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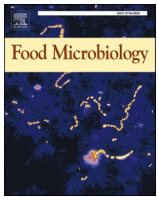

Enhancing wine malolactic fermentation: Variable effect of yeast mannoproteins on *Oenococcus oeni* strains

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ABSTRACT

Lactic acid bacteria (LAB), principally *Oenococcus oeni*, play crucial roles in wine production, contributing to the transformation of L-malic acid into L-lactic acid during malolactic fermentation (MLF). This fermentation is influenced by different factors, including the initial LAB population and wine stress factors, such as nutrient availability. Yeast mannoproteins can enhance LAB survival in wine. This study explored in model conditions the impact of a commercial mannoprotein extract on MLF dynamics in ten *O. oeni* strains. The results revealed strain-specific responses in fermentation kinetics and mannoprotein utilization. Mannoprotein addition influenced MLF outcomes, depending on the strain and concentration. The variability in MLF confirmed different technological aptitude of the strains used. The α-mannosidase enzymatic activity was determined and showed higher values in the supernatant than in whole cells. Moreover, α-mannosidase activity varied among strains, suggesting differential regulation in response to fermentation conditions. These findings highlight the importance of understanding mannoprotein interactions with *O. oeni* for optimizing MLF efficiency and enhancing wine quality. Further research under cellar conditions is needed to evaluate the potential of yeast mannoproteins to promote MLF.

1. Introduction

Lactic acid bacteria (LAB) naturally occur on grape surfaces and can persist during the initial stages of alcoholic fermentation (AF) (Bae et al., 2006; Barata et al., 2012). However, their population is relatively low, diminishing as the ethanol concentration increases and sulfur dioxide (SO_2) is introduced, except for *Oenococcus oeni*, a species that is well suited to wine conditions. *O. oeni* is the LAB main responsible for malolactic fermentation (MLF) in wine, that consists in the transformation of L-malic acid into L-lactic acid and CO_2. This process can be beneficial for certain wines, such as red and highly acidic white wines, due to the produced deacidification and the improvement of the microbiological stability and the aromatic profile (Bartowsky, 2005; Ribéreau-Gayon et al., 2006). Managing MLF under the demanding conditions of winemaking can be unpredictable, generally requiring a minimum LAB population of 10^6 cells/mL to initiate the process (Lonvaud-Funel, 1999).

LAB diversity decreases during AF due to specific nutritional requirements and various stresses (Bae et al., 2006; Terrade and Mira de Orduña, 2009). Nevertheless, certain species endure and proliferate, leading to the spontaneous occurrence of MLF. *O. oeni* is the predominant LAB species in wine. The wide intraspecific variability in the metabolic capacities of *O. oeni* strains has been demonstrated and may have a relevant impact on wine quality (Capozzi et al., 2021).

As MLF typically occurs after AF, the interactions between yeasts and *O. oeni* significantly influence the progression of this secondary fermentation (Balmaseda et al., 2018). To achieve these two fermentative processes, starter cultures of the species best adapted to each fermentation, namely, *Saccharomyces cerevisiae* and *O. oeni* for AF and MLF, respectively, can be inoculated (Paramithiotis et al., 2022).

Mannoproteins, consisting of 80%–90% mannose polysaccharides and approximately 5%–20% protein , are glycoproteins that are naturally present in the cell walls of yeast. They are typically released either at the conclusion of alcoholic fermentation or during ageing on lees. These substances have the potential to enhance the physical, chemical, organoleptic, and sensory attributes of the final product (Guadalupe

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et al., 2007; Li et al., 2018).

O. oeni glycosidase, mannosidase and peptidase activities may enable the release of sugars and amino acids from mannoproteins and other macromolecules, thereby increasing the nutritional content and survival of *O. oeni* in wine (Jamal et al., 2013). Mannose may be a phosphotransferase system (PTS) substrate and thus be involved in stimulating *O. oeni* growth in the presence of yeast mannoproteins or yeast extracts. The phosphoenolpyruvate (PEP) and PTS systems have been described as the most relevant systems for *O. oeni* sugar metabolism. Specifically, the PTS plays a significant role because it consists of integral membrane proteins capable of translocating substrates through the membrane via a phosphorylation cascade (Cibrario et al., 2016). In addition to regulate the metabolism of some polysaccharides, it also participates in stress responses and allows rapid sugar uptake from the environment without the need for a concentration gradient (Jamal et al., 2013).

