International Journal of Food Microbiology 442 (2025) 111401

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Different composition of mannoprotein extracts and their beneficial effects on *Oenococcus oeni* and wine malolactic fermentation

Paloma Toraño [a], María Oyón-Ardoiz [b], Elvira Manjón [b], M. Teresa Escribano-Bailón [b], Albert Bordons [a], Ignacio García-Estévez [b], Nicolas Rozès [c], Cristina Reguant [a,*]

[a] Universitat Rovira i Virgili, Grup de Biotecnologia Enològica, Departament de Bioquímica i Biotecnologia, Facultat d'Enologia, Tarragona, Spain
[b] Universidad de Salamanca, Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Farmacia, Salamanca, Spain
[c] Universitat Rovira i Virgili, Grup de Biotecnologia Microbiana d'Aliments, Departament de Bioquímica i Biotecnologia, Facultat d'Enologia, Tarragona, Spain

ARTICLE INFO

Keywords:
Mannoprotein
Malolactic fermentation
Oenococcus oeni
Wine

ABSTRACT

Malolactic fermentation (MLF) is a biochemical process in winemaking where lactic acid bacteria (LAB) convert L-malic acid into L-lactic acid and carbon dioxide, contributing to the deacidification and microbiological stability of wine. *Oenococcus oeni,* the main bacterium responsible for MLF, must deal with challenging conditions such as high ethanol concentrations, low pH values and low nutrient availability. In this study, the effects of commercial mannoprotein extracts derived from the yeast *Saccharomyces cerevisiae* on the MLF were investigated. Three commercial mannoprotein extracts were used to study the effects of different *O. oeni* strains on the MLF in wine-like media. The results revealed that the type and concentration of mannoproteins present in the extracts significantly influence the rate of L-malic acid consumption and the duration of MLF. The characteristics of the mannoproteins, such as their molecular weight, monosaccharide composition and protein content, varied among the products, affecting their ability to promote MLF. This study highlights not only the potential of mannoproteins to improve MLF but also the importance of the selection of an extract with adequate composition characteristics to benefit the process.

1. Introduction

The conversion of grape must into wine involves complex biochemical processes, with two primary fermentation stages standing out: alcoholic fermentation (AF), where yeasts convert sugars mainly into ethanol and CO_2; and malolactic fermentation (MLF), in which lactic acid bacteria (LAB) convert L-malic acid into L-lactic acid and CO_2 through the action of the malolactic enzyme (Ribéreau-Gayon et al., 2006). MLF is usually desirable in red wines, especially when they are meant to age in oak barrels or even in bottles, or in highly acidic white wines originating from cooler climate regions with less sun exposure (Dols-Lafargue et al., 2007; Liu, 2002; Semon et al., 2001).

This process has several significant effects on wine, including deacidification, flavour modifications and improvements in microbial stability, and contributes to the quality of final wines (Bartowsky, 2005; Ribéreau-Gayon et al., 2006).

Oenococcus oeni is the primary bacterium involved in MLF (Alexandre et al., 2004; Bartowsky, 2005; Liu et al., 2017). This bacterium is notable for its ability to tolerate adverse environmental conditions and the challenging, nutritionally poor environment found in wine; it is highly adaptable to the stress imposed by the hostile conditions of wine, which threatens the development of the MLF. These conditions include high ethanol concentrations (up to 16 % v/v), low pH (3.0–3.5), low temperatures, total sulfur dioxide (50–100 mg/L), medium-chain fatty acids produced by yeast during white wine production, and nutrient depletion caused by yeast consumption during AF (Margalef-Català et al., 2017; Olguín et al., 2009; Yang et al., 2021). The application of white wine lees (Balmaseda et al., 2024) or preadapting LAB starter cultures through the addition of mannoprotein extracts can increase LAB growth and the performance of MLF (Toraño et al., 2024).

Polysaccharides constitute one of the main groups of wine macromolecules and are divided into two families according to their origin: those originating from grape primary cell walls and those released by microorganisms (Gil et al., 2012). Mannoproteins are the second most abundant family of compounds released by yeast after AF (Bicca et al., 2023; Giovani et al., 2012; Rinaldi et al., 2021; Rodrigues et al., 2012;

* Corresponding author.
E-mail address: cristina.reguant@urv.cat (C. Reguant).

https://doi.org/10.1016/j.ijfoodmicro.2025.111401
Received 1 April 2025; Received in revised form 30 July 2025; Accepted 17 August 2025
Available online 19 August 2025

Snyman et al., 2023). The origin of these macromolecules is yeast cell walls, which are released in the early stages of fermentation and during later stages when wine ageing is performed in contact with lees (Díez et al., 2010; Dupin et al., 2000; Vejarano, 2020). Most of the yeast cell wall presents an internal layer of chitin (1–2 %) and β-glucan (50–60 %) and an external layer of mannoproteins (Lesage and Bussey, 2006), which not only provides cells with rigidity that protects them from osmotic pressure but also helps them maintain their shape, which allows them to change their shape in accordance with the cell cycle stage (Liu et al., 2017).

Mannoproteins are generally linked to proteins through covalent bonds with 80–90 % mannose, with a medium molecular weight ranging from 20 kDa to 200 kDa (Liu et al., 2017; Martínez Lapuente et al., 2019). They are highly glycosylated glycoproteins where the glycosidic fraction can present between 50 and 95 % of its weight (Lipke and Ovalle, 1998). They can be *O*-glycosylated or *N*-glycosylated, and both types of glycosylation can occur in the same molecule. *N*-glycosidase consists of eight mannose residues and two *N*-acetylglucosamine residues linked to an asparagine residue (Klis et al., 2002). The final structure has at most fifty mannose residues linked by an α (1,6) bond with short chain branches at the α (1,2) and α (1,3) positions. The sugar chains linked by *O*-glycosylated bonds to serine and threonine are short chains of up to five units of mannose, with the first two residues united by α (1,2) bonds and the next one united by α (1,3) (Lesage and Bussey, 2006).

Furthermore, the structural and chemical composition of mannoproteins is directly influenced by the yeast growth phase and environmental conditions, which vary throughout the winemaking process (Snyman et al., 2021). Mannoproteins have been associated with the stimulation of malolactic bacteria growth in wine due to the adsorption of the medium-chain fatty acids synthesized by *Saccharomyces cerevisiae*. These compounds have been shown to inhibit bacterial growth; therefore, their removal improves the MLF (Caridi, 2006; Díez et al., 2010; Guilloux-Benatier et al., 1995; Liu et al., 2017). During MLF, *O. oeni* consumes yeast mannoproteins, resulting in elevated consumption in wines. However, the degradation process cannot be explained as a generalized mechanism. Rather, it depends on the properties of the specific fermentation medium in which it occurs (Balmaseda et al., 2021).

Moreover, *O. oeni* possesses glycosidase, peptidase and α-mannosidase activities, which facilitate the liberation of sugars and amino acids from mannoproteins and other macromolecules (Aredes Fernández et al., 2004; Balmaseda et al., 2022; Olguín et al., 2011; Remize et al., 2006; Toraño et al., 2025a). This, in turn, enhances the nutritional content and promotes the survival of *O. oeni* in wine (Alexandre et al., 2004). The use of commercial preparations during winemaking is authorized by the OIV (International Organization of Vine and Wine COEI-1-MANPRO: 2004) for specific purposes, such as tartaric acid salts and/or protein stabilization.

These commercial mannoproteins are extracted either through physicochemical (heat, alkali, or sonication) or enzymatic methods. They must meet the specificities of the International Oenological Codex Resolution (Codex OIV-OENO 26–2004) and be composed of at least 60 % of the mass of polysaccharides, 70 % of which must be mannose (Bicca et al., 2022).

Over the past few decades, numerous commercial mannoproteins obtained from *S. cerevisiae* cell walls have been incorporated into wine to impart beneficial oenological characteristics. These include reducing astringency (Li et al., 2018; Manjón et al., 2020), enhancing mouthfeel sensation (Rinaldi et al., 2021; Vidal et al., 2004), increasing colour intensity (Bicca et al., 2023; Poncet-Legrand et al., 2007; Oyón-Ardoiz et al., 2022; Rinaldi et al., 2021), and ensuring stability in terms of proteins and tartrates (Caridi, 2006; Gonzalez-Ramos et al., 2008; Lubbers et al., 1994).

The aim of this study was to evaluate the potential use of different mannoprotein extracts available on the market as MLF activators. Moreover, these extracts have been structurally characterized to assess the relationships between protein structure and activity.

