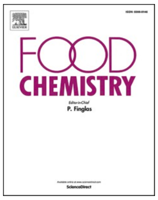
Food Chemistry 430 (2024) 137044

Contents lists available at ScienceDirect

Food Chemistry

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



Supramolecular study of the interaction between mannoproteins from *Torulaspora delbrueckii* and flavanols

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ARTICLE INFO

Keywords:
Wine
Mannoproteins
Non-*Saccharomyces* yeast
Torulaspora delbrueckii
Flavanols
Astringency

ABSTRACT

In this work, three mannoprotein extracts were obtained from *T. delbrueckii* by enzymatic and chemical treatments. The obtained mannoprotein extracts showed important differences in their molecular weight distribution and monosaccharide composition, although no significant differences were found in their protein content. In order to evaluate the possible influence of mannoprotein characteristics in the interaction with flavanols, mannoprotein-flavanol interactions were studied by HPLC-DAD-MS and ITC. The results obtained indicate that the mannoprotein extracts were able to precipitate flavanols to a different extent. Furthermore, the degree of flavanol precipitation seemed not to be related to the affinity of the interaction but to the type of intermolecular forces. In this sense, a higher proportion of hydrogen bonding could favor a greater crosslinking between aggregates promoting flavanol precipitation. This, in turn, could be related to the MP characteristics since the presence of β-glucan moieties might have an effect on the formation of hydrogen bonds.

1. Introduction

Phenolic compounds are essential for the quality of plant-derived foods and beverages such as wine. In wine, two families of phenolic compounds are especially relevant: anthocyanins, which are responsible for the color of red wine, and flavanols, who contribute to bitterness and astringency (Cheynier et al., 2006). In the last years, global climate change is having a profound impact on vine phenology and grape composition. The increasing temperatures can modify biochemical and physiological processes impacting berry levels of sugar, acids and phenolic compounds. This modification of grape composition could lead to wines with high alcohol content, low acidity, altered color and unbalanced astringency (Droulia & Charalampopoulos, 2021; Santos et al., 2020).

Astringency can be defined as a drying, roughing and puckering sensation in the oral cavity and is considered a quality attribute of red wines when is balanced with other factors (namely, alcohol and sugar content). However, high levels of astringency are not desirable since they can result in a wine that can be described as “harsh”, “unripe” or “green” (Gawel, 1998). Nowadays, flavanol-salivary protein interaction and/or precipitation is considered the main contributing factor for astringency development (Soares et al., 2017). Flavanol-salivary protein interactions can be affected by multiple factors like the structure of both the flavanol and the salivary protein, the characteristics of the wine matrix (pH and ethanol concentration) and the presence of polysaccharides (García-Estévez et al., 2018).

Polysaccharides are macromolecules found in relevant concentrations in wine (200–1500 mg/mL) that can be extracted and/or released from grapes and yeast during the winemaking process (Guadalupe et al., 2014). Mannoproteins (MPs) derived from yeast constitute one of the most abundant polysaccharides in red wine (Vidal et al., 2003b). These glycoproteins are mainly found in the outermost layer of the yeast cell wall and are characterized by being heavily glycosylated with mannose residues (Lipke & Ovalle, 1998). MPs are naturally released by yeast into the wine during fermentative and post-fermentative processes (Guadalupe et al., 2014). Moreover, the addition of MPs to wine is an oenological practice permitted by the OIV and multiple commercial products rich in MPs can be found in the market. These commercial yeast derivative products are often obtained from *Saccharomyces cerevisiae* (Pozo-Bayón et al., 2009). Given that some studies suggest that MPs can affect flavanol-salivary protein interactions (Manjón et al., 2020, 2021), the addition to wine of MPs could constitute an oenological tool to diminish the excessive astringency that can result from climate change impacts on grapevine. Once MPs are present in wine, interactions with other components of the wine matrix, mainly polyphenols such as flavanols, are expected to occur. In fact, some authors have reported that MPs can

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https://doi.org/10.1016/j.foodchem.2023.137044
Received 2 March 2023; Received in revised form 2 July 2023; Accepted 26 July 2023

effectively interact with wine tannins (Manjón et al., 2020; Nguela et al., 2016), although the affinity between MPs and flavanols is different depending on the type of MP (Manjón et al., 2020). In this sense, Manjón et al. (2020) suggested that MPs with higher protein content and of bigger size could interact more effectively with flavanols. Furthermore, some studies have shown that MPs can prevent or limit the aggregation of tannins due to the potential role of MPs as protective colloids. However, again, the stabilization of tannin particles seemed to depend on the characteristics of the MPs, since smaller MPs and MPs with a glucose/mannose ratio close to one seemed to be more effective in stabilizing tannin particles (Charpentier et al., 2004; Poncet-Legrand et al., 2007). Nguela et al. (2016) found that the interaction of MPs with wine polyphenols resulted in the formation of stable colloidal dispersions preventing aggregates precipitation and the formation of haze. Conversely, Guadalupe et al. (2007), Guadalupe and Ayestarán (2008) reported that the addition of commercial MPs and the use of MP overexpressing yeast strains conduced to wines with lower polyphenol content, indicating the precipitation of MP-polyphenol complexes. In agreement with this, Manjón et al. (2021) observed the precipitation of flavanols after the interaction with two commercial MPs and reported that the size of the aggregates formed was conditioned by the type of MP. In this way, these authors suggested that MPs with higher protein content could bind a higher amount of flavanols resulting in the formation of bigger complexes that could lead to the precipitation of flavanols over time.

In view of these studies, the mechanisms in which the MPs interact with flavanols as well as their effects on wine colloidal state remain unclear since some authors have showed that MPs can produce the precipitation of wine flavanols while others suggest that they could act as colloidal stabilizers. Establishing structure–activity relationships for MPs is of high interest for the oenologist since it would allow an effective use of MPs in the wineries. However, no simple relations are established yet possibly due to the high heterogeneity of MPs. Indeed, the characteristics of the MPs are highly variable because they depend on multiple factors like the yeast species, the yeast strain and the method of extraction (Charpentier et al., 2004; Oyón-Ardoiz et al., 2022; Snyman et al., 2021).

In grape musts, other yeast species besides *Saccharomyces cerevisiae* are naturally present. Originally, these non-*Saccharomyces* yeasts were not desired in wine since they were seen as responsible for microbial-related problems. However, in recent years, the use of non-*Saccharomyces* yeasts in mixed or sequential fermentations with *S. cerevisiae* has been reconsidered as a possible way of improving the technological and sensory properties of wine (Jolly et al., 2014). Among non-*Saccharomyces* yeasts, *Torulaspora delbrueckii* is one of the most commercialized and used in industrial fermentations since this yeast typically produces wines with lower ethanol concentrations, increases glycerol and polysaccharides content and provides better aromatic properties (Benito, 2018). Actually, some authors have shown that non-*Saccharomyces* yeast, including *T. delbrueckii*, could have a high capacity of releasing MPs to wine during alcoholic fermentation (Domizio et al., 2014; Giovani et al., 2012). In a recent work carried out in our laboratory (Oyón-Ardoiz et al., 2022), MPs from *T. delbrueckii* were, among the MPs extracted from other yeast species, the most effective ones for the stabilization of wine coloring matter, suggesting that this yeast could constitute an interesting source of MPs with potential use for the modulation of wine organoleptic properties. Moreover, the use of this yeast as a new source of MPs could provide the oenological industry with MPs that could show differential characteristics from those normally obtained from *S. cerevisiae*. However, at the present day, there is a lack of knowledge about the way in which the MPs derived from *T. delbrueckii* interact with flavanols as well as the potential modifications on wine flavanol composition that these interactions could entail. Thus, in this work different treatments were applied to *T. delbrueckii* aiming to the extraction of MP with different structure and composition. In addition, the interactions of the obtained MP extracts and a soluble grape seed flavanol extract were studied in order to deepen into the relationship between MP structure and composition and the mechanisms of MP-flavanol interactions.