The α-mannosidase activity is responsible for the hydrolysis of glycosidic bonds in the α-1,2-mannosidic portion of oligosaccharides. This enzyme is crucial for the degradation of glycoproteins and glycolipids within cells and for the maturation of specific proteins (Engström et al., 1987; Scaman et al., 1996; Winchester, 1984). These enzymes release all monosaccharides except for protein-bound GlcNAc, which is N-linked to asparagine (Tarelli et al., 1998). This specific enzyme is most likely involved in the processing of oligosaccharides during the biosynthesis of mannoproteins in *S. cerevisiae* (Jelinek-Kelly et al., 1985). In *O. oeni*, the presence of the α-mannosidase gene has been previously described (Cibrario et al., 2016).

Elucidating the use of mannoproteins by *O. oeni* during MLF has been the subject of studies in recent years. Mannoproteins can be hydrolysed by *O. oeni*, releasing mannose and other sugars that can be utilized as energy sources by the bacterium. This phenomenon is especially important in the nutrient-limiting environment of wine. Studies have shown that *O. oeni* expresses several genes related to the uptake and metabolism of mannose in response to wine conditions. These include genes such as *manA*, *manB*, *ptsI*, and *ptsH*, which are crucial for the adaptation of the bacterium and its ability to efficiently carry out MLF. Moreover, the presence of mannoproteins in wine can stimulate the growth and activity of *O. oeni*, thereby increasing the efficiency of MLF and the final quality of the wine (Balmaseda et al., 2021; Mendoza et al., 2017; Toraño et al., 2024). Given this context, it is important to understand the effects of mannoproteins on different *O. oeni* strains and their possible relationships with α-mannosidase activity. Identifying and characterizing the role of α-mannosidase in the hydrolysis of mannoproteins could reveal new strategies to increase the efficiency of *O. oeni* in winemaking environments.

2. Materials and methods

2.1. Microorganisms and culture media

Ten strains of *O. oeni* were selected for this study on the basis of their diverse characteristics. Among them, Viniflora CH11 (Chr. Hansen Holding AS, Hoersholm, Denmark) and Lalvin VP41 (Lallemand Inc., Montreal, Canada) are commercially available strains. PSU-1 (ATCC BAA-331) represents the first sequenced genome of this species (Mills et al., 2005) and serves as a model strain. 217^T (CECT217 = ATCC 23279T) is the type strain from the Spanish Type Culture Collection (CECT). Additionally, 2T2 was isolated from the Designation of Origin (DO) Tarragona, and 3P2 and 1Pw13 were isolated from the Qualified DO Priorat. Finally, MF1, MF2, and MF6 were isolated from the Mas dels Frares cellar (Universitat Rovira i Virgili, Constantí, Spain) in DO Tarragona.

Subcultures of *O. oeni* strains were grown in MRSmf medium containing MRS broth (Difco Laboratories, Detroit, MI, USA) (De Man et al., 1960) supplemented with L-malic acid (5 g/L) and fructose (4 g/L) at pH 5.0. To obtain the inocula, a colony was picked from the plates and grown in MRSmf at 27 °C in a 10% CO_2 atmosphere. *O. oeni* strains were maintained on MRSmf supplemented with 20 g/L bacteriological agar (Panreac, Química SLU, Castellar del Vallès, Spain) and stored at 4 °C. The cells were collected at the end of the exponential growth phase (OD600 nm ≈ 1) and inoculated (10%) into different fermentation media.

2.2. Mannoprotein extract

In this study, the commercial extract product (MP) Manno PLUS (Agrovin, Alcázar de San Juan, Spain) was chosen for its high purity in soluble mannoproteins (ranging between 85% and 95%). Other selection factors included pH preservation at the end of MLF and the absence of SO_2 in the extract, as elevated levels of this compound can inhibit the fermentative activity of *O. oeni*.