2. Materials and methods

2.1. Strains and culture media

Three *O. oeni* strains were used in this study: PSU-1 (ATCC BAA-331), Lalvin VP41 (Lallemand Inc., Montreal, Canada) and CECT 217^T (Spanish Type Culture Collection, CECT), which were selected from a previous screening of ten strains of this species (data in the process of publication). They were cultivated in MRSmf medium, which consisted of MRS broth (Difco Laboratories, Detroit, MI, USA) (De Man et al., 1960) enriched with L-malic acid (5 g/L) and fructose (4 g/L) at pH 5.0. *O. oeni* strains were maintained on MRSmf solid media supplemented with 20 g/L bacteriological agar (Panreac, Química SLU, Castellar del Vallès, Spain) and stored at 4 °C. To obtain the inocula, a colony was picked from the plates and grown in liquid media at 28 °C in a 10 % CO_2 atmosphere. Then, 500 μL was inoculated in 50 mL of the same freshly prepared liquid media. The cells were harvested at the end of the exponential growth phase (OD600 nm ≈ 1) and used to inoculate fermentation media at a 10 % (v/v) concentration.

2.2. Characterization of the mannoprotein extracts

In this work, three mannoprotein extracts from the cell wall of *S. cerevisiae* were selected for their purity in soluble mannoproteins (between 85 % and 95 % richness), pH preservation and minimum sulfur dioxide content (SO_2), which is inhibitory for O. oeni fermentation activity. Three different commercial mannoprotein extracts were used, named in this work MP1, MP2 and MP3, respectively.

The total protein content (w/w) in the mannoprotein products was quantified via the Lowry method (Lowry et al., 1951), which employs a DC protein assay kit (Bio-Rad Laboratories Inc., City, Country). A bovine serum albumin calibration curve was established for quantification. The protein and glycoprotein profiles of the commercial mannoproteins were examined through SDS–PAGE, employing the procedure outlined by Oyón-Ardoiz et al. (2024), with Coomassie blue staining for proteins and Schiff (pink) staining for glycoproteins.

The molecular weight distribution of the mannoproteins was assessed through HRSEC-RID using an Agilent 1260 Infinity system (Agilent Technologies, Palo Alto, CA, USA), following the procedure outlined by Manjón et al. (2020). In brief, an HPLC system equipped with two consecutive 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) was used. A calibration curve for molecular weight was established by employing various pullulan standards with molecular weights ranging from 342 Da to 805,000 Da.

Finally, the monosaccharide composition of the mannoprotein extracts was assessed in accordance with the methodology outlined by Oyón-Ardoiz et al. (2022). Briefly, the mannoprotein extracts were subjected to acid hydrolysis followed by derivatization with 1-phenyl-3-methyl-5-pyrazolone (PMP). The monosaccharide derivatives were subsequently analysed via HPLC-DAD-MS, employing an Agilent 1200 Series HPLC system fitted with an Agilent Poroshell 120 EC-18 column (2.7 μm, 4.6 mm × 150 mm) (Agilent Technologies). This HPLC system was coupled to a mass spectrometer API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany), which was equipped with an ESI source and a triple quadrupole-ion trap mass analyser. The entire setup was controlled by Analyst 5.1 software. Chromatograms were recorded at a preferred wavelength of 250 nm. A calibration curve was generated by using commercial glucose and mannose standards from Sigma–Aldrich (St. Louis, MO, USA) and ribose standards from AlfaAesar (Kandel, Germany).

2.3. Fermentation conditions

MLF was carried out in a wine-like medium (WLM) based on the protocol of Bordas et al. (2005), with 14 % ethanol (v/v), 2 g/L L-malic acid, 1.25 g/L Bacto™ casamino acids (BD, France), 0.4 g/L of fructose, and 1.25 g/L peptone (Panreac), with a pH of 3.4. At the beginning of the MLF, the WLM was supplemented with mannoprotein extract at two different concentrations, 1 and 2 g/L. O. oeni was inoculated at a concentration of 10^7 cells/mL in 50 mL tubes under anaerobic and static conditions at 20 °C, and the experiments were performed in triplicate. The progress of fermentation was monitored daily by measuring L-malic acid consumption via 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. Samples from each replicate were centrifuged and stored at −20 °C before and after MLF.

2.4. Mannoprotein consumption

The determination of the concentration of mannoprotein, as an equivalent of mannose, was mainly based on Quirós et al. (2011), but some modifications were introduced. Five mL samples were taken after cold down precipitation (−20 °C) and centrifugation (4600 ×*g* for 15 min). Five volumes of absolute ethanol were added and incubated overnight at 4 °C. Then, the samples were centrifuged at 2800 ×*g* for 10 min, and the pellet was washed with two volumes of ethanol and then 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 in a speedback vacuum (Univap 148100ECH; Progen Scientific, London, UK) for 60 min. Then, the dried samples were hydrolysed by adding 1 mL of H_2SO_4 (5 M) for 1 h at 95 °C in a thermoblock (Labnet, Madrid, Spain). Once the sample reached room temperature, 1 mL of 1 M NaOH was added to the sample (Toraño 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 had 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 H_2SO_4 and 10 % CH_3CN 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).

2.5. Amino acid composition

The amino acid (AA) consumption content was analysed via high-performance liquid chromatography (HPLC) according to the methods of Gómez-Alonso et al. (2007). The HPLC instrument (Agilent 1100) was equipped with a DAD ultraviolet detector and a fluorescence detector (Agilent Technologies). Separation was performed on a Hypersil ODS C18 column (Agilent Technologies) with a particle size of 5 μm (250 mm × 4.6 mm) at 20 °C. The mobile phase (A) consisted of 2.05 g/L anhydrous sodium acetate and 0.2 g/L sodium azide in Milli-Q water (Millipore Q-PODTM Advantage A10), adjusted to pH 5.8 with glacial acetic acid. The mobile phase (B) consisted of 80 % (v/v) acetonitrile and 20 % (v/v) methanol. Chromatograms were analysed via Agilent ChemStation Plus software (Agilent Technologies). The consumption of detected amino acids is expressed as a concentration (mg/L) and is grouped into five categories: amino acids with positively charged side chains (arginine, histidine, and lysine), amino acids with polar uncharged side chains (serine, threonine, and glutamine), amino acids with hydrophobic side chains (alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, and tryptophan), aspartic acid, and the sum of glycine and proline.

2.6. General oenological analytical parameters

The concentrations of sugars (glucose and fructose), L-malic acid, acetic acid, D- and L-lactic acids, NH_4, primary amino nitrogen (NOPA), and citric acid were determined with a Y15 analyser (Biosystems SA), and the pH was determined via a Crison micro pH 2002 pH meter (Barcelona, Spain).

2.7. Statistical analysis

Three independent biological samples were analysed for each combination of strains per condition. ANOVA (Tukey honestly significant difference (HSD) test) was performed with the XLSTAT software 2023.3 package (Addinsoft, Paris, France), with a statistical level of significance of $p < 0.05$.

3. Results and discussion

This study aimed to evaluate the impact of three commercial mannoprotein extracts on MLF in a wine-like medium using three different *O. oeni* strains. Based on a previous study (Toraño et al., 2024) in which the three mannoprotein extracts used here had been preliminarily characterized, a more exhaustive analysis was carried out in this study. A detailed physicochemical characterization of the extracts was performed, followed by an assessment of their effects on MLF duration, mannoprotein and amino acid consumption, and final wine composition.

3.1. Mannoprotein extract characterization

To better understand the structural and compositional characteristics of the commercial mannoprotein (MP) extracts, various analytical techniques were employed. Chromatographic profiles were confirmed via HRSEC-RID, the monosaccharide composition was determined via HPLC-DAD, and the protein fraction was assessed via the Lowry method. The analysis of the MP products through SDS–PAGE (Supplementary Fig. S1) revealed the presence of pure mannoproteins (see the exclusive pink colouration in MP1 and MP3) (Fig. S1). However, some non-glycosylated proteins are present in the commercial product MP2 (identified as blue bands stained with Coomassie Brilliant Blue). Notably, the glycoprotein bands are located above the 250 kDa standard, indicating the high molecular weights (MWs) of the three MP. It is difficult to detect differences in the MP profile by SDS–PAGE because this technique does not allow good resolution of proteins with such high MWs. To determine the distribution of molecular weights more accurately, a chromatographic analysis using HRSEC-RID was carried out.

The resulting HRSEC-RID chromatograms are shown in Fig. 1. The distribution of MWs from each commercial product exhibited remarkable dissimilarities, indicating structural differences among products. Different fractions were observed, four for MP1, two for MP2, and three for MP3, corresponding to molecules with diverse ranges of sizes. Table 1 provides details regarding the MW distribution, average MW of the mannoprotein extracts and total protein content. For the fractions obtained according to the MW, differences can be observed between MP1, MP2 and MP3, especially in the case of MP2, corroborating the differences observed in the SDS–PAGE gel (Fig. S1). In terms of the average MW, MP1 has the largest size (72 kDa), while MP2 has the smallest size (48 kDa), and MP3 has an intermediate size (58 kDa). Different fractions were observed for the three extracts. The profiles of MP1 and MP3 are highly similar, as both contain several fractions: a high-MW fraction (approximately 600–150 kDa), followed by a medium-MW fraction (approximately 150–10/5 kDa) and oligosaccharide fragments. However, the profile of MP2 clearly exhibited a predominant peak with a very broad MW range, from 532 to 3 kDa, whereas the percentages of small-sized fractions were more representative of those of MP1 and MP3.