2. Materials and methods

2.1. Extraction of MPs from *T. delbrueckii*

MPs were extracted from a commercial strain of *T. delbrueckii* (BIODIVA, Lallemand Inc., Montreal, Canada). The active dry yeast was rehydrated following the steps provided by the manufacturer. Briefly, the cells were rehydrated in 10 times its weight of water at 30 °C and, then, the suspension was gently agitated for 15 min at the same temperature. The suspension was centrifuged (1290 g, 5 min) and the cells were resuspended in liquid yeast extract peptone dextrose (YPD) at 25 g/hL (as the inoculation concentration in wine specified by the manufacturer). After 24 h of incubation at 30 °C, 200 μL of the liquid culture was plated in YPD plates, which were incubated at 30 °C for 48 h and then stored at 4 °C until further use. Yeasts from an isolated colony were inoculated in liquid YPD and cultivated at 30 °C with agitation (90 rpm) for 12 h to obtain a pre-culture. Then, yeasts derived from this pre-culture were inoculated at 0.01 OD_{600nm} in liquid YPD and cultivated at 30 °C with agitation (90 rpm) for 24 h until $OD_{600nm} \sim 14–16$. Cells were collected by centrifugation (1290 g, 10 min) and washed three times with distilled water. The obtained biomass was subjected to three different procedures for the extraction of MPs: induced autolysis (MP-A), Zymolyase extraction (MP-Z) and alkali extraction (MP-B).

i) Extraction of MPs by induced autolysis (MP-A). First, biomass was subjected to induced autolysis by a modification of the procedure proposed by Liu et al. (2008). The wet biomass was suspended in NaCl 3% (*w/v*) at a solid concentration of 15% (*w/v*) and the autolysis of yeast cells was performed for 24 h at 55 °C with agitation (120 rpm). Then, the suspension was heated to 80 °C for 15 min to inactivate the cellular enzymes. Autolyzed cells were collected by centrifugation (5170 g, 15 min) and washed two times with NaCl 3% (*w/v*). The supernatants were mixed and MPs liberated in the autolysis process were precipitated in 70% (*v/v*) ethanol. The precipitates were washed three times with 70% (*v/v*) ethanol and freeze-dried.

The autolyzed cells were subjected, separately, to enzymatic and chemical hydrolysis:

ii) Extraction of MPs by Zymolyase (MP-Z). Enzymatic hydrolysis was performed with Zymolyase 20T (US Biological, Salem, MA, USA) using 60 mg of enzyme per gram of wet biomass. The hydrolysis took place at 45 °C for 90 min. Then, the suspension was centrifuged (5170 g, 10 min) and the Zymolyase present in the supernatant was inactivated at 60 °C for 10 min. After enzyme inactivation, the polymeric material in the supernatant was precipitated in 70% (*v/v*) ethanol. The precipitates were washed three times with 70% (*v/v*) ethanol and freeze-dried. The freeze-dried extract was dissolved in water at 5 mg/mL and heated to 80 °C for 15 min to produce the denaturalization of the enzyme. The solutions were cooled in an ice bath in order to eliminate the Zymolyase boosting its precipitation and centrifuged twice (20,700 g, 10 min, 4 °C). The purified supernatants were freeze-dried.

iii) Extraction of MPs by chemical hydrolysis (MP-B). The extraction of MPs by chemical hydrolysis was carried out following a modification of the method by Kim and Yun (2006). The autolyzed cells were suspended in NaOH 2% (*w/v*) at a solid concentration of 15% (*w/v*) and the suspension was heated for 1 h at 90 °C. Then, the suspension was cooled at room temperature and centrifuged (5170 g, 10 min). After adjusting the pH of the supernatant to neutrality with HCl 3 M, the MPs were precipitated in 70% (*v/v*) ethanol. The precipitates were washed three times with 70% (*v/v*) ethanol and freeze-dried.

Finally, the three MP extracts obtained were dissolved in water and dialyzed in 6–8 kDa molecular weight cut off membranes for 72 h at 10 °C against 5 L of distilled water with periodic water changes.

2.2. Characterization of the MP extracts

The protein and glycoprotein profiles of the MP extracts were visualized by SDS-PAGE using 4–15% linear gradient Mini-PROTEAN® TGX™ precast gels from Bio-Rad Laboratories Inc. (Hercules, CA, USA). The extracts were dissolved in water at a concentration of 37.5 mg/mL, diluted 1:1 with sample loading buffer 2X (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. Then 16 μL of each sample were loaded into the gel and electrophoresis was carried out 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, ThermoFisher Scientific, Germany) and glycoproteins were detected following the Periodic Acid-Schiff (PAS) staining method. The result of both staining processes was scanned using a Lexmark XC4140 scanner (Lexington, KY, USA). Molecular weights (MW) were estimated using a protein ladder (PageRuler™ Plus Prestained Protein Ladder, 10–250 kDa, ThermoFisher Scientific, Germany).

Total protein concentration of the extracts was determined by Lowry method (Lowry et al., 1951) using the DC Protein assay kit (Bio-Rad Laboratories Inc.) and a bovine serum albumin calibration curve. Total protein content (*w/w*) was calculated from the concentration data.

The MW distribution of the MPs was determined by HRSEC-RID in an Agilent 1260 Infinity (Agilent Technologies, Palo Alto, CA, USA) following the method described by Manjón et al. (2020). Briefly, an HPLC system 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) was employed. The MW calibration curve was constructed using several pullulan standards of MW between 342 Da and 805,000 Da.

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

2.3. Grape seed flavanol extract

Grape seeds were manually separated from skins and pulp of *Vitis vinifera* L. Tempranillo grapes harvested at maturity. Flavanol extraction was performed following a modification of the method proposed by García-Marino et al. (2006). Briefly, grape seeds were freeze-dried and grounded to obtain a homogeneous powder. 4 g of the powder were extracted with 160 mL of ethanol/water (75:25). The mixture was homogenized for 30 s and ultrasounds were applied in an ultrasonic water bath for 15 min. The suspension was centrifuged (16500 g, 10 min, 10 °C) and the extraction process was repeated twice. The supernatants were mixed, concentrated under vacuum and freeze-dried. In order to obtain a flavanol profile similar to that present in wine, the seed extract was purified in a C18 column following a modification of the method proposed by Manjón et al. (2021). The extract was dissolved in 80 mL of ultrapure water, loaded in the C18 column and eluted with 30% ethanol (*v/v*). The purified extract was concentrated under vacuum and analyzed by HPLC-DAD-MS following the method proposed by García-Estévez et al. (2017) by using the same HPLC-DAD-MS equipment than for the monosaccharide analysis.

