23.76%) but only 1.81% in MP-B. Mpu was the strain with the lowest protein content in the extracts from the two extraction methods, with 5.51% in MP-A and 3.19% in MP-B. The protein component of mannoproteins can constitute as much as 50% (w/w) (Lipke and Ovalle, 1998), with literature reporting varying percentages of protein. Mannoproteins can be grouped into those with high protein content (~30%) or low protein content (~10%) (Manjón et al., 2020). According to this classification, we obtained high protein content mannoproteins, mainly in MP-A, and only Sc4 could be considered a high-protein content MP. These variations in the protein portions of mannoproteins among species and strains have been previously reported by different authors (Manjón et al., 2020; Oyón-Ardoiz et al., 2024; Snyman et al., 2023b).

The composition of the monosaccharides analysed through HPLC-DAD-MS varied between the extraction methods and the yeast species (Table 2). Mannose dominated as the primary monosaccharide (> 75.47%) across both types of extracts, suggesting efficient extraction of mannoproteins from diverse yeast strains and species. The proportions of mannose and glucose in the MP-A extracts, obtained by induced autolysis, were significantly different (Table 2). The strains Sc3, Sc4, and Lth presented the highest proportions of mannose, surpassing 95% of the content of this sugar. Close behind were Sc2, Mpu, and Tde, with proportions exceeding 87%, whereas Sc1 had the lowest proportion of mannose among all the strains (75.47%). In accordance with the lower mannose content, Sc1 had the highest concentration of glucose (24.91%), followed by Mpu (12.12%), Sc2 (11.22%), and Tde (8.68%). Glucose proportions below 3% were detected for Sc3, Sc4, and Lth.

Table 2

Monosaccharide composition in terms of mannose, glucose and ribose percentage (%) of the extracts from Sc1, Sc2, Sc3, Sc4 (*S. cerevisiae*) Tde (*T. delbrueckii*), Lth (*L. thermotolerans*) and Mpu (*M. pulcherrima*) obtained by induced autolysis (MP-A) and alkaline extraction (MP-B). Different lowercase letters indicate significant differences between values using the Tukey (HSD) test at $p < 0.05$. All data are expressed as the arithmetic average of three biological replicates $\pm$ standard deviation. nd: not detected.

	MPs	Mannose (%)	Glucose (%)	Ribose (%)
MP-A	Sc1	75.47 $\pm$ 0.81 ^a	24.91 $\pm$ 0.60 ^d	nd
	Sc2	88.78 $\pm$ 0.45 ^b	11.22 $\pm$ 0.45 ^c	nd
	Sc3	97.13 $\pm$ 0.40 ^d	2.87 $\pm$ 0.40 ^a	nd
	Sc4	97.46 $\pm$ 0.29 ^d	2.54 $\pm$ 0.29 ^a	nd
	Tde	91.32 $\pm$ 0.60 ^c	8.68 $\pm$ 0.60 ^b	nd
	Lth	97.57 $\pm$ 0.21 ^d	2.43 $\pm$ 0.21 ^a	nd
MP-B	Mpu	87.88 $\pm$ 0.55 ^b	12.12 $\pm$ 0.55 ^c	nd
	Sc1	76.14 $\pm$ 0.42 ^a	18.90 $\pm$ 0.45 ^c	4.96 $\pm$ 0.04 ^b
	Sc2	97.20 $\pm$ 0.20 ^e	2.74 $\pm$ 0.31 ^a	0.06 $\pm$ 0.10 ^a
	Sc3	85.64 $\pm$ 0.41 ^c	8.41 $\pm$ 0.36 ^c	5.95 $\pm$ 0.31 ^b
	Sc4	89.62 $\pm$ 0.32 ^d	9.93 $\pm$ 0.09 ^d	0.45 $\pm$ 0.23 ^a
	Tde	81.68 $\pm$ 0.06 ^b	4.14 $\pm$ 0.13 ^b	14.11 $\pm$ 0.12 ^c
	Lth	96.17 $\pm$ 0.79 ^e	3.43 $\pm$ 0.12 ^{ab}	0.39 $\pm$ 0.68 ^a
	Mpu	88.83 $\pm$ 0.49 ^d	10.46 $\pm$ 0.77 ^d	0.71 $\pm$ 1.23 ^a

With respect to the monosaccharides detected in the MP-B extracts (Table 2), the highest presence of mannose was found in Sc2 and Lth, with 97.20% and 96.17%, respectively. Additionally, proportions exceeding 80% were observed in Tde, Sc3, Mpu, and Sc4, whereas Sc1 presented the lowest proportion of mannose (76.14%), in accordance with the results observed in the MP-A extract for this strain. In line with these results, the Sc1 strain presented the highest proportion of glucose, which was also present in the MP-B extract (18.90%).

Ribose was detected in all the strains in the extracts obtained by alkali extraction (Table 2). The strain Tde stands out with a ribose content of 14.11%. The remaining strains presented significantly lower values of ribose. Glucose and ribose profiles have been associated with the release of β -glucan and nucleic acids, respectively, during cell lysis (Oyón-Ardoiz et al., 2024).

In view of these results, the characteristics of the purified mannoproteins differ depending on the yeast species and, in particular, the extraction method. Differences in protein and monosaccharide contents were observed not only between yeast species but also among different strains of the same species, *S. cerevisiae*. For these components, the differences due to the extraction method were the most remarkable, as was also the case for mannoproteins.

3.2. MLF in WLM and white and red wines

3.2.1. MLF in WLM

Based on previous results obtained when commercial mannoprotein extracts were used in WLM (Torano et al., 2024), 2 g/L MPs was added, as clear activation of MLF was observed at this concentration. The effect of MPs on the evolution of MLF was different depending on the added MPs in the WLM (Fig. 1). In the case of MP-A extracts (Fig. 1A), all the fermentations were completed faster than those of the control, demonstrating the positive effect of the extracts obtained by induced autolysis. In particular, Mpu consumed all the L-malic acid in 3 days, followed by all the other strains, which finished the process within 4 days, whereas the control condition required 9 days to complete the MLF. In the case of the addition of MP-B extracts (Fig. 1B), not all the fermentations concluded successfully. The fermentations with Sc3 and Lth extracts completed MLF at 4 and 5 days, respectively, showing faster fermentation than the control. However, the remaining fermentations started but were stuck when L-malic acid concentrations ranged between 1.60 and 1.20 g/L. This negative effect may be explained by the presence of some inhibitory compound/s in MP-B extracts from Sc1, Sc4, Tde and Mpu strains associated to the extraction methods that we couldn't identify.