The analyses demonstrated the diversity of some of the mannoprotein extracts available for winemaking. Previous works have described the specific properties of mannoproteins for oenological use, which depend on the MW. Li et al. (2018) reported that mannoproteins with MWs between 5 and 400 kDa can be used to increase protein stability, cold stability, or colour stability in wine, as well as to modify their sensory properties.

The total protein content in the MP extracts determined via the Lowry method ranged between 13 % and 15 %, with significant differences observed among the three products (Table 1). The analysed MP contain a protein content percentage similar to that reported for other MP (Klis et al., 2002; Lipke and Ovalle, 1998; Manjón et al., 2020; Orlean, 2012; Vidal et al., 2003), which seems consistent with those presented by these authors, where the characterized MP extracts had a protein content ranging from 10 % to 30 %.

Analysis of the monosaccharide composition via HPLC–DAD–MS revealed notable differences among the extracts, as presented in Table 2. Mannose was the predominant component in all the extracts, accounting for more than 50 %, and there were significant differences among the three products. Glucose, in some extracts, accounted for up to 40 %, followed by a small amount of ribose detected exclusively in the MP1 extract. The high mannose content indicates the successful isolation of mannoproteins from yeast cell walls. MP2 presented the highest mannose content (84.30 %), followed by MP1 and MP3, with values of 58.29 % and 52.79 %, respectively. In terms of glucose content, MP3 had the highest concentration (47.21 %), followed by MP1 (40.91 %) and MP2 (15.70). Glucose may be derived from mannoproteins associated with β-glucan in the yeast cell wall, suggesting its release into the medium during extraction or processing. (Klis et al., 2002; Lipke and Ovalle, 1998; Orlean, 2012). Notably, a small amount of ribose was present in MP1 (0.79 %), likely arising from yeast nucleic acid release during the extraction process.

In conclusion, there are compositional differences among the different mannoprotein extracts according to the results obtained, which may affect the properties and impact of these extracts in their oenological use, resulting in two groups: the first with a balance between the percentages of mannose and glucose (MP1 and MP3 extracts) and the second with the MP2 extract, which contained the highest percentage of mannose.

3.2. Effects of mannoprotein addition on the MLF

Three *O. oeni* strains were selected for this work based on their ability to metabolize mannoproteins: *O. oeni* PSU-1, VP41 and 217^{T,} based on previous work (Toraño et al., 2025a). This study was performed in WLM as a first approach to evaluate mannoprotein utilization by *O. oeni*, avoiding interactions with polyphenols and using reproducible conditions. Differences in the development of MLF depending on the strain and the concentration of MP added were observed. PSU-1 ended MLF in a shorter period, followed by the VP41 strain and 217^{T} strain, as a general trend in wines with the addition of MP1, MP2 and MP3 (Fig. 2). Table 3 shows the duration and consumption rate of L-malic acid during MLF in a wine-like medium (WLM). MLF was completed earlier by PSU-1 with the addition of the three products and the two different concentrations (1 g/L and 2 g/L). Compared with the control strain, strain VP41 finished MLF three days earlier with the addition of MP1 and two days earlier with the addition of MP2. The effect of the addition of the MP1 and MP2 products was neutral for strain 217^{T}, which took fifteen days to complete MLF. The addition of MP3 had a negative effect on strains VP41 and 217^{T}, finishing MLF in a longer time. The strain VP41 took two more days to complete MLF when 1 g/L of MP3 was added and five more days when the addition was 2 g/L compared with the control condition. *O. oeni* 217^{T} showed similar behaviour, and the MLF lasted fourteen days with 1 g/L added MP3 and seventeen days with 2 g/L added MP3. Nevertheless, the effect of MP3 addition was positive for PSU-1, which was fermented 3 days before the control. The rate of L-malic acid consumption was significantly different among the three strains, in accordance with the different durations of fermentation. With respect to the different mannoprotein extracts, significant differences were observed depending on the MP extract and concentration.

Significant differences were observed in MLF development depending on the mannoprotein extract, its concentration, and the specific *O. oeni* strain. These variations suggest that the mannoprotein composition, particularly the mannose content, plays a crucial role in fermentation dynamics. According to a proteomic study (Toraño 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. These differences were in accordance with the differences observed in the MLF duration with respect to the control conditions; the L-malic

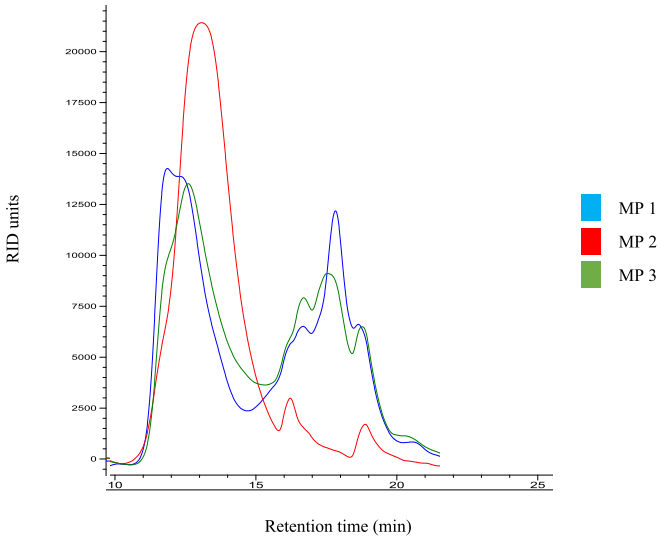

Fig. 1. HRSEC-RID chromatograms of three commercial mannoprotein extracts (MP1, MP2 and MP3). Each curve is the average of three replicates.

Table 1
Molecular weight (MW) distribution and average MW (kDa) determined by HRSEC-RID and total protein (w/w) contained in the three commercial mannoprotein extracts. Different letters within each row indicate significant differences using the Tukey (HSD) test at $p < 0.05$. All data are expressed as the arithmetic average of three biological replicates±standard deviation.

MPs	MW (kDa)	%	Average MW (kDa)	Protein content (%)
MP1	679–143	17.91 ± 0.22	72 ± 1.58^{c}	15 ± 0.80^{b}
	143–10	32.81 ± 1.52		
	10–2	9.52 ± 0.53		
	<2	39.74 ± 0.66		
MP2	532–3	91.31 ± 0.28	48 ± 1.10^{a}	13 ± 1.12^{a}
	<3	8.68 ± 0.13		
MP3	517–158	10.21 ± 0.37	58 ± 1.58^{b}	14 ± 0.18^{ab}
	158–5	42.24 ± 0.42		
	<5	47.53 ± 0.42		

Table 2
Monosaccharide composition of the three commercial mannoprotein extracts (MP1, MP2 and MP3) in terms of mannose, glucose and ribose percentage (%). Different lower-case letters indicate a significant difference between values using the Tukey (HSD) test at $p < 0.05$. n.d.: not detected. All data are expressed as the arithmetic average of three biological replicates±standard deviation.

MP extract	Mannose (%)	Glucose (%)	Ribose (%)
MP1	58.29 ± 1.39^{b}	40.91 ± 1.37^{b}	0.79 ± 0.02
MP2	84.30 ± 0.27^{c}	15.70 ± 0.27^{a}	n.d
MP3	52.79 ± 0.18^{a}	47.21 ± 0.18^{c}	n.d