2.4. Evaluation of MP-flavanol interactions by HPLC-DAD-MS^2 and HRSEC-RID

Interaction assays were carried out between the seed flavanol extract and the three MP extracts. The flavanol and the MP extracts were dissolved in water and 150 μL of each solution were mixed giving a final concentration of 0.8 and 2 mg/mL, respectively. Likewise, the corresponding controls were prepared by mixing 150 μL of flavanol solution or 150 μL of MP solution with 150 μL of ultrapure water. Interactions took place at room temperature for 10 min. Afterwards, the samples were centrifuged (13,800 g, 5 min) and the supernatants were immediately analyzed by HRSEC-RID and HPLC-DAD-MS^2 following the methodology described in the previous sections. All interactions assays were performed in triplicate.

2.5. Evaluation of MP-flavanol interactions by Isothermal Titration Calorimetry (ITC)

ITC analyses were performed in a MicroCal PEAQ-ITC system (SYS80004) (Malvern, U. K.) at a constant temperature of 298.15 K. The MP extracts were loaded into the sample cell at a concentration of 100 μM for MP-A and MP-Z and 26.7 μM for MP-B, and the flavanol extract was used as the titrant at a concentration of 4 mM. Concentrations were set to achieve the saturation of the process as well as sufficient energy signal. Blank experiments (flavanol/water and MP/water) were also performed. The titrant was injected into the sample cell in a sequence of 19 injections of 2 μL each with 150 s of space between injections and an initial delay of 300 s. The agitation of the sample cell was set at 1200 rpm. ITC data was fitted to obtain the binding isotherms with the software AFFINImeter (Software for Science Development, Santiago de Compostela, Spain) using an independent sites model with two sets of sites. Blank experiments were subtracted from the interaction experiments. All experiments were performed in triplicate.

2.6. Statistical analysis

The statistical analysis was performed using the IBM-SPSS Statistics 26 software. The statistical significance of the differences was evaluated by a one-way analysis of variance (ANOVA) followed by a post-hoc Tukey-B test. In the case of the changes in flavanol composition determined by HPLC-DAD-MS and the changes in chromatographic profile of MPs determined by HRSEC-RID, differences among the control and the interactions with the MP extracts were determined by a Student's *t*-test. In all cases, differences were considered significant when $p < 0.05$.

3. Results and discussion

3.1. Characterization of the MP extracts

The analysis of the MP extracts by SDS-PAGE (see Fig. S1 in the Supporting Information) showed that the three extraction treatments applied led to the solubilization of MPs from the cell wall of *T. delbrueckii* (as it can be seen by the pink bands stained with the Schiff reagent in the electrophoresis gel), as well as to the extraction of some non-glycosylated proteins (blue bands stained with Coomassie Brilliant Blue). The glycoprotein bands were situated above the 250 kDa standard indicating the high MW of the released MPs. It is important to note that the profile of the MPs released in the autolysis of yeast cells differ significantly from the other extracts in the sense that the glycoprotein band is concentrated in the beginning of the lane, indicating possible structural differences between MP-A and the other two MP extracts.

HRSEC-RID analysis showed differences in the MW distribution of the obtained MP extracts, corroborating the existence of important structural differences among them. Table 1 shows the MW distribution

Table 1
MW distribution and average MW (kDa) determined by HRSEC-RID of the MPs contained in the extracts and total protein content (*w/w*). MP-A: MP extract obtained by induced autolysis; MP-Z: MP extract obtained by Zymolyase extraction; MP-B: MP extract obtained by alkali extraction. Different letters within each column indicate significant differences ($p < 0.05$).

	MW (kDa)	%	Average MW (kDa)	Protein content (%)
MP-A	902–171	27.7	129 ± 2 a	3.4 ± 0.3 a
	171–58	7.7		
	58–27	11.0		
	27–2	53.6		
MP-Z	481–190	16.3	116 ± 2 b	3.5 ± 0.2 a
	190–35	70.1		
	35–4	13.6		
MP-B	376–11	94.5	94 ± 5 c	4.2 ± 0.6 a
	11–3	5.5		

and average MW of the MPs contained in the extracts as well as the total protein content. Just as it was observed in SDS-PAGE analysis (see Fig. S1 in the Supporting Information), the chromatographic profile of MP-A pointed out that this extract was the one that differed more from the rest (see Fig. S2 in the Supporting Information). According to the average MW of the extracts (Table 1), the largest MPs were found in MP-A extract (average MW *ca.* 129 kDa) whereas MP-B extract contained the smallest MPs (average MW *ca.* 94 kDa). In fact, autolysis of yeast cells led to the solubilization of an important fraction (27.7%) of MPs of high MW (between 902 and 171 kDa). However, this extraction method also resulted in a great liberation of MPs of low MW, which represent the majority of the MPs extracted (53.6%). On the contrary, in MP-Z and MP-B extracts, the MPs of low MW represent a lesser part of the total (13.6 and 5.5%, respectively). Regarding the enzymatic hydrolysis of the yeast cell wall, most MPs obtained in this process (70.1%) had an intermediate MW comprised between 190 and 35 kDa. With respect to the chemical hydrolysis of the cell wall, the great majority of the MPs extracted (94.5%) were distributed in a main peak of MW between 376 and 11 kDa.

The wide range of MW observed showed the polydispersity of the MP extracts that can be obtained and is in agreement with other authors. For example, Doco et al. (2003) showed that the MPs released during wine ageing on lees had MWs between 5 and 400 kDa. Moreover, Manjón et al. (2020) found that the MW of three commercial MP preparations ranged between 207.5 kDa and oligosaccharides of less than 10 monosaccharide units. Vidal et al. (2003b) also reported that MPs MW ranged from 50 to 530 kDa when they characterized the total polysaccharide content of red wine.

The quantification of total protein content by Lowry method (Lowry et al., 1951) (Table 1) showed low protein percentages (<4.2%) in the MP extracts with no significant differences among them. Therefore, the three extraction methods applied led to the solubilization of MPs that present a small protein fragment. The protein fraction of the MPs can represent up to 50% (*w/w*) (Lipke & Ovalle, 1998) and variable protein percentages are reported in the literature. For example, Manjón et al. (2020) showed protein percentages ranging from 10 to 30% for three commercial MPs. Giovani et al. (2012) reported that the polysaccharides released by *T. delbrueckii* during alcoholic fermentation of synthetic must exhibited 18% (*w/w*) protein. On the contrary, Domizio et al. (2014) reported lower protein content (1.5%) for the polysaccharides released by *T. delbrueckii* in the fermentation of synthetic must. The low protein content found in the extracts could result from a partial fragmentation of the protein fraction of the MPs during the extraction treatments. Indeed, autolysis is characterized by the action of proteases upon the cellular components (Alexandre & Guilloux-Benatier, 2006; Takalloo et al., 2020), Zymolyase presents protease activity besides its main action as β-1,3-glucanase and the peptide bond can be hydrolyzed under alkaline conditions.