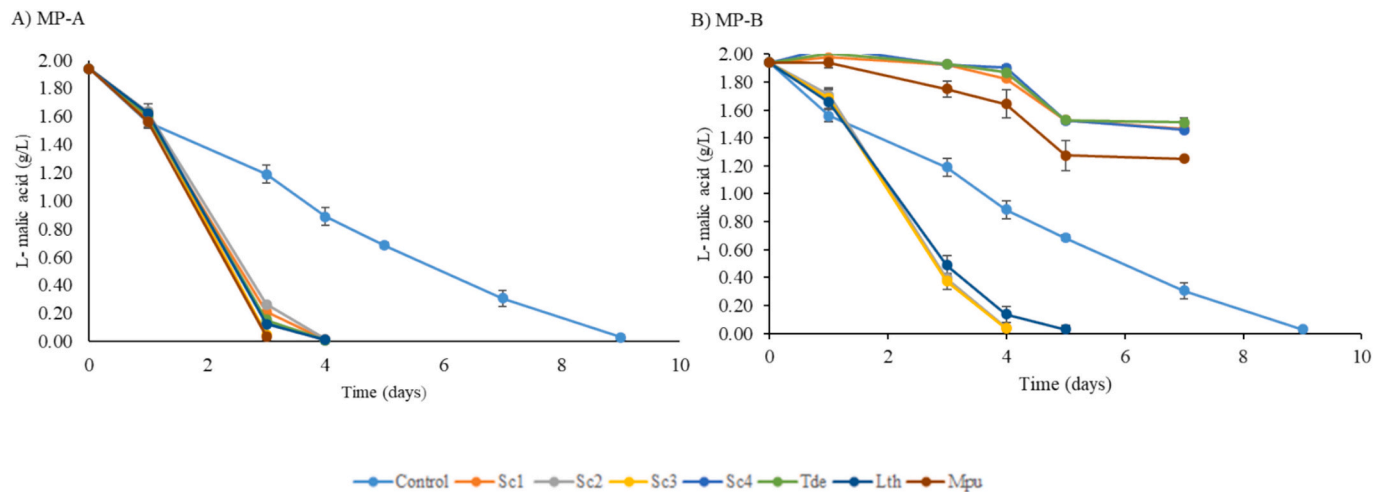


Fig. 1. Evolution of malolactic fermentation evolution in wine-like medium (WLM) fermented with *O. oeni* PSU-1 with the addition of 2 g/L of MP extract from Sc1, Sc2, Sc3, Sc4 (*S. cerevisiae*) Tde (*T. delbrueckii*), Lth (*L. thermotolerans*) and Mpu (*M. pulcherrima*) obtained by A) induced autolysis (MP-A) and B) alkaline extraction (MP-B). All data are expressed as the arithmetic average of three biological replicates $\pm$ standard deviation.

3.2.2. MLF in white and red wines

A lower concentration of extract (0.5 g/L instead of 2 g/L) was used in white and red wines because preliminary studies revealed interference with polyphenols at concentrations exceeding 0.5 g/L (data not shown), leading to the decision to use this lower concentration. Some studies have indicated that polyphenols can interact with mannoproteins, potentially affecting the stability and bioavailability of both compounds (Bicca et al., 2023; Escot et al., 2001; Manjón et al., 2020). The addition of MPs positively affected the evolution of MLF in white and red wines, resulting in faster MLF in all cases (Fig. 2). In white wine, MLFs with MP-A extracts and conducted with Sc3 and Tde were the fastest (7 days), followed by the other fermentations with MP-A, which finished a day later (Fig. 2A). Compared with the control, these fermentations were performed 3 and 2 days earlier, respectively. When the MP-B extracts were added to white wine, MLF finished one day faster than in the control in all the conditions (Fig. 2A).

In the case of red wines, all the MLFs produced with MP-A extracts finished at least 4 days earlier than the control did (Fig. 2B). Notably, the addition of MP-A extracts from Sc3 allowed the completion of MLF in 13 days, 6 days before the control. The trend with MP-B extracts was similar (Fig. 2B). In this case, all the MLFs finished 2–5 days before the control fermentation, with MP-B from Lth being the fastest MLF (13 days).

In summary, the addition of MP extracts played a positive role shortening MLF both in white and red wines. With these wines it was not observed the negative effect detected in synthetic WLM of extracts obtained by the alkaline treatment. The lower concentration of MPs added to grape wines (0.5 g/L) may have limited the negative impact of possible inhibitors from the mannoprotein extraction procedure. Notably, however, in grape wines, shorter MLFs were observed with the addition of extracts from autolysis (MP-A) than with the addition of extracts from alkali treatment (MP-B). Taken together, the studies in WLM and wine indicate that induced autolysis generates mannoprotein extracts with a more beneficial composition for *O. oeni* than those obtained by alkaline extraction. Moreover, the added concentration of MPs in the assays with white and red wine was very close to the recommended concentrations for oenological purposes (protein and tartaric stabilisation, up to 0.4 g/L). Therefore, the applicability of the addition of mannoprotein extracts to enhance MLF in wine seems to be feasible.

3.3. Mannoprotein consumption

3.3.1. Consumption of mannoproteins in WLM

With the addition of 2 g/L MP-A or MP-B extracts, the consumption

of mannoproteins was greater than 50% (Fig. 3). There was complete consumption of mannoproteins in all the MLFs with the addition of *S. cerevisiae* extracts, except for fermentation with the addition of the Sc4 extract, where mannoprotein consumption was partial. With respect to the fermentations supplemented with extracts from non-*Saccharomyces* strains, the mannoprotein consumption was also partial. There was total exhaustion of mannoproteins only with the MP-B extract from the Lth strain.

It can be concluded that *O. oeni* PSU-1 can metabolise very different mannoprotein extracts. The addition of the MPs in WLM had a positive effect on MLF in most of the cases, except in some of the fermentations with the extract obtained from alkaline treatment (MP-B). However, as discussed previously, the inhibitory effect of NaCl accumulation from this type of extract may have counteracted the positive effect of mannoprotein consumption on *O. oeni* PSU-1 performance.

3.3.2. Consumption of mannoproteins in white and red wine

Mannoprotein consumption was also observed in the control fermentations without the addition of MPs (data not shown). These wines contain mannoproteins released during alcoholic fermentation (AF), with concentrations of 150 mg/L in white wine and 100 mg/L in red wines, with consumption rates of 39% and 15%, respectively (Fig. 4).