2.3. Fermentation conditions

Fermentation was conducted in a wine-like medium (WLM) according to (Bordas et al., 2015), with 14% ethanol (v/v), 2 g/L L-malic acid, 1.25 g/L Bacto™ casamino acids (BD, France), 0.4 g of fructose and 1.25 g/L peptone (Panreac), with a pH of 3.4. The WLM was supplemented with commercial mannoprotein products at two different concentrations, 1 and 2 g/L, at the beginning of MLF. *O. oeni* was inoculated at a density of 10^7 cells/ml in 50 mL tubes under anaerobic and static conditions at 20 °C in triplicate. The progress of fermentation was monitored daily by following L-malic acid consumption with an enzymatic kit using a Y15 autoanalyzer (Biosystems S.A., Barcelona, Spain) and bacterial population evolution. MLF was considered complete when the L-malic acid concentration dropped below 0.1 g/L. Samples from each triplicate experiment were centrifuged and stored at −20 °C before and after MLF.

2.4. Mannoprotein consumption

The determination of mannoproteins was based on previous methods Quirós et al. (2011), but some modifications were introduced (Toraño et al., 2024). Five mL samples were taken after cold precipitation (−20 °C) and centrifugation (4600×*g* for 15 min). Five volumes of absolute ethanol (Panreac) were added, and the mixture was incubated overnight at 4 °C. Then, the samples were centrifuged at 2300×*g* for 10 min, and the pellet was washed with two volumes of ethanol. The pellet was resuspended in 1 mL of ethanol to move the sample to a 2 mL tube. Then, the samples were centrifuged at 6600×*g* for 5 min, and the pellet was dried under speed back vacuum (Univap 148 100ECH; Progen Scientific, London, UK) for 60 min. The dried samples were then hydrolysed by adding 1 mL of 5 M H_2SO_4 for 1 h at 95 °C in a thermoblock (Labnet, Madrid, Spain). Once the sample reached room temperature, 1 mL of 10 M NaOH was added for neutralization. Finally, the sample was centrifuged (6600×*g*, 5 min), and the supernatant was retained for analysis. The free sugar (D-glucose, D-fructose and D-mannose) content was then determined in accordance with the manufacturer's instructions via a D-mannose and D-glucose assay kit K-MANGL (Megazyme, Wicklow, Ireland).

The mannoprotein content of the WLM before and after MLF was quantified as equivalents of mannose. Free mannose from the supernatant was also quantified with the same enzymatic kit at the same time points.

2.5. Determination of α-mannosidase activity

The determination of α-mannosidase activity was based on a protocol to measure β-glucosidase activity in *O. oeni* (Olguín et al., 2011) modifying the substrate for the enzymatic reaction. The mannosidase activity of the ten *O. oeni* strains in the fermentation medium used in this study (WLM) was characterized. Two-milliliters samples were centrifuged (10,000×*g*, 5 min), and both the pellets (corresponding to 2 × 10^7 cell/mL)

Table 1
Duration of MLF (days), L-malic acid consumption rate (g/L·day) and effect on MLF (+, shorter; =, no changes; -, longer) of different MP additions (0, 1 and 2 g/L) in WLM inoculated with *O. oeni* 217^T, 2T2, 3P2, 1Pw13, PSU-1, CH11 VP41, MF1, MF2 and MF6. Mean ± standard deviation (n = 3). Different lower-case letters indicate a significant difference between *O. oeni* strains and uppercases letters between the different concentrations of MP using the Tukey (HSD) test at $p < 0.05$. MLF was considered complete when L-malic acid concentration was below 0.1 g/L.