Monosaccharide composition analysis performed by HPLC-DAD-MS revealed important differences among the extracts (Table 2). It should be noted the predominance of mannose (>74.6%) in all the extracts obtained, indicating the release of MPs from the cell wall of *T. delbrueckii* by the three treatments conducted. However, there were significant differences in the proportion of mannose of the extracts. MP-B had the highest presence of mannose (96%) followed by MP-Z (86.3%) and MP-A (74.6%). In the case of MP-Z, it should be highlighted the higher content of glucose (12.5%) in comparison with MP-A and MP-B (<3%). The higher presence of glucose in MP-Z could be due to the β-1,3-glucanase activity of Zymolyase that can release fragments of β-glucan from the cell wall and/or fragments composed of a MP connected to a portion of β-glucan. With regard to MP-A, the autolysis of *T. delbrueckii* resulted in an important presence of ribose (22.1%), which could be explained by the release of nucleic acids during the autolytic process (Alexandre & Guilloux-Benatier, 2006; Takalloo et al., 2020). On the contrary, only traces of ribose can be found in MP-Z and this monosaccharide was not detected in MP-B. Xylose was also detected in all the extracts obtained, but in low proportion (<1%).

Overall, the results of the MPs characterization point to the existence of important differences in the structure and composition of the MPs extracted from *T. delbrueckii* by the applied treatments.

3.2. *Evaluation of MP-flavanol interactions by HPLC-DAD-MS^2 and HRSEC-RID*

The HPLC-DAD-MS analysis of the seed flavanol extract allowed the identification of a total of 42 procyanidins: 2 monomers, 9 dimers (4 of them galloylated), 14 trimers (6 of them galloylated), 14 tetramers (5 of them galloylated) and 3 pentamers. The flavanol extract was mainly composed of oligomers, representing the dimers and trimers 67.08% and 25.76% of the total, respectively (Table S1 in the Supporting Information). The galloylated flavanols accounted for 12.55% and the mean degree of polymerization (mDP) of the extract was 2.29, similar to that present in Tempranillo red wines (Quijada-Morín et al., 2012).

In the interaction assays with MPs (Fig. 1), a significant decrease in total flavanol concentration was observed. This could be due to the formation of insoluble MP-flavanol aggregates that precipitate. Indeed, Manjón et al. (2021) reported the precipitation of flavanols caused by the interaction with two commercial MPs and detected the formation of MP-flavanol aggregates by Dynamic Light Scattering. However, it is important to note significant differences in the effect of the MP extracts assayed. MP-Z was the extract that produced the highest precipitation of flavanols (20.5%). Conversely, the interaction with MP-A only led to the precipitation of 4.4% of total flavanols, while MP-B had an intermediate effect (13.8%). The effect of MP extracts on flavanol concentration indicate that they could act as fining agents in wine removing flavanols from solution (mainly MP-Z and MP-B), which could contribute to modulate wine astringency, due to the positive correlation between tannins concentration and astringency that has been reported (Pavez et al., 2022). In the interaction assays with the three MP extracts, changes in the content of non-galloylated flavanols in comparison with

Table 2
Monosaccharide composition of the MP extracts. Man: mannose; Glc: glucose; Rib: ribose; Xyl: xylose. MP-A: MP extract obtained by induced autolysis; MP-Z: MP extract obtained by Zymolyase extraction; MP-B: MP extract obtained by alkali extraction. Different letters within each row indicate significant differences ($p < 0.05$).

	MP-A	MP-Z	MP-B
Man (%)	74.6 ± 0.4 c	86.3 ± 0.2 b	96.0 ± 0.1 a
Glc (%)	2.2 ± 0.2 c	12.5 ± 0.2 a	3.0 ± 0.04 b
Rib (%)	22.1 ± 0.3 a	0.2 ± 0.1 b	–
Xyl (%)	1.1 ± 0.1 a	1.1 ± 0.2 a	1.0 ± 0.1 a

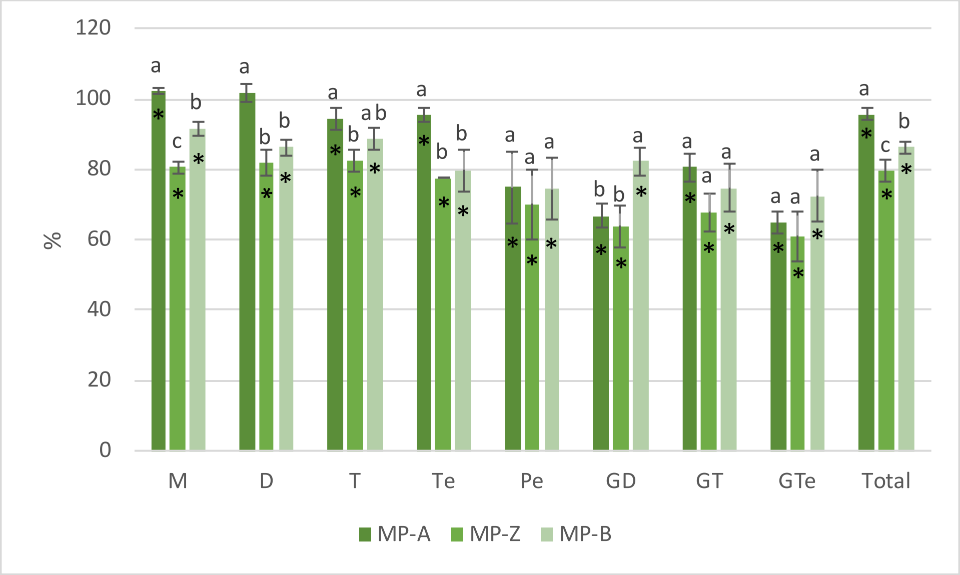

Fig. 1. Percentage (%) of flavanols with respect to the control after the interaction with the MP extracts. M: monomers; D: dimers; T: trimers; Te: tetramers; Pe: pentamers; GD: galloylated dimers; GT: galloylated trimers; GTe: galloylated tetramers. Different letters within each type of flavanols indicate significant differences ($p < 0.05$) among MP extracts. Significant differences ($p < 0.05$) between the control and the MP-flavanol interactions are indicated with an asterisk.

the control followed a similar trend than those produced in the total flavanol content, since this type of flavanols represented 87.45% of the total. However, this trend was not maintained when it comes to galloylated flavanols (Fig. 2). In this way, MP-A did not lead to significant changes in the concentration of non-galloylated flavanols with respect to the control, but produced a significant decrease in the content of galloylated flavanols that was even more pronounced than the produced by MP-B. Unlike MP-A, MP-B had similar effects in the content of both types of flavanols, with no significant differences (data not shown). MP-Z also significantly reduced the content of both types of flavanols with respect to the control, being the precipitation of galloylated flavanols after the interaction with this extract higher than that produced by MP-B. The differential effect of the MP extracts over galloylated and non-galloylated flavanols highlights the importance of flavanol structure in the interaction with MPs and suggests differences in the affinity of the extracted MPs for both types of flavanols. In this sense, MP-B would have less substrate specificity (since the precipitation of galloylated and non-galloylated flavanols is similar) than MP-Z and, specially, MP-A (that precipitated preferentially galloylated flavanols). Manjón et al. (2021) have reported that two commercial MPs bound preferentially to non-galloylated flavanols and proposed that this fact could be explained by the aminoacid composition of MPs since salivary proteins interact effectively with galloylated flavanols and are characterized by being rich in proline, glycine and glutamine residues. In line with this hypothesis, the different selectivity of the obtained MPs for non-galloylated and galloylated flavanols could result from a different aminoacidic composition of their protein fragment. On the other hand, the higher precipitation of galloylated flavanols produced by MP-A and MP-Z could indicate that the addition of these extracts to red wines might favor a decrease in wine astringency, since flavanol esterification with gallic acid enhances the interaction with salivary proteins and, therefore, the developing of astringency sensation (Soares et al., 2017, 2018).