In summary, *O. oeni* PSU-1 has the capacity to metabolise mannoproteins with different characteristics in both white and red wines. Even if mannoprotein consumption was lower in grape wines than in synthetic WLM, the impact on MLF was more evident in white and red wines since all MLFs were shorter due to the addition of MPs, independent of the yeast species and extraction method used. These results reinforce the potential oenological applicability of extracts enriched with mannoproteins as MLF activators.

3.4. Chemical composition of wines after MLF

3.4.1. Changes in composition in WLM

No significant differences were observed due to the addition of both types of extracts at the end of MLF in the contents of nitrogen compounds, such as inorganic nitrogen (NH_4^+) and organic nitrogen (NOPA) (Table 3). Citric acid was consumed to a greater extent in the presence of the MPs obtained by both methods than in the control. However, in the wines in which MLF was arrested (MP-B: Sc1, Sc2, Sc4, Tde, and Mpu) no citric acid consumption was observed due to the absence of *O. oeni* metabolic activity. Regarding D-lactic acid, the concentration was slightly greater at the end of MLF in the presence of the extracts. This

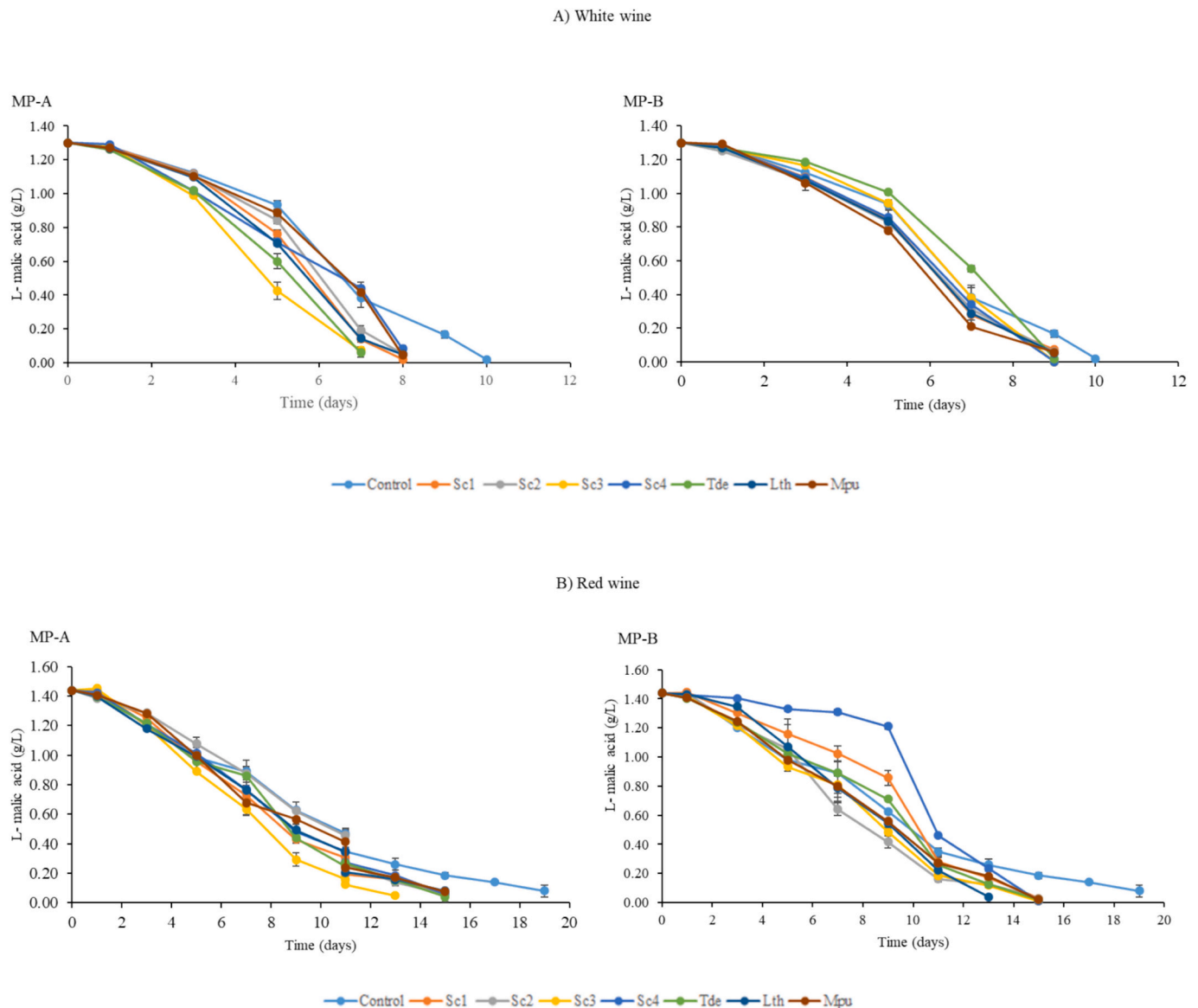


Fig. 2. Evolution of malolactic fermentation with different MP from Sc1, Sc2, Sc3, Sc4 (*S. cerevisiae*) Tde (*T. delbrueckii*), Lth (*L. thermotolerans*) and Mpu (*M. pulcherrima*) in white wine (A) and red wine (B) fermented with *O. oeni* strain PSU-1 with the addition of 0.5 g/L of extracts from induced autolysis (MP-A) and alkaline extraction (MP-B). All data are expressed as the arithmetic average of three biological replicates $\pm$ standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

could be associated with the consumption of mannose (released from mannoproteins) by heterolactic fermentation. However, the differences in the concentrations of D-lactic acid are less than 0.1 g/L, which does not explain the utilisation of mannose from mannoproteins. Moreover, an increase in acetic acid would be expected from the heterolactic fermentation of sugars, which was not the case (Table 3).

As expected, the consumption of L-malic acid was correlated with an increase in L-lactic acid, which was lower when fermentation did not occur (Table 3).

3.4.2. Changes in the composition of white and red wines

In both white and red wines (Tables 4 and 5), there were no significant differences observed in the concentrations of inorganic nitrogen (NH_4^+) or D- or L-lactic acid. However, NOPA was significantly greater in the fermentations with the added extracts from the Sc strains than in the control, possibly due to the release of the protein portion of mannoprotein in white wines. In contrast, fermentations with non-*Saccharomyces* cell walls presented lower concentrations. Towards the end of

fermentation, D-lactic acid and acetic acid levels increased in wines where yeast cell walls were added, which could be related to the amounts of sugars they consumed. Moreover, the concentration of G/F increased in wines with non-*Saccharomyces* cell walls, potentially because of the retained portion of this analyte. Citric acid consumption varied depending on the added strain and the mannoprotein extract. Although the differences were significant in many cases (Tables 4 and 5), the variation did not exceed 50 mg/L, and these differences were not reflected in the increase in metabolites from citric acid, such as acetate.