O. oeni strain	Concentration MP (g/L)	MLF duration (days)	L-malic acid consumption rate (g/L day)	Effect on MLF
217^T	0	7	0.29 ± 0.01 ^{Aabc}	
	1	6	0.35 ± 0.01 ^{Babc}	+
	2	6	0.34 ± 0.01 ^{Babc}	+
2T2	0	6	0.48 ± 0.01 ^{Cabc}	
	1	11	0.13 ± 0.02 ^{Aabc}	–
	2	6	0.43 ± 0.02 ^{Babc}	=
3P2	0	7	0.27 ± 0.02 ^{Aab}	
	1	7	0.26 ± 0.01 ^{ABab}	=
	2	6	0.29 ± 0.01 ^{Bab}	+
1Pw13	0	6	0.31 ± 0.01 ^{Cab}	
	1	6	0.18 ± 0.01 ^{Aab}	=
	2	6	0.26 ± 0.01 ^{Aab}	=
PSU-1	0	4	0.34 ± 0.01 ^{Ac}	
	1	4	0.44 ± 0.01 ^{Bc}	=
	2	3	0.58 ± 0.01 ^{Cc}	+
CH11	0	4	0.53 ± 0.01 ^{Ba}	
	1	15	0.06 ± 0.01 ^{Aa}	–
	2	15	0.06 ± 0.01 ^{Aa}	–
VP41	0	6	0.43 ± 0.01 ^{Ac}	
	1	5	0.46 ± 0.01 ^{Bc}	+
	2	4	0.63 ± 0.01 ^{Cc}	+
MF1	0	7	0.24 ± 0.07 ^{Abc}	
	1	5	0.61 ± 0.01 ^{Cbc}	+
	2	6	0.37 ± 0.01 ^{Bbc}	+
MF2	0	6	0.36 ± 0.01 ^{Aabc}	
	1	5	0.39 ± 0.01 ^{Babc}	+
	2	5	0.34 ± 0.04 ^{Aabc}	+
MF6	0	5	0.46 ± 0.01 ^{Bbc}	
	1	5	0.34 ± 0.01 ^{Abc}	=
	2	5	0.46 ± 0.01 ^{Bbc}	=

and the supernatant fractions were retained for analysis. For whole-cell activity, the pellets were resuspended in 1 mL of 0.1 M sodium acetate buffer, pH 5.1. For the enzymatic activity assay, a 25 μL sample (from the supernatants or from the whole-cell suspensions) was added to 75 μL of substrate (25 mM p-nitrophenyl-α-D-mannopyranoside [p-NMan, (Sigma–Aldrich, Barcelona, Spain)] diluted in 0.1 M sodium acetate buffer, pH 5.1) and incubated for 30 min at 37 °C. Next, 100 μL of 1 M Na_2CO_3 was added to stop the reaction and allow the p-nitrophenolate anion to turn yellow. The reaction tubes were centrifuged, and the assay was read against the blank at 400 nm using a Spectro Star Nano (BMG). From this measurement, the concentration of liberated p-nitrophenol (p-NP) was determined from a calibration curve taken from standard p-NP, where one unit of α-mannosidase activity (U) corresponded to 1 μmol of p-nitrophenol released per minute. The enzymatic activity (U) data were normalized to 1 L of culture. All the samples were collected to obtain the same population of 2×10^7 CFU/mL, considering the growth curve (OD 600 nm *vs*. CFU/mL) of each strain. The viability of the samples was confirmed via colony counting on MRSmf plates.

3. Results and discussion

3.1. Effects of mannoprotein addition on malolactic fermentation

The commercial mannoprotein extract (MP) used in this work was characterized in a previous study (Toraño et al., 2024). According to the chromatograms obtained via high-resolution size exclusion chromatography (HRSEC) via a refractive index detector (RID), MP is a mannoprotein extract rich in polysaccharides of 98.06% (w/w) and has a notable content of the medium/high-molecular-weight (M/HMW) polysaccharide fraction at 74.15% (w/w). Additionally, 23.09% (w/w) of the MP content was in the low-molecular-weight (LMW) polysaccharide fraction. The protein content of MP was low at 1.94% (w/w). This extract could be included in the medium/high-molecular-weight (M/HMW) polysaccharide fraction, i.e., between 3 and 440 kDa (Toraño et al., 2024). The addition of MP affected the evolution of the MLFs differently depending on the added concentration and the *O. oeni* strain used (Table 1). This variability is in accordance with previous studies (Balmaseda et al., 2023; Brizuela et al., 2017; Díez et al., 2010; El Khoury et al., 2017). A shorter MLF was observed for both MP concentrations for strains 217^T, VP41, MF1 and MF2 than for the control condition without MP. For strains 3P2 and PSU-1, the positive effect on the MLF duration was evident only when 2 g/L MP was added. The MLF duration was negatively affected for strain 2T2 when 1 g/L mannoprotein extract was added; however, with 2 g/L added MP, there was no effect on the MLF duration with respect to the control condition. Strain CH11 was the only strain that had a very clear negative effect on the MLF at the two MP concentrations. No effect on the MLF performance was observed for strains 1Pw13 and MF6.