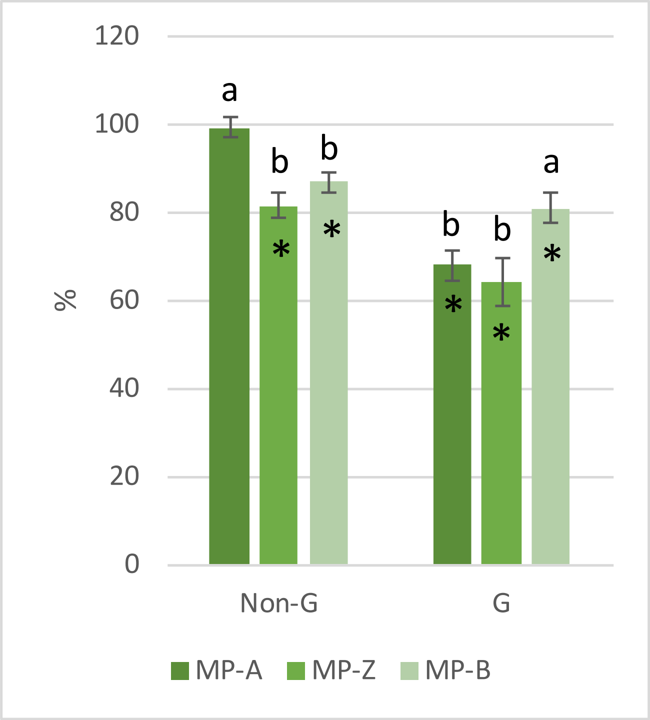

Fig. 2. Percentage (%) of non-galloylated (non-G) and galloylated (G) flavanols with respect to the control after the interaction with the MP extracts. Different letters within each type of flavanols indicate significant differences ($p < 0.05$) among MP extracts. Significant differences ($p < 0.05$) between the control and the MP-flavanol interactions are indicated with an asterisk.

Regarding flavanol degree of polymerization (DP), some differences in the effect of the MP extracts on the content of proanthocyanidin monomers, dimers, trimers, tetramers and pentamers can be observed (Fig. 1). The interaction with MP-A led to a slight increase in the content of monomers and dimers, that was statistically significant in the case of monomers. The interaction with this MP also produced a significant precipitation of trimers, tetramers and pentamers. The decrease in the content of trimers and tetramers was slight (~5%), being more pronounced in the case of pentamers (25.2%). In contrast to MP-A, MP-Z and MP-B caused a significant precipitation of all families of non-galloylated flavanols. The precipitation of non-galloylated monomers, dimers and trimers by MP-Z was similar (19.4, 18.2 and 17.6%, respectively), increasing in the case of tetramers (22.5%) and, specially, pentamers (29.9%). Unlike MP-Z, MP-B only precipitated 8.4% of monomers. With respect to dimers and trimers, the decrease in the content of these flavanols after the interaction with MP-B was similar (13.6 and 11.4%, respectively), being greater the precipitation of tetramers (20.2%) and pentamers (25.6%). The greater precipitation of tetramers and pentamers could be explained by the increase in the number of interaction sites with the increase in the polymer weight, leading to a higher crosslinking between aggregates and, therefore, the formation of larger complexes that would precipitate. In the case of galloylated proanthocyanidins, MP-A and MP-Z followed a similar trend, since the interaction with these MPs caused a greater precipitation of galloylated dimers (33.1 and 36.1%, respectively) than galloylated trimers (19.4 and 32.2%, respectively). However, the precipitation of galloylated flavanols after the interaction with MP-B increased linearly with the DP. Some authors have found that the perceived astringency increase with the DP of proanthocyanidins (Sun et al., 2013; Vidal et al., 2003a). In this sense, the higher precipitation of pentamers by all MP extracts and also tetramers by MP-Z and MP-B suggests that the addition to wines of the assayed extracts could lead to a decrease in astringency sensation by removing high DP proanthocyanidins from solution. Nevertheless, some authors point out that the relation between flavanol DP and the perceived astringency is not clear (Quijada-Morín et al., 2014).

Besides studying the changes in flavanol content, the changes in the MPs profile caused by the interaction with flavanols were analyzed by HRSEC-RID (Table 3). The results of this analysis were in agreement with the previous findings. After the interaction with proanthocyanidins, MP-Z was the MP that precipitated the most (3.41%) followed by MP-B (1.19%). On the contrary, after the interaction with flavanols, a slight but significant increase in the total content of MPs from MP-A extract was observed (1.85%) as well as in the MPs of MW between 58 and 27 kDa (10%). This was accompanied by a slight but significant increase in the content of monomers, suggesting that MP-A could enhance the stability in solution of flavanol monomers. Poncet-Legrand et al. (2007) found that MPs are able to stabilize tannin particles, being low (51 kDa) and medium (62 kDa) MW MPs more efficient than high MW (337 kDa) polymers as these first polymers would cover the surface of tannin colloids inhibiting their aggregation. Therefore, the significant increase in the area of MPs of MW 58–27 kDa could suggest that these low MW MPs could be able to inhibit tannin aggregation (specially, monomers) to some extent. However, MP-A also presented an important content (27.7%) of high MW MPs (902–171 kDa) and its average MW was higher than that of MP-Z, being this latter extract the one that led to the highest flavanol precipitation. This indicates that there must be other factors implicated besides the MW of the MPs that lead to the inhibition of tannin aggregation. MP-Z presented a significantly higher content of glucose (12.5%) than MP-B (3.0%) indicating the release of β-glucan from the cell wall. The presence of β-glucan and/or MPs connected to a fragment of β-glucan could result in a higher formation of insoluble MP-flavanol aggregates. In a recent work carried out in our laboratory (Oyón-Ardoiz et al., 2022), MP extracts with intermediate content of glucose were more effective for the colloidal stabilization of wine pigments than MP extracts with low presence of β-glucan. Moreover, Charpentier et al. (2004) found that MPs with a mannose/glucose ratio close to one were more efficient for the stabilization of tannins. Therefore, it seems that there is not a simple relation between the content of glucose in MP extracts and their effect on the stability in solution of wine polyphenols. Regarding the protein fragment of the MPs, Manjón et al. (2021) suggested that MPs with higher protein content (~30%) could be more effective in precipitating flavanols (specially, non-galloylated flavanols with low DP) than MPs with lower protein percentages (~10%). In the present work, no significant differences were found in the protein content of the MP extracts, but their ability to precipitate flavanols differed notably. This point out that other MP characteristics, besides their protein percentage, could also constitute important factors affecting the formation of MP-flavanol insoluble aggregates.

Table 3
Percentage (%) of the chromatographic areas determined by HRSEC-RID with respect to the control after the interaction of the MP extracts with flavanols. MP-A: MP extract obtained by induced autolysis; MP-Z: MP extract obtained by Zymolyase extraction; MP-B: MP extract obtained by alkali extraction. Significant differences ($p < 0.05$) between the control and the MP-flavanol interactions are indicated with an asterisk.