In the different media studied, the final pH remained unaffected by the addition of MPs in all cases, increasing from 0.2 to 0.3.

Taken together, the changes in the composition of the metabolites analysed provide little information on the use of mannoproteins by *O. oeni*. On one hand, mannose (released from consumed mannoproteins) can be transformed into 6-P-fructose by *O. oeni* and then to pyruvate, which can be redirected to different catabolic and anabolic pathways depending on the needs of the cell. On the other hand, the observed mannoprotein consumption could also be associated with a

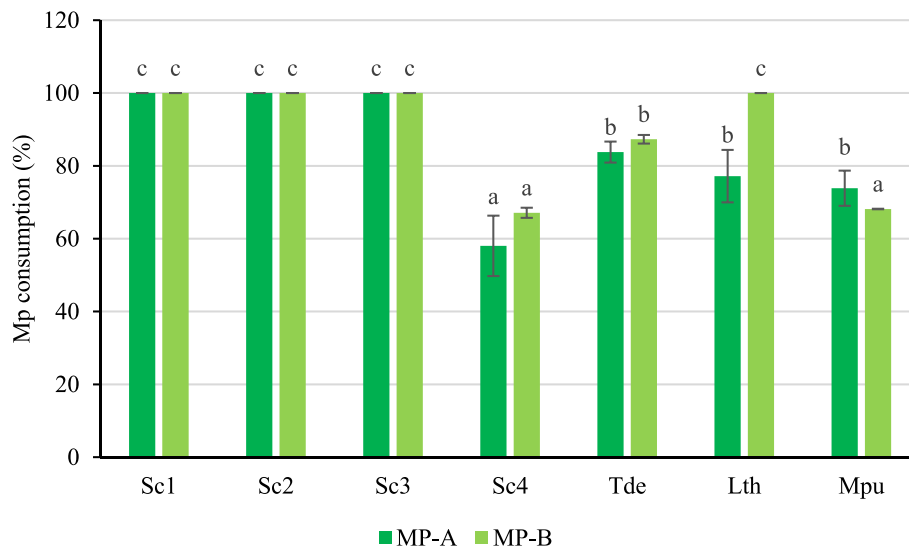


Fig. 3. Consumption of mannoproteins (%) by *O. oeni* PSU-1 at the end of MLF in wine-like medium (WLM) with 2 g/L of the different MP from Sc1, Sc2, Sc3, Sc4 (*S. cerevisiae*) Tde (*T. delbrueckii*), Lth (*L. thermotolerans*) and Mpu (*M. pulcherrima*) obtained by A) induced autolysis (MP-A) and B) by alkaline extraction (MP-B). Different lowercase letters indicate significant differences between values for the MP for each extraction method using the Tukey (HSD) test at $p < 0.05$. All data are expressed as the arithmetic average of three biological replicates $\pm$ standard deviation.

phenomenon of coprecipitation with other compounds, such as yeast medium chain fatty acids that are inhibitory for MLF, so that mannoproteins would perform a detoxifying function, as described by some authors (Guilloux-Benatier et al., 2001). The activating effect of mannoprotein extracts on MLF seems to be unequivocal. In a previous work (data in the process of publication), the use of amino acids from yeast mannoprotein extracts was evaluated, and no differences were observed that could correlate the use of the protein portion of mannoproteins with MLF improvement. Therefore, the polysaccharide fraction of mannoproteins seems to be metabolised mainly by *O. oeni*, with the possible detoxifying role of these molecules also to be considered. Mannose oligosaccharides (MOSs) promote the growth of some probiotic LAB species. As prebiotics, MOSs can increase the population of probiotic microorganisms, such as bifidobacteria and lactobacilli, and support the in vitro growth of probiotics, such as *Lactiplantibacillus plantarum* (Zhang et al., 2021). According to a proteomic study of our research group (Torano et al., 2025b), mannoproteins activate carbohydrate transport and metabolism in *O. oeni*, with some proteins associated with this metabolic pathway undergoing significant changes upon the addition of these extracts. The same study showed the higher abundance of proteins that had been associated to mannan recognition.

The positive effects of mannoproteins on MLF observed in this study could also be present in fermentations where non-*Saccharomyces* yeasts are used alongside *S. cerevisiae*. In these scenarios, a greater amount of mannoproteins are released during AF (Balmaseda et al., 2018, 2021; Ruiz-de-Villa et al., 2023a, Ruiz-de-Villa et al., 2023b). This finding reinforces the idea that mannoproteins could be key factors in yeast–bacteria interactions in wine.

3.5. Effect of mannoprotein addition on wine colour

Mannoproteins increase the colour stability of red wines by adsorbing phenolic compounds such as anthocyanins and tannins, protecting them from degradation and oxidation. This interaction helps maintain the colour intensity and tone of wine, stabilizes its structure, and prevents the precipitation of coloured matter (Alcalde-Eon et al., 2019; Poncet-LeGrand et al., 2007; Guadalupe et al., 2007; Guadalupe and Ayestarán, 2008; Snyman et al., 2023). In this work, colour parameters were analysed in red wines to evaluate the addition of mannoprotein extracts.

The CIELab coordinates L^* (Lightness) and C^* (Chroma) provided quantitative information about colour characteristics. No significant differences were observed when MP-A or MP-B extracts were added to either of the two parameters. In terms of colour intensity (CI), which is typically negatively correlated with L^* , no significant differences were detected. Thus, the colour remained stable at the end of MLF without any effect due to the addition of 0.5 g/L MPs. When considering the H^* coordinate (hue) of CIELab, which is related to qualitative aspects of colour and is calculated as the ratio of the absorbances obtained at 420 and 520 nm ($A_{420/520}$), no significant differences were observed. However, MLF with extracts maintained the same hue compared to the control condition in accordance with previous studies that described no changes in colour associated with higher mannoprotein concentrations (Olguín et al., 2020; Rodrigues et al., 2012).

With respect to the total polyphenol index and the ethanol index, no significant differences were found between the conditions. Thus, it could be concluded that during MLF, the added mannoproteins did not form more complexes than those formed under the control conditions did, and the total polyphenol content of the wine remained unchanged (Nguela et al., 2023). For the tannins analysed using the methyl cellulose method, the ethanol index did not significantly differ from that of the control. Therefore, the addition of mannoproteins did not alter their composition or increase their binding with polysaccharides (Fig. S3).