In summary, the addition of 1 g/L MP resulted in a positive effect for 40% of the strains, a negative effect for another 20%, and a neutral effect for the remaining 40% (Table 1). With the addition of 2 g/L MP, the percentage of strains with a positive effect increased to 60%, while the percentage of strains with a negative effect decreased to 10%, and the percentage of strains with a neutral effect was 30%. The fermentation kinetics of PSU-1, VP41, and 217^T are shown in Fig. 1 as examples of differences in the effects of MP addition among strains. The MLF development of the rest of the strains is represented in Supplementary Fig. S1.

As expected, there was a relationship between the positive, neutral, and negative effects on the duration of MLF and the rate of L-malic acid consumption. Compared with the control, strains 217^T, VP41, MF1 and MF2 had positive effects on both the concentration of MP and the L-malic acid consumption rate. In the case of 3P2 and PSU-1, this relationship also occurred, although in these cases, the positive effect of MP was observed only with the addition of 2 g/L.

3.2. Mannoprotein consumption

The consumption of mannoproteins was observed with the addition of either 1 g/L or 2 g/L MP, with significant differences between strains for each addition, indicating that the capacity of *O. oeni* to metabolize mannoproteins and use them as a source of nutrients is strain dependent in this species (Fig. 2). This variability is in accordance with previous findings (Balmaseda et al., 2021; Toraño et al., 2024).

With 1 g/L added MP, the consumption of mannoprotein ranged from undetectable to 30.77%, with the exception of strain PSU-1, whose consumption was greater than 90%. For this concentration of MP, it was not possible to establish a clear correlation between mannoprotein consumption and the effect on the duration of MLF. However, when 2 g/L MP was added, a greater proportion of mannoprotein consumption (more than 30%) was observed only in those strains that presented a shorter MLF (217^T, 3P2, PSU-1, VP41 and MF1), with the exception of strain MF2, which did not consume MP at all. Among all the studied strains, the capacity of PSU-1 to metabolize mannoproteins (90 and 80%, with 1 and 2 g/L, respectively) was previously reported (Balmaseda et al., 2021; Toraño et al., 2024). In this study, *O. oeni* PSU-1 consumed a higher proportion of the added mannoprotein (Fig. 2) with 1 g/L MP (around 90%) than with 2 g/L MP (around 65%). However, the total amount of mannoprotein consumed was higher with 2 g/L added MP.

Previous studies have correlated the utilization of mannoproteins with increased survival under wine conditions, particularly when the preferred substrate of *O. oeni* in wine, L-malic acid, is depleted (Balmaseda et al., 2021). *O. oeni* has a highly conserved set of