	MW (kDa)	%
MP-A	902–171	99.3 ± 3.6
	171–58	111.5 ± 8.6
	58–27	110.0 ± 1.7*
	27–2	100.1 ± 1.8
	Total	101.8 ± 1.1*
MP-Z	481–190	95.0 ± 3.4
	190–35	99.2 ± 0.5*
	35–4	85.1 ± 8.4*
	Total	96.6 ± 1.8*
MP-B	376–11	97.9 ± 1.2*
	11–3	113.8 ± 7.6*
	Total	98.8 ± 1.1

Overall, these results indicate that the three MP extracts obtained exhibit a different behavior depending not only on flavanol structure (galloylation and DP) but also on the composition and structure of the MPs. Therefore, it could be expected that the addition of the obtained MP extracts could be useful for wine astringency modulation, although possibly having different impacts on this organoleptic property.

3.3. *Evaluation of MP-flavanol interactions by ITC*

In order to characterize the thermodynamics of MP-flavanol interactions that could help explaining the precipitation previously observed, ITC experiments were carried out. From ITC data, free energy change (ΔG), apparent binding constant (K), change in enthalpy (ΔH) and change in entropy ($-T\Delta S$) for the interaction of flavanols with the MP extracts were determined and are shown in Table 4. The negative ΔG value for all interactions indicate that they take place spontaneously. The more negative ΔG value for the interaction of flavanols with MP-B could indicate the higher affinity of this MP extract for proanthocyanidins. On the contrary, MP-Z interacted with flavanols with lesser affinity

Table 4
Thermodynamic parameters for the interactions between flavanols and the MP extracts. MP-A: MP extract obtained by induced autolysis; MP-Z: MP extract obtained by Zymolyase extraction; MP-B: MP extract obtained by alkali extraction. Different letters within each row indicate significant differences ($p < 0.05$).

	MP-A	MP-Z	MP-B
ΔG_{tot} (cal/mol)	$(-15.711 \pm 0.086) \times 10^3$ b	$(-12.080 \pm 0.134) \times 10^3$ a	$(-27.721 \pm 0.142) \times 10^3$ c
	Set 1		
K_1 (M^{-1})	$(2.209 \pm 0.264) \times 10^6$ b	$(1.362 \pm 0.125) \times 10^5$ b	$(3.051 \pm 0.156) \times 10^{10}$ a
ΔH_1 (cal/mol)	$(-6.490 \pm 0.399) \times 10^2$ a	$(-1.802 \pm 0.254) \times 10^3$ b	$(-2.484 \pm 0.139) \times 10^3$ c
$-T\Delta S_1$ (cal/mol)	$(-8.002 \pm 0.107) \times 10^3$ b	$(-5.200 \pm 0.267) \times 10^3$ a	$(-1.182 \pm 0.087) \times 10^4$ c
	Set 2		
K_2 (M^{-1})	$(1.514 \pm 0.288) \times 10^5$ b	$(5.317 \pm 0.766) \times 10^3$ b	$(6.879 \pm 0.354) \times 10^9$ a
ΔH_2 (cal/mol)	$(-3.090 \pm 0.487) \times 10^2$ b	$(-5.386 \pm 0.786) \times 10^2$ c	$(-6.781 \pm 0.255) \times 10^1$ a
$-T\Delta S_2$ (cal/mol)	$(-6.750 \pm 0.150) \times 10^3$ b	$(-4.539 \pm 0.161) \times 10^2$ a	$(-1.335 \pm 0.069) \times 10^4$ c

than the other two MP extracts though it led to the greatest flavanol precipitation. Hence, it seems that a greater affinity of the interaction was not linked to a greater formation of insoluble aggregates. Indeed, MP-A only produced the precipitation of 4.4% flavanols but interacted with flavanols more effectively than MP-Z. As aforementioned, MP-A seems to slightly promote the stability of flavanol monomers. This could indicate that this MP extract could form soluble complexes with low DP flavanols in wine that remain stable in solution. However, further studies will be necessary to determine the characteristics of the aggregates formed.

The different affinities observed for the interactions between the three MP extracts and flavanols could be related to differences in the characteristics of the MPs. This is in agreement with Manjón et al. (2020), who studied the interactions between a grape seed flavanol extract and three commercial MPs by ITC and found that the affinity of each commercial MP for flavanols depended on the type of MP. Furthermore, these authors suggested that a larger protein portion in the MP could be related to a greater affinity for flavanols. The fact that the MP extracts assayed had no significant differences in their protein content but their affinity for flavanols differed significantly indicate that, as explained above, other MP characteristics seem also to play a role in MP affinity for flavanols. Since the protein part of MPs is likely a link between tannins and the MPs polysaccharidic part (Charpentier et al., 2004; Nguela et al., 2016), a different aminoacidic composition of the MP protein fragment could explain the different affinities observed. Other characteristics of the MPs like the degree of branching could also explain the differences in the interaction affinity. Nguela et al. (2023) found that the degree of branching impacted polyphenol adsorption by yeast cell walls and suggested that the absence of backbone branching could allow polyphenols to access sites of interactions more easily within the protein part of the molecule. Fernandes et al. (2020) studied the interaction of arabinan-rich pectic polysaccharides with polyphenols and suggested that, in the case of more branched polysaccharides, interactions decreased due to steric hindrance. In this sense, a higher degree of branching of MP-Z could explain the lesser affinity of this MP extract for flavanols. In the yeast cell wall, MPs are covalently bound to β-1,3-glucan or β-1,6-glucan through their protein fragment (Orlean, 2012). As explained before, cell wall hydrolysis with Zymolyase could result in the liberation of MPs connected to a portion of β-glucan. The presence of a fragment of β-glucan bound to the protein moiety in the MPs could also explain the lesser affinity of MP-Z for flavanols since it may limit the access of flavanols to the binding sites of MPs due to steric hindrance. On the other hand, Nguela et al. (2016) found negligible interactions between grape skin and wine tannins with laminarin (a low MW β-(1,3)-glucan polymer with β-(1,6)-linkages). Therefore, the lesser affinity of MP-Z for flavanols could also be due to the fact that the β-glucan fragment may not be an active site for the interaction with flavanols.

The driving forces of MP-flavanol interactions can be deduced from ΔH and $-T\Delta S$ values. Positive values for ΔH and negative for $-T\Delta S$ are related to an entropic driven process where hydrophobic interactions are the main forces present. On the other hand, an enthalpy driven process (where hydrogen bonding predominate) is characterized by negative values for ΔH and positive for $-T\Delta S$. In view of Table 4, hydrophobic interactions predominate over hydrogen bonding in all interactions, except for the set 2 of the interaction with MP-Z. This MP extract had a higher contribution of hydrogen bonding than MP-B and MP-A. MP-flavanol interaction could initiate with the formation of hydrophobic interactions followed by the stabilization of the complexes formed by hydrogen bonding, in a similar way to what occur in the interaction of flavanols with salivary proteins (García-Estévez et al., 2018). The further self-association of the MP-flavanol complexes by hydrogen bonds could lead to the formation of larger aggregates that precipitate. Consequently, the higher contribution of hydrogen bonding in the interaction with MP-Z could explain the greatest precipitation of flavanols produced by this MP extract. This suggests that the formation of insoluble aggregates seemed more dependent on the types of intermolecular forces present (*i.e.* the ratio of hydrophobic interactions/hydrogen bonding) than on the affinity of the interaction. The different proportion of hydrophobic interactions and hydrogen bonding could also be related to the characteristics of the MPs. Fernandes et al. (2020) proposed that for more branched polysaccharides interactions are mostly established via multiple hydrogen bonding. Thus, a higher degree of branching and/or the presence of a fragment of β-glucan bound to the protein moiety in MP-Z could promote the interaction with flavanols through hydrogen bonding. In addition, a different aminoacidic composition of MP-Z (for example, a higher proportion of polar residues) could also promote the formation of hydrogen bonds with proanthocyanidins.