However, significant differences were observed in the concentration of total anthocyanins, where the control conditions and fermentation with the Sc1 and Sc2 extracts resulted in lower concentrations than fermentation with the Sc3, Sc4, and non-*Saccharomyces* extracts. Acylated and nonacylated anthocyanins presented relatively high concentrations in the control and various *S. cerevisiae* strains and relatively low concentrations in the fermentations with non-*Saccharomyces* extracts. A- and B-type vitisins, as well as the studied pyranoanthocyanins, presented higher values in the control samples than in all the other conditions. These results could be explained by binding to the protein portion of the mannoprotein (Wu et al., 2015) (Table S1).

4. Conclusions

Considerable differences have been observed in the mannoproteins extracted from ADY depending on the yeast used, especially depending on the extraction method. All the extracts had beneficial effects on MLF,

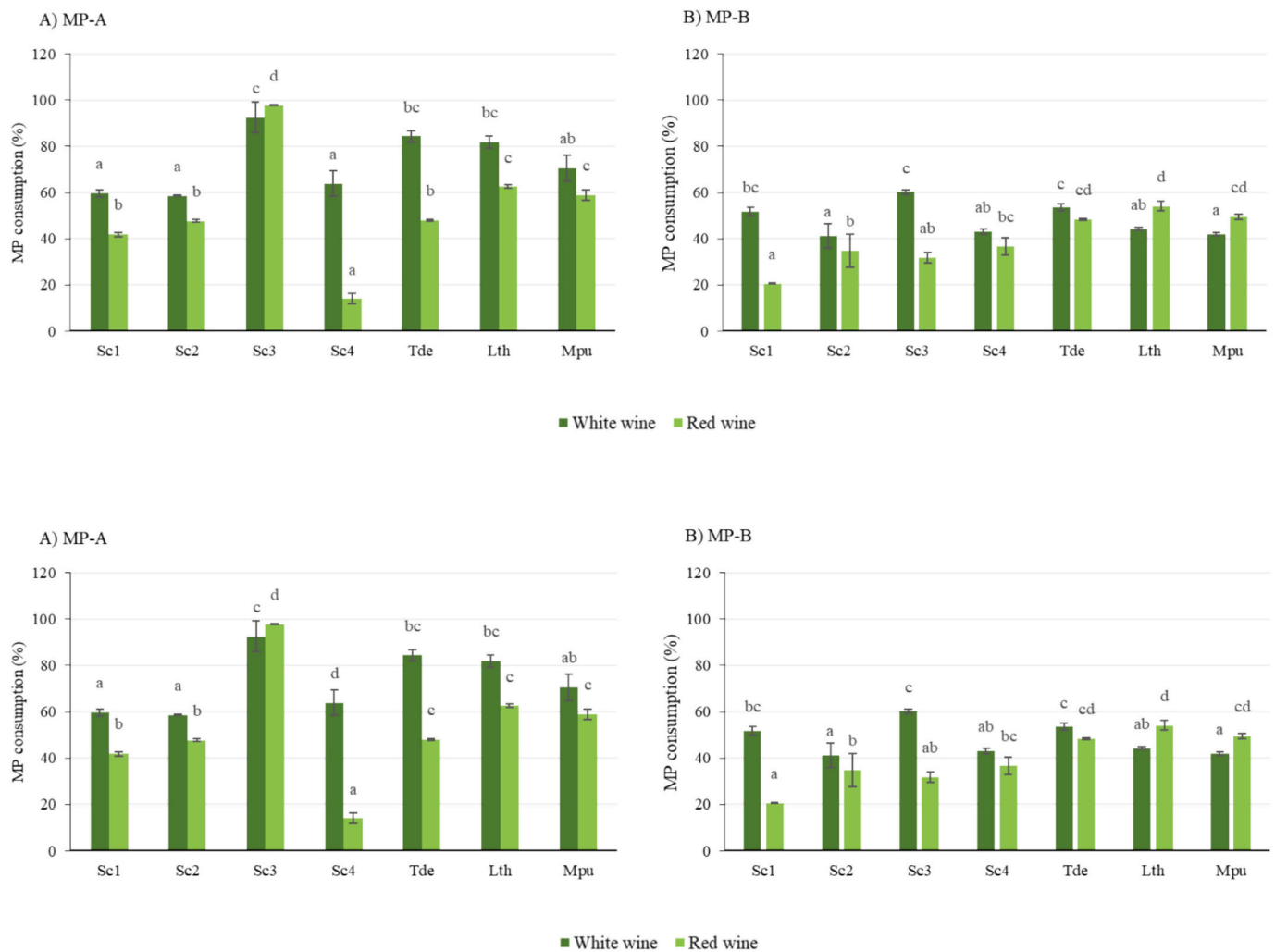


Fig. 4. Consumption of mannoproteins (%) by *O. oeni* PSU-1 at the end of MLF in white (dark green colour) and red wines (light green) with the addition of 0.5 g/L of the different MP from Sc1, Sc2, Sc3, Sc4 (*S. cerevisiae*) Tde (*T. delbrueckii*), Lth (*L. thermotolerans*) and Mpu (*M. pulcherrima*) obtained by induced autolysis (MP-A) and by alkaline extraction (MP-B). Different lowercase letters indicate a significant difference between the different values for the MP for white and red wine using the Tukey (HSD) test at $p < 0.05$. All data are expressed as the arithmetic average of three biological replicates $\pm$ standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3

Chemical composition of wine-like medium (WLM) after MLF with the addition of MP-A (induced autolysis) and MP-B (alkaline extraction) in a concentration of 0.5 g/L for *O. oeni* PSU-1 strain with MP from Sc1, Sc2, Sc3, Sc4, Tde, Lth and Mpu. Average $\pm$ standard deviation ($n = 3$). Different lowercase letters indicate a significant difference between Mps from Sc1, Sc2, Sc3, Sc4 (*S. cerevisiae*) Tde (*T. delbrueckii*), Lth (*L. thermotolerans*) and Mpu (*M. pulcherrima*) cell wall. Glu/Fru: Glucose + Fructose. NOPA: Primary Amino Nitrogen. *: Unfinished fermentations.