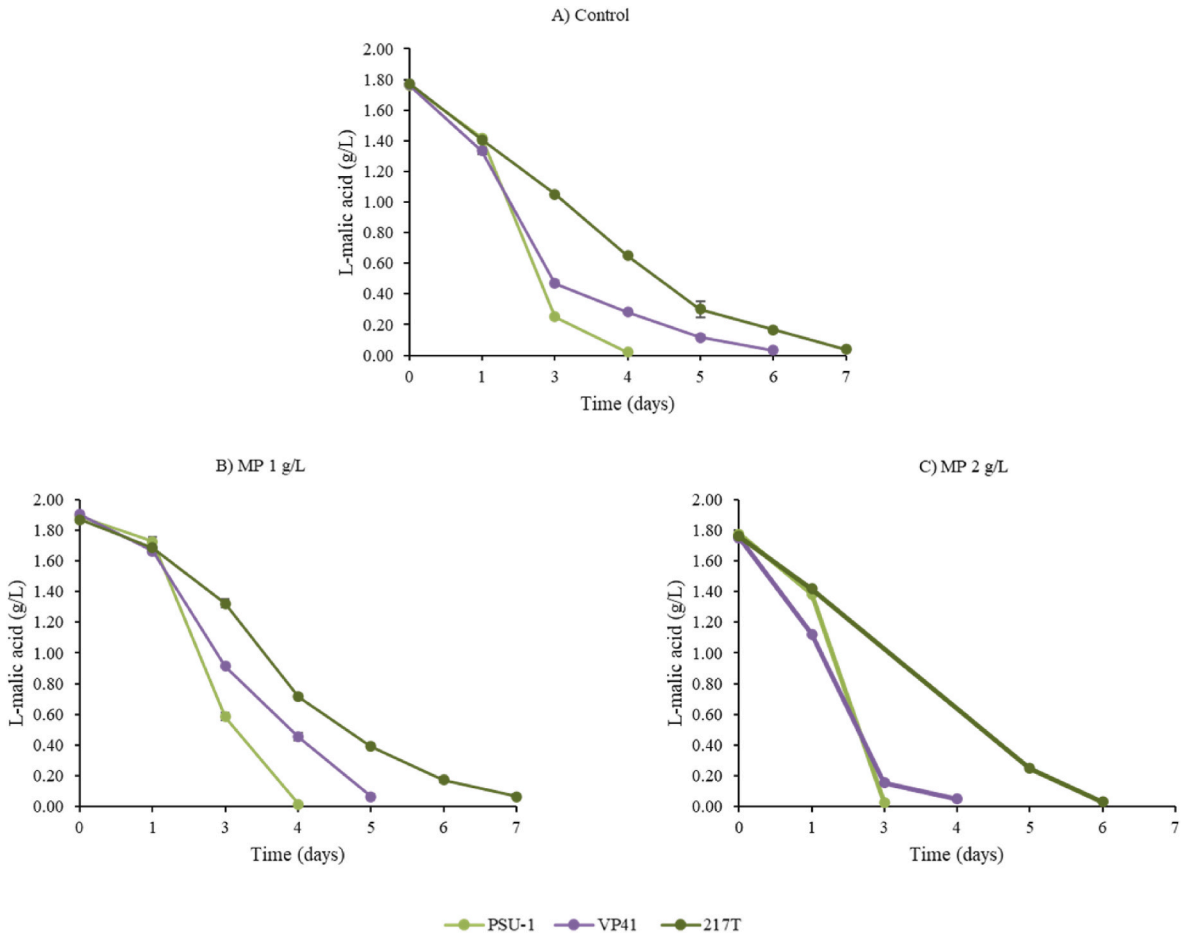

Fig. 1. Evolution of MLF in WLM fermented with *O. oeni* PSU-1, VP41 and 217^T in A) the control condition without MP addition and with the addition of B) 1 g/L and C) 2 g/L of MP.

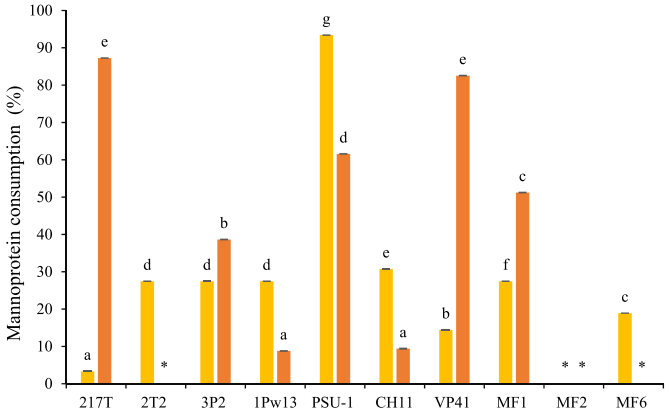

Fig. 2. Mannoproteins consumption (%) by *O. oeni* 217^T, 2T2, 3P2, 1Pw13, PSU-1, CH11. VP41, MF1, MF2 and MF6 with the additions of 1 g/L (yellow) and 2 g/L (orange) of MP. Different letters indicate a significant difference between values of each condition using the Tukey (HSD) test at $p < 0.05$. *Not detected. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