4. Conclusion

The three treatments applied to *T. delbrueckii* lead to obtaining MPs that presented important structural and compositional differences. In order to deepen into the structure–activity relationships of MPs, the interactions of the three MP extracts with a grape seed flavanol extract were studied and the results obtained point out that both the structure of the flavanol and the characteristics of the MPs were relevant for the interaction. The three MP extracts produced the precipitation of proanthocyanidins to a different extent, being MP-Z the extract that removed more flavanols from solution. Moreover, MP-A and MP-Z precipitated preferentially galloylated flavanols, indicating some substrate specificity, while MP-B produced a similar precipitation of non-galloylated and galloylated flavanols. Regarding flavanol DP, the MP extracts were more effective in precipitating flavanols of higher DP (*i.e.* tetramers and pentamers). The observed effects of the MP extracts suggest that their addition to wine during winemaking or ageing could be useful to reduce by precipitation the quantity of flavanols, possibly leading to a modulation of wine astringency.

Despite leading to the greatest precipitation of flavanols, MP-Z was the MP extract that showed the least binding affinity for this substrate. Indeed, the extent of formation of insoluble MP-flavanol aggregates seemed not to depend on the affinity of the interaction but on the type of intermolecular forces present. The higher formation of hydrogen bonds in the interaction of flavanols with MP-Z could lead to a further growth of the aggregates until they reach a size in which they may become unstable and precipitate. However, further studies are necessary in order to characterize the size of the aggregates formed.

Altogether, these results showed that the formation of insoluble complexes as well as the affinity and driven forces of the interaction with flavanols depended on the type of MP. Nevertheless, no simple relationships were found between the behavior of the MPs and their protein percentage, size and monosaccharide composition.

CRediT authorship contribution statement

María Oyón-Ardoiz: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. **Elvira Manjón:** Conceptualization, Methodology, Investigation, Formal analysis, Validation, Supervision, Writing – review & editing. **María Teresa Escribano-Bailón:** Conceptualization, Methodology, Validation, Supervision, Writing – review & editing, Project administration, Funding acquisition. **Ignacio García-Estévez:** Conceptualization, Methodology, Formal analysis, Validation, Supervision, Writing – review & editing, Project administration, Funding acquisition.

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.

Data availability

Data will be made available on request.

Acknowledgements

This research was financially supported by Grant PID2021-127126OB-C21 funded by MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe". E. Manjón thanks Junta de Castilla y León-FEDER Programme her postdoctoral contract (Project Ref. SA0093P20) and M. Oyón-Ardoiz thanks Junta de Castilla y León, cofunded by Consejería de Educación and Fondo Social Europeo Plus (FSE+), her pre-doctoral contract. Thanks are also due to Junta de Castilla y León-FEDER Programme for the Strategic Research Programs for Units of Excellence (Escalera de Excelencia CLU-2O18-04).

Appendix A. Supplementary data

Supplementary data (Fig. S1. SDS-PAGE of the MP extracts obtained from T. delbrueckii. MP-A: MP extract obtained by induced autolysis; MP-Z: MP extract obtained by Zymolyase extraction; MP-B: MP extract obtained by alkali extraction. Fig. S2. Chromatographic profiles of the MPs contained in the extracts. MP-A: MP extract obtained by induced autolysis; MP-Z: MP extract obtained by Zymolyase extraction; MP-B: MP extract obtained by alkali extraction. Table S1. Flavanol composition of the grape seed extract.) to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.137044.

References

Alexandre, H., & Guilloux-Benatier, M. (2006). Yeast autolysis in sparkling wine – a review. *Australian Journal of Grape and Wine Research, 12*(2), 119–127. https://doi.org/10.1111/j.1755-0238.2006.tb00051.x

Benito, S. (2018). The impact of *Torulaspora delbrueckii* yeast in winemaking. *Applied Microbiology and Biotechnology, 102*(7), 3081–3094. https://doi.org/10.1007/s00253-018-8849-0

Charpentier, C., Escot, S., Gonzalez, E., Dulau, L., & Feuillat, M. (2004). The influence of yeast glycosylated proteins on tannins aggregation in model solution. *OENO One, 38*(4), 209. https://doi.org/10.20870/oeno-one.2004.38.4.911

Cheynier, V., Dueñas-Paton, M., Salas, E., Maury, C., Souquet, J.-M., Sarni-Manchado, P., & Fulcrand, H. (2006). Structure and Properties of Wine Pigments and Tannins. *American Journal of Enology and Viticulture, 57*(3), 298–305. https://doi.org/10.5344/ajev.2006.57.3.298

Doco, T., Vuchot, P., Cheynier, V., & Moutounet, M. (2003). Structural modification of wine arabinogalactans during aging on lees. *American Journal of Enology and Viticulture, 54*(3), 150–157. https://doi.org/10.5344/ajev.2003.54.3.150

Domizio, P., Liu, Y., Bisson, L. F., & Barile, D. (2014). Use of non-*Saccharomyces* wine yeasts as novel sources of mannoproteins in wine. *Food Microbiology, 43*, 5–15. https://doi.org/10.1016/j.fm.2014.04.005

Droulia, F., & Charalampopoulos, I. (2021). Future climate change impacts on european viticulture: A review on recent scientific advances. *Atmosphere, 12*(4), 495. https://doi.org/10.3390/atmos12040495

Fernandes, P. A. R., Le Bourvellec, C., Renard, C. M. G. C., Wessel, D. F., Cardoso, S. M., & Coimbra, M. A. (2020). Interactions of arabinan-rich pectic polysaccharides with polyphenols. *Carbohydrate Polymers, 230*, Article 115644. https://doi.org/10.1016/j.carbpol.2019.115644

García-Estévez, I., Alcalde-Eon, C., & Escribano-Bailón, M. T. (2017). Flavanol quantification of grapes via multiple reaction monitoring mass spectrometry. Application to differentiation among clones of *Vitis vinifera* L. cv. Rufete grapes. *Journal of Agricultural and Food Chemistry, 65*(31), 6359–6368. https://doi.org/10.1021/acs.jafc.6b05278

García-Estévez, I., Ramos-Pineda, A. M., & Escribano-Bailón, M. T. (2018). Interactions between wine phenolic compounds and human saliva in astringency perception. *Food & Function, 9*(3), 1294–1309. https://doi.org/10.1039/C7FO02030A

García-Marino, M., Rivas-Gonzalo, J. C., Ibáñez, E., & García-Moreno, C. (2006). Recovery of catechins and proanthocyanidins from winery by-products using subcritical water extraction. *Analytica Chimica Acta, 563*(1–2), 44–50. https://doi.org/10.1016/j.aca.2005.10.054

Gawel, R. (1998). Red wine astringency: A review. *Australian Journal of Grape and Wine Research, 4*(2), 74–95. https://doi.org/10.1111/j.1755-0238.1998.tb00137.x

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

Guadalupe, Z., & Ayestarán, B. (2008). Effect of commercial mannoprotein addition on polysaccharide, polyphenolic, and color composition in red wines. *Journal of Agricultural and Food Chemistry, 56*(19), 9022–9029. https://doi.org/10.1021/jf801535k

Guadalupe, Z., Ayestarán, B., Williams, P., & Doco, T. (2014). Determination of must and wine polysaccharides by gas chromatography-mass spectrometry (GC-MS) and size-exclusion chromatography (SEC). In K. G. Ramawat, & J.-M. Mérillon (Eds.), *Polysaccharides* (pp. 1–28). Springer International Publishing. https://doi.org/10.1007/978-3-319-03751-6_56-2.