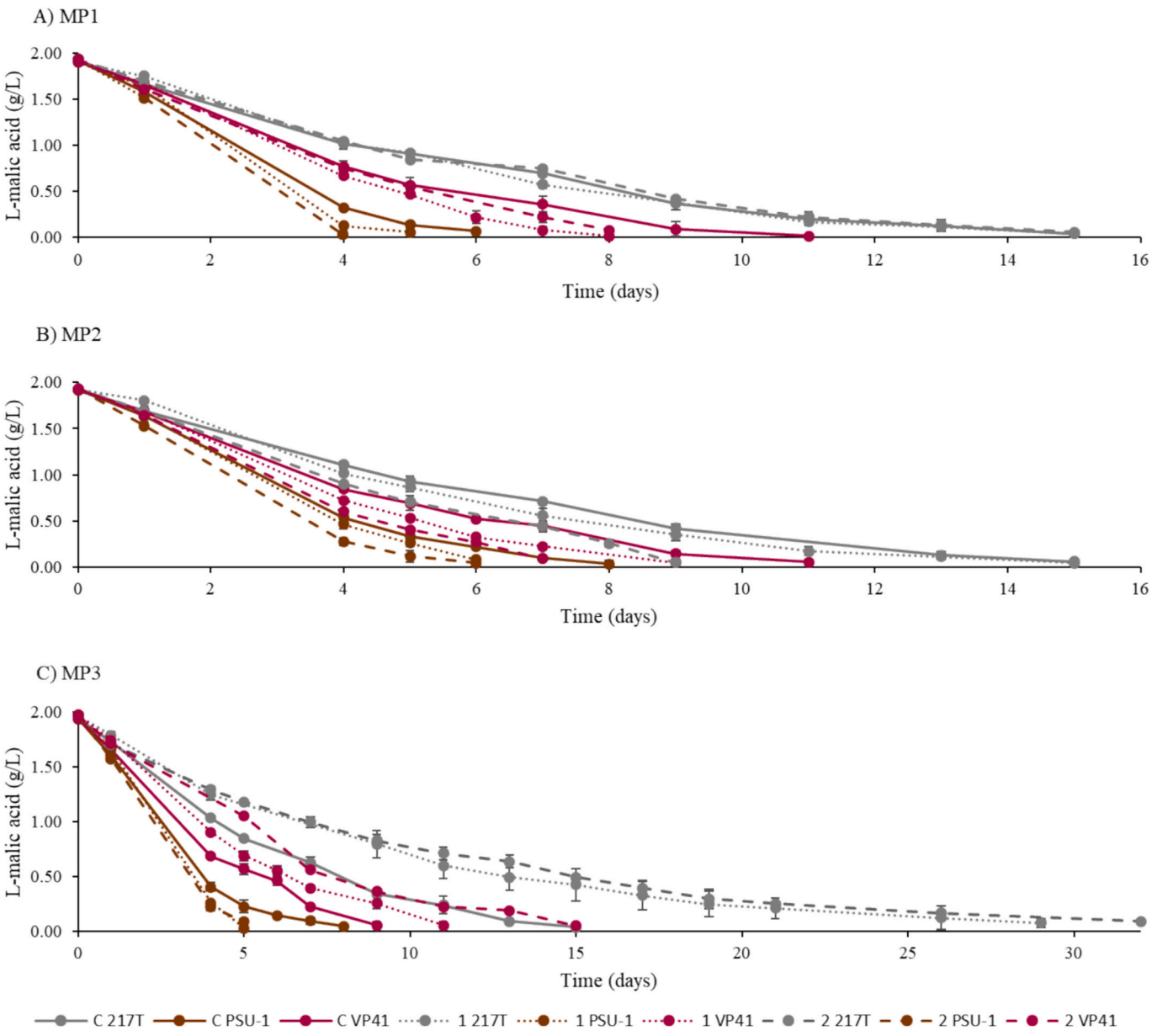

Fig. 2. Evolution of the kinetics of L-malic acid consumption with the three commercial mannoprotein extracts MP1 (A), MP2 (B) and MP3 (C) in wine-like medium (WLM) fermented with *O. oeni strains* VP41, PSU-1 and 217^{T} with the additions of 1 g/L (1) and 2 g/L (2) of each mannoprotein extract. All data are expressed as the arithmetic average of three biological replicates±standard deviation.

Table 3
Time of consumption of 100 % of L-malic acid in MLF and L-malic acid consumption rate (g/L·day) of wines after MLF for the three different strains of *O. oeni* (217^{T}, PSU-1 and VP41) and the additions of two different concentration of mannoproteins extracts (1 g/L and 2 g/L). Average ± standard deviation (n = 3). Different lowercase letters indicate a significant difference between MP1, MP2 and MP3 and uppercase letters between the *O. oeni* strains for each condition values using the Tukey (HSD) test at $p < 0.05$.

Mannoprotein extracts	Concentration (g/L)	Duration (days)			Rate (g/L-malic consumed/day)		
		O. oeni strains			*O. oeni* strains		
		217^{T}	PSU-1	VP41	217^{T}	PSU-1	VP41
MP1	Control	15	6	11	0.23 ± 0.01^{Ad}	0.40 ± 0.01^{Bd}	0.29 ± 0.02^{Cd}
	1 g/L	15	5	8	0.22 ± 0.01^{Ae}	0.44 ± 0.02^{Be}	0.31 ± 0.01^{Ce}
	2 g/L	15	4	8	0.22 ± 0.00^{Ae}	0.47 ± 0.01^{Be}	0.30 ± 0.01^{Ce}
MP2	Control	15	8	11	0.20 ± 0.01^{Ab}	0.35 ± 0.01^{Bb}	0.27 ± 0.01^{Cb}
	1 g/L	15	6	9	0.23 ± 0.01^{Acd}	0.36 ± 0.01^{Bcd}	0.30 ± 0.02^{Ccd}
	2 g/L	9	6	7	0.26 ± 0.01^{Ae}	0.41 ± 0.01^{Be}	0.33 ± 0.01^{Ce}
MP3	Control	15	8	9	0.23 ± 0.02^{Ad}	0.38 ± 0.01^{Bd}	0.31 ± 0.01^{Cd}
	1 g/L	29	5	11	0.18 ± 0.01^{Ac}	0.43 ± 0.02^{Bc}	0.27 ± 0.01^{Cc}
	2 g/L	32	5	15	0.17 ± 0.01^{Aa}	0.43 ± 0.01^{Ba}	0.12 ± 0.01^{Ca}

consumption rate was significantly greater when the MLF duration was shorter and significantly lower when the fermentation duration was longer. The cell viability was maintained on the order of 10^7 CFU/mL throughout the MLF in all the cases (data not shown), indicating that the different effects of the MP extracts on the MLF duration were not due to the cell concentration.

3.3. Consumption of mannoprotein by O. oeni strains

Analysis of the consumption of commercial MP extracts by the three strains of *O. oeni* at two concentrations, 1 and 2 g/L, during the MLF revealed that the consumption was complete for all strains at 2 g/L (data not shown). However, when 1 g/L was added, mannoprotein consumption consistently remained above 45 % for all *O. oeni* strains (Fig. 3). Significant differences in mannoprotein consumption depending on the strain and the MP extract were detected. The complete consumption of these molecules was observed for most of the evaluated conditions. However, partial consumption was observed when 1 g/L of MP1 was added to WLM with 217^T (52.7 %) and PSU-1 (62.7 %), whereas 100 % of the consumption with VP41 occurred. The strain VP41 consumed 52.3 % of the mannoprotein content when 1 g/L MP2 was added. When 1 g/L MP3 was added, PSU-1 alone did not affect the mannoprotein content (46.7 %).

O. oeni can hydrolyse mannoproteins, thus increasing the nutritional content of the medium and stimulating its activity (Caridi, 2006; Díez et al., 2010). LAB may degrade wine polysaccharides and thus decrease the total polysaccharide content and may also synthesize new polysaccharides (Dols-Lafargue et al., 2007). However, further evidence is needed to better understand the role of mannoprotein metabolism by *O. oeni* in wine. The beneficial effects of mannoproteins, particularly those with molecular weights between 6 and 22 kDa, on *O. oeni* growth have been reported (Díez et al., 2010). Although little information is available about the positive impact of mannoproteins on *O. oeni*, other LAB yeast mannose oligosaccharides (MOSs) that promote beneficial responses in some probiotic LAB species have been described. When considered prebiotics, MOSs demonstrate the ability to increase populations of probiotic microorganisms, such as bifidobacteria and lactobacilli (Rodrigues et al., 2012), as well as support the in vitro growth of the probiotic *Lactiplantibacillus plantarum* in vitro (Zang et al., 2019).

In this work, we observed clear activation of fermentation activity when MP1 and MP2 were added; however, fermentation activity could not be directly associated with differences in mannoprotein consumption. All the strains presented a high capacity for mannoprotein consumption, especially when 2 g/L MP was added. However, in some cases, such as MP3, the observed effect on the MLF was negative for some strains. Most likely, not determining the compounds remaining in the extracts from the extraction process could interfere with the effect of MP on malolactic activity.

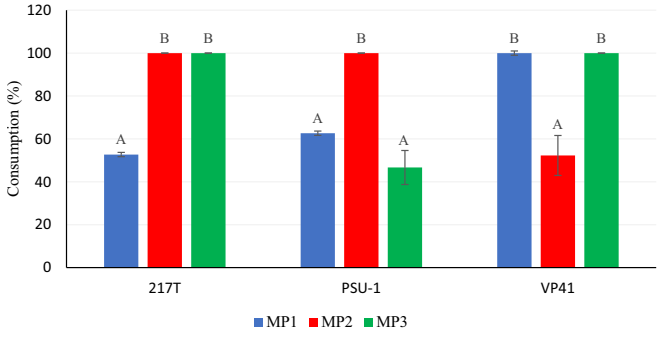

Fig. 3. Mannoprotein consumption (%) by *O. oeni* 217^T, PSU-1 and VP41 at the end of MLF with the addition of 1 g/L of each mannoprotein extract. Different uppercase letters indicate a significant difference between values using the Tukey (HSD) test at $p < 0.05$. All data are expressed as the arithmetic average of three biological replicates ± standard deviation.