	MP	Glu/Fru (g/L)	Acetic acid (g/L)	D- Lactic (g/L)	L- Lactic (g/L)	Citric acid (mg/L)	NH ₄ ⁺ (mg/L)	NOPA (mg/L)
MP-A	Control	0.42 $\pm$ 0.02 ^a	0.65 $\pm$ 0.03 ^{ab}	0.10 $\pm$ 0.02 ^a	1.73 $\pm$ 0.01 ^a	257.50 $\pm$ 2.12 ^f	28.33 $\pm$ 4.04 ^a	133.50 $\pm$ 2.12 ^a
	Sc1	0.45 $\pm$ 0.03 ^{ab}	0.67 $\pm$ 0.02 ^{ab}	0.14 $\pm$ 0.01 ^b	1.74 $\pm$ 0.01 ^a	213.33 $\pm$ 11.59 ^{de}	22.00 $\pm$ 1.00 ^a	135.67 $\pm$ 5.13 ^a
	Sc2	0.43 $\pm$ 0.02 ^a	0.66 $\pm$ 0.02 ^{ab}	0.13 $\pm$ 0.01 ^b	1.76 $\pm$ 0.01 ^{ab}	228.00 $\pm$ 18.08 ^e	23.33 $\pm$ 2.08 ^a	137.00 $\pm$ 6.08 ^a
	Sc3	0.42 $\pm$ 0.01 ^a	0.62 $\pm$ 0.02 ^a	0.13 $\pm$ 0.02 ^b	1.75 $\pm$ 0.01 ^{ab}	199.67 $\pm$ 5.13 ^{cd}	22.00 $\pm$ 1.73 ^a	139.00 $\pm$ 2.31 ^a
	Sc4	0.46 $\pm$ 0.04 ^{ab}	0.64 $\pm$ 0.03 ^{ab}	0.14 $\pm$ 0.01 ^b	1.79 $\pm$ 0.01 ^{bc}	137.00 $\pm$ 7.07 ^a	23.67 $\pm$ 3.06 ^a	129.50 $\pm$ 2.12 ^a
	Tde	0.48 $\pm$ 0.02 ^{ab}	0.65 $\pm$ 0.03 ^{ab}	0.14 $\pm$ 0.01 ^b	1.81 $\pm$ 0.01 ^{cd}	157.50 $\pm$ 0.71 ^{ab}	23.67 $\pm$ 4.62 ^a	128.00 $\pm$ 5.66 ^a
	Lth	0.52 $\pm$ 0.02 ^b	0.70 $\pm$ 0.03 ^b	0.15 $\pm$ 0.01 ^b	1.82 $\pm$ 0.02 ^d	140.33 $\pm$ 5.51 ^a	25.00 $\pm$ 2.00 ^a	143.33 $\pm$ 4.93 ^a
	Mpu	0.47 $\pm$ 0.04 ^{ab}	0.70 $\pm$ 0.04 ^b	0.14 $\pm$ 0.01 ^b	1.84 $\pm$ 0.01 ^d	173.50 $\pm$ 4.95 ^{bc}	25.33 $\pm$ 2.52 ^a	141.00 $\pm$ 2.65 ^a
MP-B	Control	0.42 $\pm$ 0.02 ^{bc}	0.65 $\pm$ 0.03 ^a	0.10 $\pm$ 0.02 ^c	1.73 $\pm$ 0.01 ^a	257.50 $\pm$ 2.12 ^c	28.33 $\pm$ 4.04 ^a	133.50 $\pm$ 2.12 ^a
	Sc1	0.35 $\pm$ 0.03 ^{ab}	0.83 $\pm$ 0.03 ^b	0.03 $\pm$ 0.01 ^a	0.62 $\pm$ 0.01 [*]	512.00 $\pm$ 4.90 ^{fg}	27.60 $\pm$ 0.58 ^a	139.50 $\pm$ 0.71 ^a
	Sc2	0.33 $\pm$ 0.02 ^a	0.81 $\pm$ 0.02 ^b	0.03 $\pm$ 0.05 ^a	0.62 $\pm$ 0.02 [*]	508.56 $\pm$ 2.08 ^{ef}	25.52 $\pm$ 1.00 ^a	139.33 $\pm$ 1.50 ^a
	Sc3	0.45 $\pm$ 0.01 ^c	0.76 $\pm$ 0.04 ^b	0.14 $\pm$ 0.02 ^d	1.81 $\pm$ 0.01 ^b	137.58 $\pm$ 4.93 ^a	28.00 $\pm$ 1.00 ^a	140.33 $\pm$ 0.58 ^a
	Sc4	0.35 $\pm$ 0.03 ^{ab}	0.80 $\pm$ 0.03 ^b	0.06 $\pm$ 0.02 ^b	0.63 $\pm$ 0.02 [*]	498.63 $\pm$ 3.32 ^{de}	29.00 $\pm$ 1.00 ^a	140.00 $\pm$ 1.00 ^a
	Tde	0.46 $\pm$ 0.03 ^c	0.77 $\pm$ 0.04 ^a	0.05 $\pm$ 0.03 ^b	0.58 $\pm$ 0.02 [*]	530.50 $\pm$ 4.63 ^g	28.67 $\pm$ 1.53 ^a	141.67 $\pm$ 1.53 ^a
	Lth	0.48 $\pm$ 0.01 ^c	0.79 $\pm$ 0.03 ^b	0.14 $\pm$ 0.06 ^d	1.80 $\pm$ 0.01 ^b	206.00 $\pm$ 5.69 ^b	29.67 $\pm$ 2.08 ^a	146.33 $\pm$ 2.51 ^a
	Mpu	0.34 $\pm$ 0.05 ^a	0.81 $\pm$ 0.02 ^b	0.07 $\pm$ 0.08 ^b	0.85 $\pm$ 0.03 [*]	480.67 $\pm$ 5.03 ^d	28.33 $\pm$ 1.53 ^a	141.02 $\pm$ 1.73 ^a

except for the extract obtained through alkaline treatment in WLM,

which was probably due to the accumulation of possible inhibitors from

Table 4

Chemical composition of white wine after MLF with the addition of MP-A (induced autolysis) and MP-B (alkaline extraction) in a concentration of 0.5 g/L for *O. oeni* PSU-1 strain with MP from Sc1, Sc2, Sc3, Sc4 (*S. cerevisiae*) Tde (*T. delbrueckii*), Lth (*L. thermotolerans*) and Mpu (*M. pulcherrima*). Average $\pm$ standard deviation ($n = 3$). Different lowercase letters indicate a significant difference between MP extracts. Glu/Fru: Glucose + Fructose. NOPA: Primary Amino Nitrogen.