Guadalupe, Z., Palacios, A., & Ayestarán, B. (2007). Maceration enzymes and mannoproteins: A possible strategy to increase colloidal stability and color extraction in red wines. *Journal of Agricultural and Food Chemistry, 55*(12), 4854–4862. https://doi.org/10.1021/jf063585a

Jolly, N. P., Varela, C., & Pretorius, I. S. (2014). Not your ordinary yeast: Non-*Saccharomyces* yeasts in wine production uncovered. *FEMS Yeast Research, 14*(2), 215–237. https://doi.org/10.1111/1567-1364.12111

Kim, K. S., & Yun, H. S. (2006). Production of soluble β-glucan from the cell wall of *Saccharomyces cerevisiae*. *Enzyme and Microbial Technology, 39*(3), 496–500. https://doi.org/10.1016/j.enzmictec.2005.12.020

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

Liu, X., Wang, Q., Cui, S., & Liu, H. (2008). A new isolation method of β-d-glucans from spent yeast Saccharomyces cerevisiae. *Food Hydrocolloids, 22*(2), 239–247. https://doi.org/10.1016/j.foodhyd.2006.11.008

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry, 193*(1), 265–275.

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. *Journal of Agricultural and Food Chemistry, 68*(47), 13459–13468. https://doi.org/10.1021/acs.jafc.9b08083

Manjón, E., Recio-Torrado, A., Ramos-Pineda, A. M., García-Estévez, I., & Escribano-Bailón, M. T. (2021). Effect of different yeast mannoproteins on the interaction between wine flavanols and salivary proteins. *Food Research International, 143*, Article 110279. https://doi.org/10.1016/j.foodres.2021.110279

Nguela, J. M., Poncet-Legrand, C., Sieczkowski, N., & Vernhet, A. (2016). Interactions of grape tannins and wine polyphenols with a yeast protein extract, mannoproteins and β-glucan. *Food Chemistry, 210*, 671–682. https://doi.org/10.1016/j.foodchem.2016.04.050

Nguela, J. M., Teuf, O., Assuncao Bicca, S., & Vernhet, A. (2023). Impact of mannoprotein N-glycosyl phosphorylation and branching on the sorption of wine polyphenols by yeasts and yeast cell walls. *Food Chemistry, 403*, Article 134326. https://doi.org/10.1016/j.foodchem.2022.134326

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*, Article 114219. https://doi.org/10.1016/j.lwt.2022.114219

Pavez, C., González-Muñoz, B., O'Brien, J. A., Laurie, V. F., Osorio, F., Núñez, E., … Brossard, N. (2022). Red wine astringency: Correlations between chemical and sensory features. *LWT, 154*, Article 112656. https://doi.org/10.1016/j.lwt.2021.112656

Poncet-Legrand, C., Doco, T., Williams, P., & Vernhet, A. (2007). Inhibition of grape seed tannin aggregation by wine mannoproteins: Effect of polysaccharide molecular weight. *American Journal of Enology and Viticulture, 58*(1), 87–91. https://doi.org/10.5344/ajev.2007.58.1.87

Pozo-Bayón, M. A., Andújar-Ortiz, I., & Moreno-Arribas, M. V. (2009). Scientific evidences beyond the application of inactive dry yeast preparations in winemaking. *Food Research International, 42*(7), 754–761. https://doi.org/10.1016/j.foodres.2009.03.004

Quijada-Morín, N., Regueiro, J., Simal-Gándara, J., Tomás, E., Rivas-Gonzalo, J. C., & Escribano-Bailón, M. T. (2012). Relationship between the sensory-determined astringency and the flavanolic composition of red wines. *Journal of Agricultural and Food Chemistry, 60*(50), 12355–12361. https://doi.org/10.1021/jf3044346

Quijada-Morín, N., Williams, P., Rivas-Gonzalo, J. C., Doco, T., & Escribano-Bailón, M. T. (2014). Polyphenolic, polysaccharide and oligosaccharide composition of Tempranillo red wines and their relationship with the perceived astringency. *Food Chemistry, 154*, 44–51. https://doi.org/10.1016/j.foodchem.2013.12.101

Santos, J. A., Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., Dinis, L.-T., Correia, C., … Schultz, H. R. (2020). A review of the potential climate change impacts and adaptation options for European viticulture. *Applied Sciences, 10*(9), 3092. https://doi.org/10.3390/app10093092

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

Soares, S., Brandão, E., Mateus, N., & de Freitas, V. (2017). Sensorial properties of red wine polyphenols: Astringency and bitterness. *Critical Reviews in Food Science and Nutrition, 57*(5), 937–948. https://doi.org/10.1080/10408398.2014.946468

Soares, S., García-Estévez, I., Ferrer-Galego, R., Brás, N. F., Brandão, E., Silva, M., … de Freitas, V. (2018). Study of human salivary proline-rich proteins interaction with food tannins. *Food Chemistry, 243*, 175–185. https://doi.org/10.1016/j.foodchem.2017.09.063

Sun, B., de Sá, M., Leandro, C., Caldeira, I., Duarte, F. L., & Spranger, I. (2013). Reactivity of polymeric proanthocyanidins toward salivary proteins and their contribution to young red wine astringency. *Journal of Agricultural and Food Chemistry, 61*(4), 939–946. https://doi.org/10.1021/jf303704u

Takalloo, Z., Nikkhah, M., Nemati, R., Jalilian, N., & Sajedi, R. H. (2020). Autolysis, plasmolysis and enzymatic hydrolysis of baker's yeast (Saccharomyces cerevisiae): A comparative study. *World Journal of Microbiology and Biotechnology, 36*(5), 68. https://doi.org/10.1007/s11274-020-02840-3

Vidal, S., Francis, L., Guyot, S., Marnet, N., Kwiatkowski, M., Gawel, R., … Waters, E. J. (2003a). The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. *Journal of the Science of Food and Agriculture, 83*(6), 564–573. https://doi.org/10.1002/jsfa.1394

Vidal, S., Williams, P., Doco, T., Moutounet, M., & Pellerin, P. (2003b). The polysaccharides of red wine: Total fractionation and characterization. *Carbohydrate Polymers, 54*(4), 439–447. https://doi.org/10.1016/S0144-8617(03)00152-8