3.4. Amino acid consumption during the MLF

The peptidase activity of *O. oeni* can release amino acids from mannoproteins and other macromolecules, thus increasing the nutritional content and survival of *O. oeni* in wine (Jamal et al., 2013; Jin et al., 2014). The preference of *O. oeni* for peptides as a nitrogen source has been reported. LAB can breakdown these peptides, releasing free amino acids into the wine, which they subsequently consume (Remize et al., 2006). Consequently, they can also utilize preexisting free amino acids in wine, as reported by Roca-Mesa et al. (2020). The protein parts of *S. cerevisiae* mannoproteins (representing up to 10 % of the molecule) are rich in serine and threonine (Klis et al., 2002; Lipke and Ovalle, 1998; Orlean, 2012). To evaluate the possible metabolism of the protein moiety of MP extracts by *O. oeni*, the concentration of amino acids was determined by HPLC at the beginning and end of the MLF. The differences found in amino acid concentrations at the end of the MLF with respect to the initial wine were very similar for the three *O. oeni* strains in each fermentation condition. For this reason, the average of all the values obtained for *O. oeni* 217^T, PSU-1 and VP41 were considered to illustrate the common behaviour of all the strains, represented as amino acid consumption (Fig. 4). For a more understandable interpretation of the results, the detected amino acids were grouped into five categories according to their physicochemical characteristics (see Fig. 4, A–E). Significant differences in amino acid consumption and release (shown as negative values) with respect to the control conditions were mainly found for aspartic acid (Fig. 4D). In all the fermentations with the addition of MP extracts, there was a very slight increase in this amino acid at the end of the MLF with respect to the initial wine (between 2 and 5 mg/L released), whereas in the control without MP addition, there was a decrease in aspartic acid content, indicating its consumption, at the end of the MLF (approximately 5 mg/L). Aspartic acid is present in some components of the WLM (casamino acids and peptone) used and might be used by *O. oeni* in control fermentations without MP, which are relatively poor in nitrogen. However, even if the differences observed are statistically significant, the detected values are quite low, meaning that aspartic acid is not one of the most relevant amino acid sources of nitrogen. In fact, other amino acids were much more consumed (Fig. 4), but in most cases, there were no differences between the fermentations with MP addition and the control. Punctual differences were observed for some fermentations with added MP2 and MP1. With the addition of 2 g/L MP2, the consumption of positively charged (Fig. 4A) and uncharged (Fig. 4B) polar amino acids slightly differed from that of the control. However, it should be noted that the consumption of these amino acids was observed in all the cases, including the control. When 1 g/L of added MP1 was added, hydrophobic amino acids (Fig. 4E) were released, whereas the control showed no changes in the concentrations of these amino acids, and the other fermentations with added MP showed some consumption.

Taken together, the observed differences in amino acid utilization cannot be correlated with mannoprotein addition and metabolism, as most amino acids did not significantly differ from those in the control condition. Proteomic analysis revealed that proteins related to amino acid metabolism in *O. oeni* generally decreased in abundance in the presence of mannoprotein extracts (Artículo proteómica). These findings suggest that, although mannoproteins contain an amino acid fraction, they do not activate amino acid transport or metabolism in *O. oeni*. In detail, threonine and serine are present mostly in mannoproteins (in Fig. 4B, approximately 35 mg/L of consumption), and there were no significant changes in their consumption due to the addition of any of the three MP products. Therefore, we can conclude that the amino acids present in MP are not responsible for the differences observed in MLF duration. The utilization of the polysaccharide part of the MP would be the most feasible explanation for the improvement in *O. oeni* metabolic

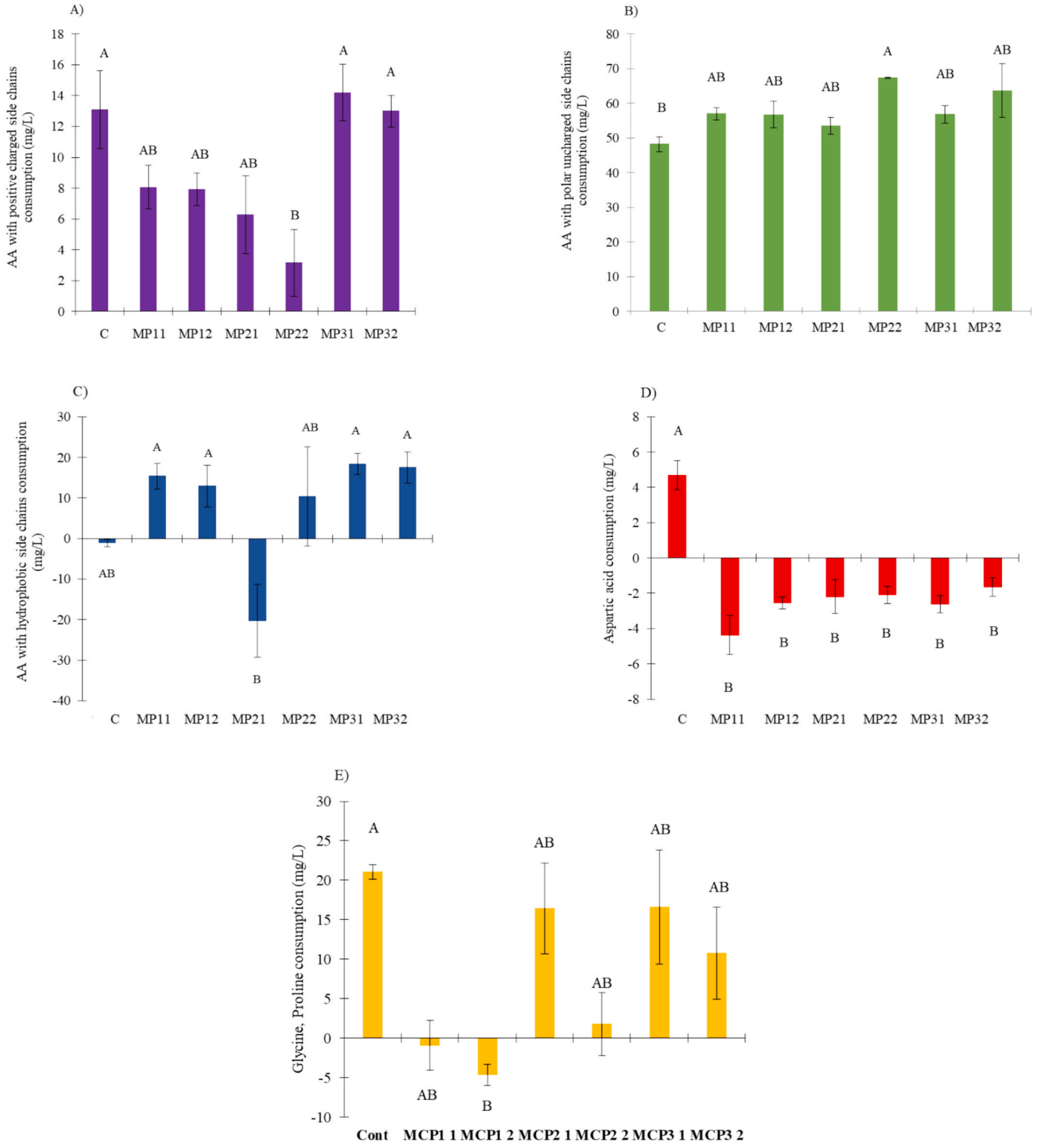

Fig. 4. Average of the differences in amino acid concentration present at the end of the MLF with respect to the initial wine, indicated as amino acid consumption (mg/L), for the three *O. oeni* strains (217^{T} (1), PSU-1 (2) and VP41 (3)) with the addition of 1 g/L (1) or 2 g/L (2) of the commercial mannoprotein extracts. The detected amino acids are grouped into five categories: A) amino acids (AA) with positive charged side chain (Arg, His and Lys), B) amino acids (AA) with polar uncharged side chain (Ser, Thr and Gln), C) amino acids (AA) hydrophobic side chain (Ala, Val, Ile, Leu, Met, Phe, Tyr and Trp), D) aspartic acid and E) glycine and proline. Negative values indicate release of amino acids. In each category (A–E), the represented value is the sum of concentrations of the amino acids included in the group. Different uppercases letters indicate a significant difference between values using the Tukey (HSD) test at p-value < 0.05. All data are expressed as the arithmetic average of three biological replicates ± standard deviation.

performance in the MLF.

3.5. Chemical composition of wines at the end of the MLF

The addition of MP extracts did not particularly modify any of the analysed chemical parameters at the end of the MLF (Table 4). However, there were some significant differences between the three *O. oeni* strains for D-lactic acid and for the final pH with 217^T, which was slightly greater for this strain, but any difference could not be associated with the addition of MP.