	MP	Glu/Fru (g/L)	Acetic acid (g/L)	D- Lactic (g/L)	L- Lactic (g/L)	Citric acid (mg/L)	NH ₄ ⁺ (mg/L)	NOPA (mg/L)
MP- A	Control	5.75 $\pm$ 0.15 ^a	0.43 $\pm$ 0.01 ^a	0.12 $\pm$ 0.02 ^a	1.11 $\pm$ 0.02 ^a	251.67 $\pm$ 6.11 ^d	8 $\pm$ 3.06 ^{ab}	93 $\pm$ 1.53 ^b
	Sc1	5.96 $\pm$ 0.22 ^a	0.45 $\pm$ 0.02 ^a	0.21 $\pm$ 0.03 ^b	1.14 $\pm$ 0.02 ^a	223.33 $\pm$ 5.03 ^{bc}	10 $\pm$ 0.58 ^b	226 $\pm$ 3.51 ^d
	Sc2	6.04 $\pm$ 0.18 ^a	0.43 $\pm$ 0.01 ^a	0.16 $\pm$ 0.03 ^{ab}	1.19 $\pm$ 0.04 ^a	274.00 $\pm$ 6.00 ^e	9 $\pm$ 1.00 ^{ab}	246 $\pm$ 6.11 ^e
	Sc3	5.88 $\pm$ 0.44 ^a	0.43 $\pm$ 0.03 ^a	0.19 $\pm$ 0.04 ^{ab}	1.16 $\pm$ 0.04 ^a	205.00 $\pm$ 7.55 ^a	4 $\pm$ 0.58 ^a	317 $\pm$ 8.50 ^f
	Sc4	6.06 $\pm$ 0.26 ^a	0.45 $\pm$ 0.02 ^a	0.16 $\pm$ 0.01 ^{ab}	1.14 $\pm$ 0.08 ^a	220.00 $\pm$ 6.56 ^{ab}	9 $\pm$ 1.53 ^{ab}	174 $\pm$ 4.36 ^c
	Tde	6.12 $\pm$ 0.16 ^a	0.42 $\pm$ 0.01 ^a	0.16 $\pm$ 0.03 ^{ab}	1.22 $\pm$ 0.07 ^a	234.33 $\pm$ 8.74 ^{bcd}	8 $\pm$ 2.00 ^{ab}	60 $\pm$ 3.51 ^a
	Lth	4.76 $\pm$ 1.47 ^a	0.42 $\pm$ 0.02 ^a	0.15 $\pm$ 0.03 ^b	1.15 $\pm$ 0.06 ^a	239.00 $\pm$ 2.65 ^{cd}	5 $\pm$ 0.58 ^{ab}	91 $\pm$ 7.02 ^b
	Mpu	6.06 $\pm$ 0.26 ^a	0.42 $\pm$ 0.02 ^a	0.14 $\pm$ 0.03 ^b	1.18 $\pm$ 0.08 ^a	276.67 $\pm$ 6.03 ^e	5 $\pm$ 1.00 ^{ab}	78 $\pm$ 6.43 ^b
MP- B	Control	5.75 $\pm$ 0.15 ^a	0.43 $\pm$ 0.01 ^a	0.12 $\pm$ 0.02 ^a	1.11 $\pm$ 0.02 ^a	251.67 $\pm$ 6.11 ^{ab}	8 $\pm$ 3.06 ^a	93 $\pm$ 1.53 ^b
	Sc1	5.62 $\pm$ 0.12 ^a	0.39 $\pm$ 0.08 ^a	0.27 $\pm$ 0.09 ^b	1.18 $\pm$ 0.04 ^{ab}	257.00 $\pm$ 2.65 ^b	9 $\pm$ 0.58 ^a	230 $\pm$ 15.28 ^d
	Sc2	5.84 $\pm$ 0.23 ^a	0.43 $\pm$ 0.03 ^a	0.20 $\pm$ 0.03 ^{ab}	1.15 $\pm$ 0.03 ^{ab}	267.00 $\pm$ 7.00 ^{bc}	10 $\pm$ 1.53 ^a	277 $\pm$ 3.61 ^e
	Sc3	5.83 $\pm$ 0.16 ^a	0.42 $\pm$ 0.01 ^a	0.15 $\pm$ 0.02 ^{ab}	1.17 $\pm$ 0.03 ^{ab}	260.67 $\pm$ 8.02 ^b	9 $\pm$ 3.77 ^a	268 $\pm$ 4.51 ^e
	Sc4	5.92 $\pm$ 0.15 ^a	0.42 $\pm$ 0.02 ^a	0.19 $\pm$ 0.02 ^{ab}	1.19 $\pm$ 0.08 ^{ab}	252.00 $\pm$ 7.00 ^{ab}	8 $\pm$ 0.58 ^a	120 $\pm$ 4.36 ^c
	Tde	5.88 $\pm$ 0.26 ^a	0.43 $\pm$ 0.02 ^a	0.17 $\pm$ 0.02 ^{ab}	1.25 $\pm$ 0.02 ^b	282.00 $\pm$ 1.00 ^c	5 $\pm$ 1.06 ^a	58 $\pm$ 2.65 ^a
	Lth	5.83 $\pm$ 0.16 ^a	0.43 $\pm$ 0.01 ^a	0.16 $\pm$ 0.03 ^{ab}	1.21 $\pm$ 0.05 ^{ab}	240.00 $\pm$ 5.57 ^a	7 $\pm$ 3.00 ^a	86 $\pm$ 1.53 ^b
	Mpu	5.95 $\pm$ 0.13 ^a	0.43 $\pm$ 0.01 ^a	0.15 $\pm$ 0.02 ^{ab}	1.25 $\pm$ 0.01 ^b	253.67 $\pm$ 3.06 ^{ab}	7 $\pm$ 2.52 ^a	93 $\pm$ 4.36 ^b

Table 5

Chemical composition of red wine after MLF with the addition of MP-A (induced autolysis) and MP-B (alkaline extraction) in a concentration of 0.5 g/L for *O. oeni* PSU-1 strain with MP from Sc1, Sc2, Sc3, Sc4 (*S. cerevisiae*) Tde (*T. delbrueckii*), Lth (*L. thermotolerans*) and Mpu (*M. pulcherrima*). Average $\pm$ standard deviation ($n = 3$). Different lowercase letters indicate a significant difference between MP. Glu/Fru: Glucose + Fructose. NOPA: Primary Amino Nitrogen.