phosphotransferase system (PTS) genes. This system enables *O. oeni* to phosphorylate sugars such as glucose, cellobiose, trehalose, and mannose via phosphoenolpyruvate (PEP). These findings suggest that the PTS plays an important role in the adaptation of this bacterium to its ecological niche in wine fermentation, where mannose and other sugars are present at low concentrations after AF (Jamal et al., 2013). Notably, yeast mannoproteins play a significant role in the growth and metabolic activities of *O. oeni*, a key bacterium in wine fermentation. Specifically, one study highlights that yeast mannoproteins, particularly those of intermediate molecular weight (6–22 kDa), can be beneficial additives in wine fermentation to support bacterial growth under the stressful conditions typical in winemaking, such as high ethanol levels (Díez et al., 2010). Another possible beneficial effect of mannoproteins on *O. oeni* that has been described is the medium detoxification thanks to the adsorption of medium-chain fatty acids released from yeast that are toxic for *O. oeni* (Guilloux-Benatier et al., 1995, 1998).

The mechanisms by which mannoproteins can be beneficial are not entirely clear, but they may also aid in biofilm formation and possess prebiotic capacities in other lactic acid bacteria. *O. oeni* exhibits various biosynthetic pathways for exopolysaccharides (EPS), which are fundamental for its adaptation to the wine environment (Dimopoulou et al., 2018) In the context of winemaking, this biofilm-forming ability allows *O. oeni* to colonize and persist on the surfaces of barrels or fermentation tanks, facilitating malolactic fermentation (MLF) and contributing to wine quality by reducing volatile aromatic compounds during ageing, such as furfural, guaiacol and eugenol (Bastard et al., 2016). However, further investigations are needed to understand the mechanisms involved in mannoprotein utilization by *O. oeni.*

3.3. Mannosidase activity

Initially, the α-mannosidase activity was characterized in the ten *O. oeni* strains in WLM without added MP. The α-mannosidase activity

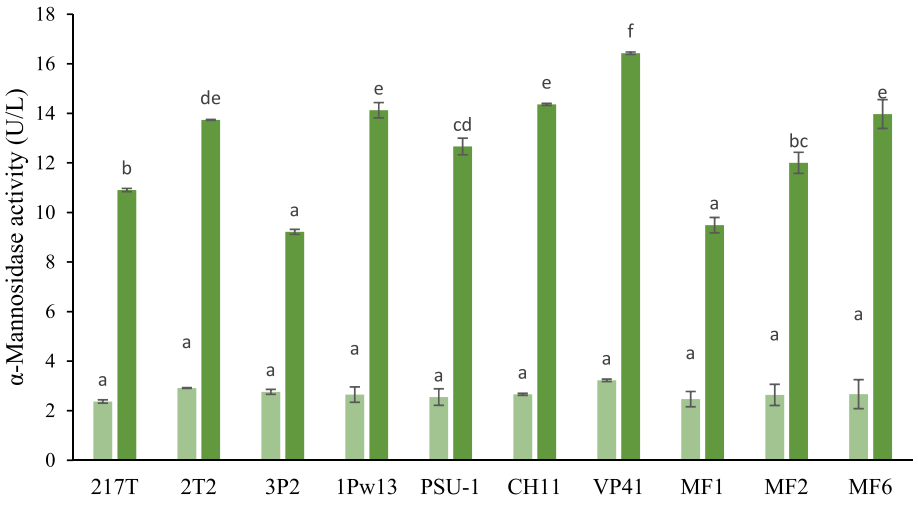

Fig. 3. α-Mannosidase activity at the middle of MLF of the different *O. oeni* strains in WLM without MP added. Enzymatic activity was measured in whole cells collected by centrifugation(light green) and in the WLM supernatant (darker green) for each strain. Data are the mean values of triplicate assays expressed in units of enzymatic activity per liter (U/L). Letters indicate a significant difference between values of each condition using the Tukey (HSD) test at $p < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