Variations in the final citric acid consumption were observed, primarily associated with the malolactic fermentation rate rather than the direct consumption of mannoproteins. A slower fermentation rate correlated with higher citric acid consumption (Bartowsky and Henschke, 2004), which was particularly evident for the *O. oeni* 217 T strain when MP3 extract was added. In contrast, PSU-1 strain, which exhibited faster malolactic fermentation with the addition of extracts compared to the control, showed lower citric acid consumption. The VP41 *O. oeni* strain displayed the greatest variability in citric acid consumption relative to the control.

There were no significant differences between strains for L-lactic acid, acetic acid, NOPA or NH_4^+. Mannose released from mannoprotein consumption did not result in an increase in D-lactic acid or acetic acid, as could be expected due to *O. oeni* heterofermentative metabolism. This sugar can be converted into fructose-6-P and then into glucose-6-P (Cibrario et al., 2016; Jamal et al., 2013), after which it can be further metabolized to other central metabolites, such as pyruvate, which can be directed to different pathways depending on the needs of the cell. However, more exhaustive studies are needed to elucidate the metabolic fate of mannose from mannoprotein consumption in *O. oeni*.

4. Conclusions

This study provides a detailed characterization of different mannoprotein extracts for oenological use and their impact on MLF and different *O. oeni* strains in WLM. Characterization of the mannoprotein extracts revealed significant differences in the MW distribution and mannose content among the studied products. The profile of MP2 clearly exhibited a predominant peak with a very broad MW range, from 532 to 3 kDa, whereas the percentages of small-sized fractions were more representative of those of MP1 and MP3. These differences in mannoprotein composition influenced the development of the MLF, with the effect of mannoprotein addition being dependent on both the *O. oeni* strain and the concentration of the added extract.

While *O. oeni* PSU-1 completed MLF faster with the addition of all the mannoprotein extracts, *O. oeni* VP41 and 217^T showed different responses, with MLF activation occurring when MP1 and MP2 were added but being inhibited with the addition of MP3. Mannoprotein consumption also differed with 1 g/L addition but was complete at a concentration of 2 g/L for all strains, with significant differences between strains and products.

In summary, this work provides new information about the potential use of yeast mannoprotein extracts as activators of the MLF. The results emphasize the varying impacts on the MLF depending on both the strain and the MP extract. Structural variations among mannoprotein extracts, particularly in molecular weight and composition, influence the metabolic activity of *O. oeni* strains during MLF. These differences are evident in the duration and efficiency of malic acid consumption, underscoring

Table 4
Chemical composition of wines after MLF with the addition of MP1, MP2 and MP3 (1 g/L and 2 g/L) for the three *O. oeni* strains. Average ± standard deviation (n = 3). Different lower-case letters indicate a significant difference between MP1, MP2 and MP3 and uppercases letters between the *O. oeni* (217^T, PSU-1 and VP41) strains values using the Tukey (HSD) test at $p < 0.05$. pHf: final pH. NOPA: Primary Amino Nitrogen.

	pH_f	L-Lactic (g/L)	D-Lactic (g/L)	Citric acid (mg/L)	Acetic acid (g/L)	NOPA (mg/L)	NH_4^+ (mg/L)
O. oeni strain 217^T							
Control	3.91 ± 0.01^{Abc}	1.50 ± 0.09^{Abc}	0.14 ± 0.01^{Aab}	88.00 ± 1.00^{Aa}	0.44 ± 0.02^{Abc}	126 ± 2.65^{Aa}	13 ± 1.15^{Aab}
MP1							
1	3.89 ± 0.00^{Aab}	1.64 ± 0.26^{Ac}	0.16 ± 0.02^{Ab}	103.33 ± 2.08^{Aab}	0.47 ± 0.07^{Ac}	131 ± 4.00^{Abc}	11 ± 3.79^{Aab}
2	3.90 ± 0.01^{Ac}	1.54 ± 0.24^{Ac}	0.17 ± 0.02^{Aab}	134.33 ± 2.08^{Ac}	0.46 ± 0.06^{Ac}	143 ± 1.00^{Ad}	9 ± 2.65^{Aab}
MP2							
1	3.90 ± 0.03^{Aab}	1.47 ± 0.18^{Abc}	0.13 ± 0.01^{Aa}	119.67 ± 1.53^{Aabc}	0.43 ± 0.04^{Aabc}	129 ± 1.15^{Aab}	5 ± 1.73^{Aa}
2	3.85 ± 0.02^{Aa}	1.39 ± 0.04^{Abc}	0.12 ± 0.01^{Aa}	102.00 ± 2.65^{Aabc}	0.41 ± 0.01^{Abc}	126 ± 0.58^{Ab}	11 ± 2.31^{Aab}
MP3							
1	3.88 ± 0.00^{Aab}	1.49 ± 0.03^{Aab}	0.13 ± 0.02^{Aab}	74.67 ± 5.03^{Abc}	0.36 ± 0.06^{Aab}	154 ± 7.00^{Acd}	19 ± 1.53^{Ab}
2	3.83 ± 0.00^{Aab}	1.44 ± 0.05^{Aa}	0.13 ± 0.02^{Aab}	50.00 ± 2.00^{Aabc}	0.34 ± 0.01^{Aa}	155 ± 3.06^{Ad}	16 ± 3.06^{Aab}
O. oeni strain PSU-1							
Control	3.91 ± 0.01^{Bbc}	1.66 ± 0.20^{Abc}	0.08 ± 0.01^{Bab}	88.33 ± 1.15^{Ba}	0.46 ± 0.06^{Abc}	125 ± 2.00^{Aa}	9 ± 2.65^{Aab}
MP1							
1	3.80 ± 0.00^{Bab}	1.53 ± 0.06^{Ac}	0.12 ± 0.01^{Bb}	127.67 ± 2.52^{Bab}	0.47 ± 0.02^{Ac}	137 ± 2.65^{Abc}	10 ± 3.00^{Aab}
2	3.83 ± 0.01^{Bc}	1.64 ± 0.15^{Ac}	0.10 ± 0.01^{Bab}	159.33 ± 8.39^{Bc}	0.46 ± 0.02^{Ac}	145 ± 3.06^{Ad}	10 ± 2.00^{Aab}
MP2							
1	3.78 ± 0.01^{Bab}	1.34 ± 0.08^{Abc}	0.05 ± 0.02^{Ba}	102.00 ± 2.65^{Babc}	0.37 ± 0.03^{Aabc}	123 ± 0.58^{Aab}	8 ± 3.51^{Aa}
2	3.83 ± 0.05^{Ba}	1.55 ± 0.03^{Abc}	0.07 ± 0.01^{Ba}	98.67 ± 1.16^{Babc}	0.45 ± 0.01^{Abc}	132 ± 4.04^{Ab}	11 ± 1.16^{Aab}
MP3							
1	3.77 ± 0.06^{Bab}	1.56 ± 0.4^{Aab}	0.07 ± 0.01^{Bab}	163.67 ± 5.51^{Bbc}	0.43 ± 0.10^{Aab}	131 ± 2.08^{Acd}	10 ± 4.73^{Ab}
2	3.81 ± 0.02^{Bab}	1.33 ± 0.03^{Aa}	0.08 ± 0.01^{Bab}	133.00 ± 2.65^{Babc}	0.39 ± 0.01^{Aa}	142 ± 2.52^{Ad}	11 ± 2.52^{Aab}
O. oeni strain VP41							
Control	3.91 ± 0.02^{Bbc}	1.43 ± 0.04^{Abc}	0.12 ± 0.01Cab	115.00 ± 1.00^{Aa}	0.40 ± 0.02^{Abc}	123 ± 0.58^{Aa}	12 ± 1.00^{Aab}
MP1							
1	3.83 ± 0.02^{Bab}	1.62 ± 0.13^{Ac}	0.14 ± 0.01^{Cb}	168.33 ± 7.64^{Aab}	0.44 ± 0.04^{Ac}	138 ± 2.65^{Abc}	14 ± 2.65^{Aab}
2	3.90 ± 0.03Bc	1.59 ± 0.12^{Ac}	0.13 ± 0.01^{Cab}	151.67 ± 0.58^{Ac}	0.44 ± 0.02^{Ac}	145 ± 1.00^{Ad}	11 ± 2.00^{Aab}
MP2							
1	3.90 ± 0.01^{Bab}	1.56 ± 0.09^{Abc}	0.11 ± 0.02^{Ca}	97.67 ± 2.52^{Aabc}	0.43 ± 0.02^{Aabc}	131 ± 1.00^{Ab}	8 ± 0.58^{Aa}
2	3.82 ± 0.03^{Ba}	1.49 ± 0.05^{Abc}	0.11 ± 0.01^{Ca}	99.33 ± 2.31^{Aabc}	0.42 ± 0.01^{Abc}	134 ± 3.79^{Ab}	10 ± 4.93^{Aab}
MP3							
1	3.90 ± 0.01^{Bab}	1.45 ± 0.02^{Aab}	0.12 ± 0.02^{Cab}	95.00 ± 5.00^{Abc}	0.37 ± 0.01^{Aab}	136 ± 2.31^{Acd}	12 ± 1.15^{Ab}
2	3.91 ± 0.01^{Bab}	1.48 ± 0.03^{Aa}	0.11 ± 0.01^{Cab}	97.33 ± 2.08^{Aabc}	0.37 ± 0.01^{Aa}	147 ± 1.00^{Ad}	8 ± 4.51^{Aab}

the importance of selecting mannoproteins with suitable structural characteristics to optimize the MLF process.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2025.111401.