	MP	Glu/Fru (g/L)	Acetic acid (g/L)	D- Lactic acid (g/L)	L- Lactic acid (g/L)	Citric acid (mg/L)	NH ₄ ⁺ (mg/L)	NOPA (mg/L)
MP- A	Control	0.03 $\pm$ 0.01 ^a	0.38 $\pm$ 0.01 ^a	0.18 $\pm$ 0.02 ^a	1.26 $\pm$ 0.01 ^a	164.67 $\pm$ 9.50 ^{cd}	39 $\pm$ 4.36 ^a	40 $\pm$ 4.51 ^a
	Sc1	0.05 $\pm$ 0.02 ^a	0.40 $\pm$ 0.01 ^{ab}	0.25 $\pm$ 0.02 ^{bc}	1.27 $\pm$ 0.02 ^a	206.50 $\pm$ 11.53 ^e	45 $\pm$ 3.06 ^a	48 $\pm$ 6.43 ^a
	Sc2	0.05 $\pm$ 0.02 ^a	0.40 $\pm$ 0.02 ^a	0.22 $\pm$ 0.01 ^b	1.21 $\pm$ 0.03 ^a	205.00 $\pm$ 2.00 ^e	39 $\pm$ 7.37 ^a	43 $\pm$ 6.24 ^a
	Sc3	0.03 $\pm$ 0.02 ^a	0.41 $\pm$ 0.01 ^{abc}	0.23 $\pm$ 0.01 ^{bc}	1.21 $\pm$ 0.02 ^a	163.67 $\pm$ 5.86 ^{cd}	41 $\pm$ 1.73 ^a	38 $\pm$ 1.15 ^a
	Sc4	0.15 $\pm$ 0.02 ^{bc}	0.43 $\pm$ 0.06 ^{abc}	0.22 $\pm$ 0.02 ^{bc}	1.18 $\pm$ 0.05 ^a	168.00 $\pm$ 5.57 ^{cd}	41 $\pm$ 4.36 ^a	47 $\pm$ 4.73 ^a
	Tde	0.16 $\pm$ 0.05 ^{bc}	0.47 $\pm$ 0.01 ^{abc}	0.25 $\pm$ 0.02 ^{bc}	1.16 $\pm$ 0.01 ^a	124.67 $\pm$ 4.04 ^a	45 $\pm$ 3.06 ^a	45 $\pm$ 4.65 ^a
	Lth	0.20 $\pm$ 0.04 ^c	0.48 $\pm$ 0.02 ^c	0.26 $\pm$ 0.02 ^{bc}	1.18 $\pm$ 0.01 ^a	144.33 $\pm$ 5.51 ^b	39 $\pm$ 4.00 ^a	47 $\pm$ 1.53 ^a
	Mpu	0.07 $\pm$ 0.06 ^{ab}	0.45 $\pm$ 0.03 ^{abc}	0.26 $\pm$ 0.01 ^c	1.17 $\pm$ 0.02 ^a	182.67 $\pm$ 3.06 ^d	45 $\pm$ 1.53 ^a	37 $\pm$ 1.00 ^a
MP- B	Control	0.03 $\pm$ 0.01 ^a	0.38 $\pm$ 0.01 ^a	0.18 $\pm$ 0.02 ^a	1.26 $\pm$ 0.01 ^a	164.67 $\pm$ 9.5 ^c	39 $\pm$ 4.36 ^a	40 $\pm$ 4.51 ^a
	Sc1	0.03 $\pm$ 0.01 ^a	0.40 $\pm$ 0.02 ^a	0.24 $\pm$ 0.01 ^b	1.24 $\pm$ 0.02 ^a	158.33 $\pm$ 5.03 ^{bc}	38 $\pm$ 10.02 ^a	33 $\pm$ 1.15 ^a
	Sc2	0.09 $\pm$ 0.02 ^{bc}	0.39 $\pm$ 0.01 ^a	0.23 $\pm$ 0.03 ^{ab}	1.19 $\pm$ 0.01 ^a	194.67 $\pm$ 1.53 ^d	42 $\pm$ 4.04 ^a	42 $\pm$ 13.43 ^a
	Sc3	0.06 $\pm$ 0.01 ^{ab}	0.40 $\pm$ 0.01 ^a	0.25 $\pm$ 0.04 ^b	1.21 $\pm$ 0.15 ^a	167.00 $\pm$ 2.00 ^c	41 $\pm$ 3.00 ^a	46 $\pm$ 9.07 ^a
	Sc4	0.14 $\pm$ 0.03 ^c	0.50 $\pm$ 0.05 ^b	0.25 $\pm$ 0.02 ^b	1.22 $\pm$ 0.03 ^a	109.67 $\pm$ 5.51 ^a	40 $\pm$ 7.37 ^a	51 $\pm$ 7.94 ^a
	Tde	0.23 $\pm$ 0.04 ^d	0.52 $\pm$ 0.01 ^b	0.26 $\pm$ 0.02 ^d	1.22 $\pm$ 0.03 ^a	109.00 $\pm$ 5.00 ^a	41 $\pm$ 5.51 ^a	44 $\pm$ 7.64 ^a
	Lth	0.12 $\pm$ 0.01 ^{bc}	0.49 $\pm$ 0.01 ^b	0.22 $\pm$ 0.03 ^{ab}	1.23 $\pm$ 0.01 ^a	147.00 $\pm$ 10.15 ^b	41 $\pm$ 2.00 ^a	38 $\pm$ 2.08 ^a
	Mpu	0.08 $\pm$ 0.03 ^{abc}	0.42 $\pm$ 0.01 ^a	0.22 $\pm$ 0.01 ^{ab}	1.21 $\pm$ 0.04 ^a	199.00 $\pm$ 1.00 ^d	39 $\pm$ 3.21 ^a	40 $\pm$ 3.21 ^a

the extraction procedure in MPs extracts.

Overall, the extracts obtained through autolysis appear to favour MLF in more cases. However, it is important to note that in wines enriched with 0.5 g/L extract, all the extracts had positive effects. This highlights their potential as effective activators of MLF. Nevertheless, further investigations are needed to understand the metabolism of mannoproteins by *O. oeni* and the molecular mechanisms underlying the activation of MLF. The possible detoxifying effect of mannoproteins cannot be ruled out either.

In conclusion, this work provides new insights into the role of mannoproteins in wine, which, whether added or naturally present in wine, can have a positive effect on *O. oeni*, which often faces challenges in its development.

CRedit authorship contribution statement

Paloma Torano: Writing – original draft, Investigation, Formal analysis, Data curation. **María Oyón-Ardoiz:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Elvira Manjón:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Ignacio García-Estévez:** Writing – review & editing, Supervision, Investigation, Formal analysis, Data curation. **Albert Bordon:** Writing – review & editing. **Nicolas Rozès:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization. **M. Teresa Escribano-Bailón:** Writing – review & editing,

Supervision, Resources, Formal analysis, Data curation. **Cristina Reguant:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijfoodmicro.2026.111755>.

Data availability

No data was used for the research described in the article.

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