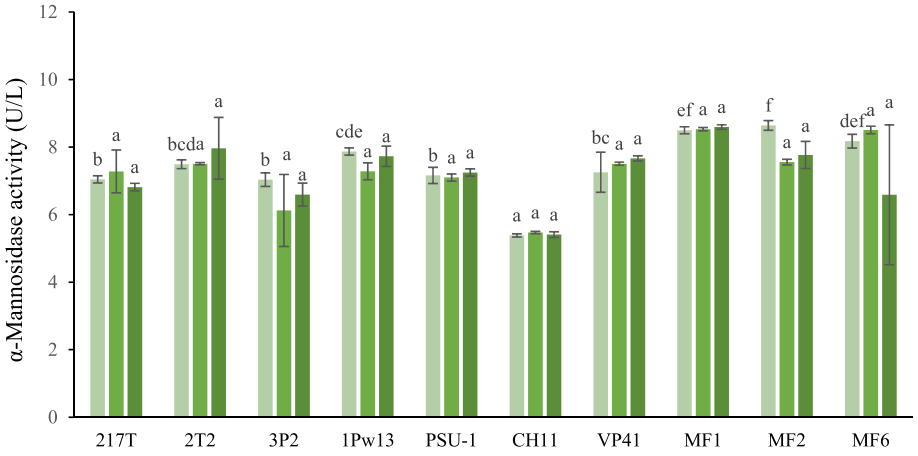

Fig. 4. α-Mannosidase activity at the end of MLF in the supernatant of WLM inoculated with the different *O. oeni* strains without MP addition (control: light green) and with the additions of 1 g/L (medium green) and 2 g/L (darker green) of MP. Data are the mean values of triplicate assays in units of enzymatic activity per liter (U/L). Letters indicate a significant difference between values of each condition using the Tukey (HSD) test at $p < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

measured in cultured whole cells with a population of 2×10^7 cells/ml did not significantly differ among strains, with values ranging from 2.37 to 3.23 U/L (Fig. 3). In contrast, significant differences in α-mannosidase activity were observed in the supernatant of synthetic wine (WLM). Strain VP41 showed the highest enzymatic activity at 16.43 U/L, followed by CH11 at 14.36 U/L and MF6 at 13.97 U/L. Strains 3P2 (9.22 U/L) and MF1 (9.49 U/L) exhibited the lowest activity. These findings suggest that the strains may secrete the enzyme at different levels or that the extracellular enzymatic activity is influenced by specific factors in the WLM medium (Fig. 3). The highest enzymatic activity in the supernatant was also observed for β-glucosidase activity in *O. oeni* by Olguín et al. (2011).

The α-mannosidase activity with the addition of 1 and 2 g/L of MP was evaluated at the end of MLF. The enzyme activity decreased at the end of the fermentation, with significant differences observed among the strains compared with the control conditions (Fig. 4). Strains such as MF2 (8.64 U/L) and MF1 (8.49 U/L) have a greater capacity for α-mannosidase secretion than CH11 (5.38 U/L), 3P2 (7.03 U/L), or 217T (7.04 U/L). These enzymatic activity values are lower than those observed in whole cells and their supernatants, suggesting a loss of activity over time. However, no differences in α-mannosidase activity associated with mannoprotein addition were observed and neither associated to a faster MLF. This indicates that other metabolic aspects associated with mannoprotein utilization, rather than this enzymatic activity, may be responsible for the observed effects of these molecules on certain *O. oeni* strains.

4. Conclusions

MLF in wine production, facilitated by *O. oeni*, is influenced by various factors, including the presence of mannoproteins derived from the yeast cell wall. Mannoproteins serve as nutrient sources for *O. oeni*, impacting its growth and metabolic activities under wine conditions. This study evaluated the effects of commercial mannoprotein extracts on ten strains of *O. oeni* in WLM. Fermentation was monitored for MLF progression, mannoprotein consumption, and α-mannosidase activity. The results indicated strain-specific responses to mannoprotein addition, with some strains showing increased MLF efficiency, whereas others experienced delays. Mannoprotein consumption varied significantly among strains, suggesting differential metabolic capabilities.

Moreover, the activity of α-mannosidase, which is crucial for mannoprotein utilization, showed strain variability and was greater in the supernatants. These findings highlight the complex interplay between mannoproteins and *O. oeni* in wine fermentation, underscoring the potential of mannoprotein supplementation to modulate MLF outcomes and improve wine quality. Further research under cellar conditions is needed to evaluate this possible benefits.

CRediT authorship contribution statement

Paloma Toraño: Writing – original draft, Investigation, Formal analysis, Data curation. **Alba Martín-García:** Investigation, Data curation. **Albert Bordons:** Writing – review & editing, Funding acquisition, Conceptualization. **Nicolas Rozès:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **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.fm.2024.104689.

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