CRediT authorship contribution statement

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

Acknowledgments

This work was supported by the Ministry of Science, Innovation and Universities (project PID2021-124943OB-I00/AEI/10.13039/501100011033/FEDER, UE). PT is grateful for the predoctoral fellowship 2019PMF-PIPF-44 from the Martí Franquès program at Universitat Rovira i Virgili (Tarragona, Spain). MOA thanks Junta de Castilla y León, cofunded by Consejería de Educación and Fondo Social Europeo Plus (FSE +), for her predoctoral contract.

Data availability

Data will be made available on request.

References

Alexandre, H., Costello, P.J., Remize, F., Guzzo, J., Guilloux-Benatier, M., 2004. *Saccharomyces cerevisiae–Oenococcus oeni* interactions in wine: current knowledge and perspectives. Int. J. Food Microbiol. 93 (2), 141–154. https://doi.org/10.1016/j.ijfoodmicro.2003.10.013.

Aredes Fernández, P.A., Saguir, F.M., Manca de Nadra, M.C., 2004. Effect of dipeptides on the growth of *Oenococcus oeni* in synthetic medium deprived of amino acids. Curr. Microbiol. 49 (5), 361–365. https://doi.org/10.1007/s00284-004-4367-7.

Balmaseda, A., Aniballi, L., Rozès, N., Bordons, A., Reguant, C., 2021. Use of yeast mannoproteins by *Oenococcus oeni* during malolactic fermentation under different oenological conditions. Foods 10 (7), 1540. https://doi.org/10.3390/foods10071540.

Balmaseda, A., Rozès, N., Bordons, A., Reguant, C., 2022. Molecular adaptation response of *Oenococcus oeni* in non-*Saccharomyces* fermented wines: a comparative multi-omics approach. Int. J. Food Microbiol. 362, 109490. https://doi.org/10.1016/j.ijfoodmicro.2021.109490.

Balmaseda, A., Miot-Sertier, C., Lytra, G., Poulain, B., Reguant, C., Lucas, P., Nioi, C., 2024. Application of white wine lees for promoting lactic acid bacteria growth and malolactic fermentation in wine. Int. J. Food Microbiol. 413, 110583. https://doi.org/10.1016/j.ijfoodmicro.2024.110583.

Bartowsky, E.J., 2005. *Oenococcus oeni* and malolactic fermentation – moving into the molecular arena. Aust. J. Grape Wine Res. 11 (2), 174–187. https://doi.org/10.1111/j.1755-0238.2005.tb00286.x.

Bartowsky, E.J., Henschke, P.A., 2004. The 'buttery' attribute of wine—diacetyl—desirability, spoilage and beyond. Int. J. Food Microbiol. 96 (3), 235–252. https://doi.org/10.1016/j.ijfoodmicro.2004.05.013.

Bicca, S., Poncet-Legrand, C., Williams, P., Mekoue Nguela, J., Doco, T., Vernhet, A., 2022. Structural characteristics of *Saccharomyces cerevisiae* mannoproteins: impact of their polysaccharide part. Carbohydr. Polym. 277, 118758.

Bicca, S., Poncet-Legrand, C., Roi, S., Mekoue, J., Doco, T., Vernhet, A., 2023. Exploring the influence of *S. cerevisiae* mannoproteins on wine astringency and color: Impact of their polysaccharide part. Food Chem. 422, 136160. https://doi.org/10.1016/j.foodchem.2023.136160.

Bordas, M., Araque, I., Bordons, A., Reguant, C., 2005. Differential expression of selected *Oenococcus oeni* genes for adaptation in wine-like media and red wine. Ann Microbiol 65, 2277–2285. https://doi.org/10.1007/s13213-015-1069-2.

Caridi, A., 2006. Enological functions of parietal yeast mannoproteins. Antonie Van Leeuwenhoek 89 (3–4), 417–422. https://doi.org/10.1007/s10482-005-9050-x.

Cibrario, A., Peanne, C., Lailheugue, M., Campbell-Sills, H., Dols-Lafargue, M., 2016. Carbohydrate metabolism in *Oenococcus oeni*: a genomic insight. BMC Genomics 17 (1), 984. https://doi.org/10.1186/s12864-016-3338-2.

De Man, J.C., Rogosa, M., Sharpe, M.E., 1960. A medium for the cultivation of Lactobacilli. J. Appl. Bacteriol. 23 (1), 130–135. https://doi.org/10.1111/j.1365-2672.1960.tb00188.x.

Díez, L., Guadalupe, Z., Ayestarán, B., Ruiz-Larrea, F., 2010. Effect of yeast mannoproteins and grape polysaccharides on the growth of wine lactic acid and acetic acid bacteria. J. Agric. Food Chem. 58 (13), 7731–7739. https://doi.org/10.1021/jf100199n.

Dols-Lafargue, M., Gindreau, E., Le Marrec, C., Chambat, G., Heyraud, A., Lonvaud-Funel, A., 2007. Changes in red wine soluble polysaccharide composition induced by malolactic fermentation. J. Agric. Food Chem. 55 (23), 9592–9599. https://doi.org/10.1021/jf071677+.

Dupin, I.V., Stockdale, V.J., Williams, P.J., Jones, G.P., Markides, A.J., Waters, E.J., 2000. *Saccharomyces cerevisiae* mannoproteins that protect wine from protein haze: Evaluation of extraction methods and immunolocalization. J. Agric. Food Chem. 48 (4), 1086–1095. https://doi.org/10.1021/jf9905020.

Gil, M., Kontoudakis, N., González, E., Esteruelas, M., Fort, F., Canals, J.M., Zamora, F., 2012. Influence of grape maturity and maceration length on color, polyphenolic composition, and polysaccharide content of Cabernet Sauvignon and Tempranillo wines. J. Agric. Food Chem. 60 (32), 7988–8001. https://doi.org/10.1021/jf302064n.

Giovani, G., Rosi, I., Bertuccioli, M., 2012. Quantification and characterization of cell wall polysaccharides released by non-*Saccharomyces* yeast strains during alcoholic fermentation. Int. J. Food Microbiol. 160 (2), 113–118. https://doi.org/10.1016/j.ijfoodmicro.2012.10.007.

Gómez-Alonso, S., Hermosín-Gutiérrez, I., García-Romero, E., 2007. Simultaneous HPLC analysis of biogenic amines, amino acids, and ammonium ion as aminoenone derivatives in wine and beer samples. J. Agric. Food Chem. 55 (3), 608–613. https://doi.org/10.1021/jf062820m.

Gonzalez-Ramos, D., Cebollero, E., Gonzalez, R., 2008. A recombinant *Saccharomyces cerevisiae* strain overproducing mannoproteins stabilizes wine against protein haze. Appl. Environ. Microbiol. 74 (17), 5533–5540. https://doi.org/10.1128/AEM.00302-08.

Guilloux-Benatier, M., Guerreau, J., Feuillat, M., 1995. Influence of initial colloid content on yeast macromolecule production and on the metabolism of wine microorganisms. Am. J. Enol. Vitic. 46 (4), 486–492. https://doi.org/10.5344/ajev.1995.46.4.486.

Jamal, Z., Miot-Sertier, C., Thibau, F., Dutilh, L., Lonvaud-Funel, A., Ballestra, P., Le Marrec, C., Dols-Lafargue, M., 2013. Distribution and functions of phosphotransferase system genes in the genome of the lactic acid bacterium *Oenococcus oeni*. Appl. Environ. Microbiol. 79 (11), 3371–3379. https://doi.org/10.1128/AEM.00380-13.

Jin, G., Wang, H., Zhang, C., Li, C., Du, L.-Y., Grbin, P., Li, H., 2014. Characterization and amino acid metabolism performances of indigenous *Oenococcus oeni* isolated from Chinese wines. Eur. Food Res. Technol. 238, 597–605. https://doi.org/10.1007/s00217-013-2112-9.

Klis, F.M., Mol, P., Hellingwerf, K., Brul, S., 2002. Dynamics of cell wall structure in *Saccharomyces cerevisiae*. FEMS Microbiol. Rev. 26 (3), 239–256. https://doi.org/10.1111/j.1574-6976.2002.tb00613.x.

Lesage, G., Bussey, H., 2006. Cell wall assembly in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. 70 (2), 317–343. https://doi.org/10.1128/MMBR.00038-05.

Li, S., Wilkinson, K.L., Bindon, K.A., 2018. Compositional variability in commercial tannin and mannoprotein products. Am. J. Enol. Vitic. 69 (2), 176–181. https://doi.org/10.5344/ajev.2017.17057.

Lipke, P.N., Ovalle, R., 1998. Cell wall architecture in yeast: new structure and new challenges. J. Bacteriol. 180 (15), 3735–3740. https://doi.org/10.1128/JB.180.15.3735-3740.1998.

Liu, S.-Q., 2002. Malolactic fermentation in wine – beyond deacidification. J. Appl. Microbiol. 92 (4), 589–601. https://doi.org/10.1046/j.1365-2672.2002.01589.x.

Liu, Y., Rousseaux, S., Tourdot-Maréchal, R., Sadoudi, M., Gougeon, R., Schmitt-Kopplin, P., Alexandre, H., 2017. Wine microbiome: a dynamic world of microbial interactions. Crit. Rev. Food Sci. Nutr. 57 (4), 856–873. https://doi.org/10.1080/10408398.2014.983591.

Lowry, H., Rosebrough, N.J., Farr, A.L., Randall, RoseJ., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193 (1), 265–275. https://doi.org/10.1016/S0021-9258(19)52451-6.

Lubbers, S., Voilley, A., Feuillat, M., Charpentier, C., 1994. Influence of mannaproteins from yeast on the aroma intensity of a model wine. LWT Food Sci. Technol. 27 (2), 108–114. https://doi.org/10.1006/fstl.1994.1025.

Manjón, E., Brás, N.F., García-Estévez, I., Escribano-Bailón, M.T., 2020. Cell wall mannoproteins from yeast affect salivary protein–flavanol interactions through different molecular mechanisms. J. Agric. Food Chem. 68 (47), 13459–13468. https://doi.org/10.1021/acs.jafc.9b08083.

Margalef-Català, M., Felis, G.E., Reguant, C., Stefanelli, E., Torriani, S., Bordons, A., 2017. Identification of variable genomic regions related to stress response in

Oenococcus oeni. Food Res. Int. 102, 625–638. https://doi.org/10.1016/j.foodres.2017.09.039. Ottawa, Ont.
Martínez Lapuente, L., Guadalupe, Z., Ayestarán, B., 2019. Properties of Wine Polysaccharides. https://doi.org/10.5772/intechopen.85629.
Olguín, N., Bordons, A., Reguant, C., 2009. Influence of ethanol and pH on the gene expression of the citrate pathway in *Oenococcus oeni*. Food Microbiol. 26 (2), 197–203. https://doi.org/10.1016/j.fm.2008.09.004.
Olguín, N., Alegret, J.O., Bordons, A., Reguant, C., 2011. β-Glucosidase activity and bgl gene expression of *Oenococcus oeni* strains in model media and Cabernet Sauvignon wine. Am. J. Enol. Vitic. 62 (1), 99–105. https://doi.org/10.5344/ajev.2010.10044.
Orlean, P., 2012. Architecture and biosynthesis of the *Saccharomyces cerevisiae* cell wall. Genetics 192 (3), 775–818. https://doi.org/10.1534/genetics.112.144485.
Oyón-Ardoiz, M., Manjón, E., Escribano-Bailón, M.T., García-Estévez, I., 2022. Effect of mannoproteins from different oenological yeast on pigment composition and color stability of red wine. LWT 172, 114219. https://doi.org/10.1016/j.lwt.2022.114219.
Oyón-Ardoiz, M., Manjón, E., Escribano-Bailón, M.T., García-Estévez, I., 2024. Potential use of *Torulaspora delbrueckii* as a new source of mannoproteins of oenological interest. J. Agric. Food Chem. 72 (20), 11606–11616. https://doi.org/10.1021/acs.jafc.4c01001.
Poncet-Legrand, C., Doco, T., Williams, P., Vernhet, A., 2007. Inhibition of grape seed tannin aggregation by wine mannoproteins: effect of polysaccharide molecular weight. Am. J. Enol. Vitic. 58 (1), 87–91. https://doi.org/10.5344/ajev.2007.58.1.87.
Quirós, M., Morales, P., Pérez-Través, L., Barcenilla, J.M., González, R., 2011. A new methodology to determine cell wall mannoprotein content and release in wine yeasts. Food Chem 125 (2), 760–766. https://doi.org/10.1016/j.foodchem.2010.08.066.
Remize, F., Gaudin, A., Kong, Y., Guzzo, J., Alexandre, H., Krieger, S., Guilloux-Benatier, M., 2006. *Oenococcus oeni* preference for peptides: qualitative and quantitative analysis of nitrogen assimilation. Arch. Microbiol. 185 (6), 459–469. https://doi.org/10.1007/s00203-006-0116-6.
Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., Lonvaud, A., 2006. Handbook of Enology, Volume 1: The Microbiology of Wine and Vinifications. John Wiley & Sons.
Rinaldi, A., Gonzalez, A., Moio, L., Gambuti, A., 2021. Commercial mannoproteins improve the mouthfeel and colour of wines obtained by excessive tannin extraction. Molecules 26 (14), 14. https://doi.org/10.3390/molecules26144133.
Roca-Mesa, H., Sendra, S., Mas, A., Beltran, G., Torija, M.-J., 2020. Nitrogen preferences during alcoholic fermentation of different non-*Saccharomyces* yeasts of oenological interest. Microorganisms 8 (2), 157. https://doi.org/10.3390/microorganisms8020157.
Rodrigues, A., Ricardo-Da-Silva, J.M., Lucas, C., Laureano, O., 2012. Effect of commercial mannoproteins on wine colour and tannins stability. Food Chem. 131 (3), 907–914. https://doi.org/10.1016/j.foodchem.2011.09.075.
Semon, M.J., Edwards, C.G., Forsyth, D., Dinn, C., 2001. Inducing malolactic fermentation in Chardonnay musts and wines using different strains of *Oenococcus oeni*. Aust. J. Grape Wine Res. 7 (1), 52–59. https://doi.org/10.1111/j.1755-0238.2001.tb00194.x.
Snyman, C., Mekoue Nguela, J., Sieczkowski, N., Marangon, M., Divol, B., 2021. Optimised extraction and preliminary characterisation of mannoproteins from non-*Saccharomyces* wine yeasts. Foods 10 (5), 924. https://doi.org/10.3390/foods10050924.
Snyman, C., Mekoue Nguela, J., Sieczkowski, N., Divol, B., Marangon, M., 2023. Characterization of mannoprotein structural diversity in wine yeast species. J. Agric. Food Chem. 71 (49), 19727–19738. https://doi.org/10.1021/acs.jafc.3c05742.
Toraño, P., Gombau, J., Mejías, I., Bordons, A., Rozès, N., Reguant, C., 2024. Evaluation of the addition of yeast mannoprotein to *Oenococcus oeni* starter cultures to improve wine malolactic fermentation. Fermentation 10 (1), 52. https://doi.org/10.3390/fermentation10010052.
Toraño, P., Martín-García, A., Bordons, A., Rozès, N., Reguant, C., 2025a. Enhancing wine malolactic fermentation: variable effect of yeast mannoproteins on *Oenococcus oeni* strains. Food Microbiol. 127, 104689. https://doi.org/10.1016/j.fm.2024.104689.
Toraño, P., Balmaseda, A., Bordons, A., Rozès, N., Reguant, C., 2025b. Proteomic insight into the beneficial effect of mannoproteins on *Oenococcus oeni* in wine malolactic fermentation. Food Microbiol. 129, 104754. https://doi.org/10.1016/j.fm.2025.104754.
Vejarano, R., 2020. Non-*Saccharomyces* in winemaking: source of mannoproteins, nitrogen, enzymes, and antimicrobial compounds. Fermentation 6 (3), 3. https://doi.org/10.3390/fermentation6030076.
Vidal, S., Williams, P., Doco, T., Moutounet, M., Pellerin, P., 2003. The polysaccharides of red wine: total fractionation and characterization. Carbohydr. Polym. 54 (4), 439–447. https://doi.org/10.1016/S0144-8617(03)00152-8.
Vidal, S., Francis, L., Williams, P., Kwiatkowski, M., Gawel, R., Cheynier, V., Waters, E., 2004. The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. Food Chem. 85 (4), 519–525. https://doi.org/10.1016/S0308-8146(03)00084-0.
Yang, K., Dai, X., Fan, M., Zhang, G., 2021. Influences of acid and ethanol stresses on *Oenococcus oeni* SD-2a and its proteomic and transcriptional responses. J. Sci. Food Agric. 101 (7), 2892–2900. https://doi.org/10.1002/jsfa.10921.
Zang, H., Xie, S., Zhu, B., Yang, X., Gu, C., Hu, B., Gao, T., Chen, Y., Gao, X., 2019. Mannan oligosaccharides trigger multiple defence responses in rice and tobacco as a novel danger-associated molecular pattern. Mol. Plant Pathol. 20, 1067–1079. https://doi.org/10.1111/mpp.12811.