# MANNOPROTEINS EXTRACTS FROM WINE LEES: CHARACTERIZATION AND IMPACT ON WINE PROPERTIES

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## Introduction

Wine lees are a sludge-like material mainly containing living and dead yeast cells and their residues and other insoluble particles that settle at the bottom of fermentation vessels (Fia, 2016). Unlike grape skins and seeds, this by-product is currently not sufficiently exploited to add value to the winemaking sector, as its treatment and disposal generally represents a cost for wineries.
In addition to the always applied ethanol extraction, researchers have recently proposed new wine lees valorisation strategies involving the recovery of tartaric acid, polyphenols and the production of microbial media supplements (De Iseppi et al., 2020; Kopsahelis et al., 2018). Nevertheless, only a few of these strategies have targeted the lees' yeast biomass, and none attempted to extract mannoproteins from it. Mannoproteins (MPs) are yeast cell wall polysaccharides containing a small quantity of protein which are increasingly applied in winemaking as they have been shown to enhance wine foam characteristics, and to stabilize wines against protein and tartrate precipitations (Blasco et al., 2011; Moine-Ledoux & Dubourdieu, 1999; Moine-Ledoux & Dubourdieu, 2002), as well as to improve wine mouthfeel. MPs-based oenological additives are currently produced starting from pure yeast biomasses grown in industrial bioreactors following an efficient but costly process. On the other hand, yeast-based by-products derived from the fermentation of beer and other alcoholic beverages were tested and proposed as an alternative source of commercial MPs (Dikit et al., 2010, 2012; Silva Araújo et al., 2014). In those studies, MPs were recovered following relatively simple and scalable extraction approaches involving physical and/or enzymatic treatments to destabilise the yeast cell walls and solubilise MPs.

In order to propose a new approach for the valorisation of wine lees, this study aimed at developing efficient methods for yeast MPs extraction from white wine lees and to assess their impacts when added back to wine.

## Methods

Three extraction protocols evaluated, whose schematic overview is reported in Figure 1. For each extraction, 5 g of freeze-dried lees deriving from the fermentation of a white wine (Sauvignon blanc) were dissolved in 40 mL of McIlvaine buffer prepared at different pH values (see below) and containing 20 mM potassium metabisulphite.
The suspension was extracted following three different treatments: *Autoclave* (pH 3.4, 121°C, 20 min), *Ultrasonication* (pH 5, 50% power, 5 min), and *Glucanex* (an enzymatic preparation with glucanase activity, Novozymes) (pH 5, 37°C, 3 h).
At the end of each treatment, the suspensions were centrifuged (5000 rpm, 4°C, 10 min) and the supernatant frozen (-18°C). After thawing, an insoluble fraction, containing tartrate crystals, was separated by a second centrifugation (5000 rpm, 4°C, 10 minutes). The obtained supernatant was

then added with pure Ethanol reaching 70% concentration (v/v) and placed overnight at -18°C. Then, the samples were centrifuged, and the resulting pellets freeze-dried.

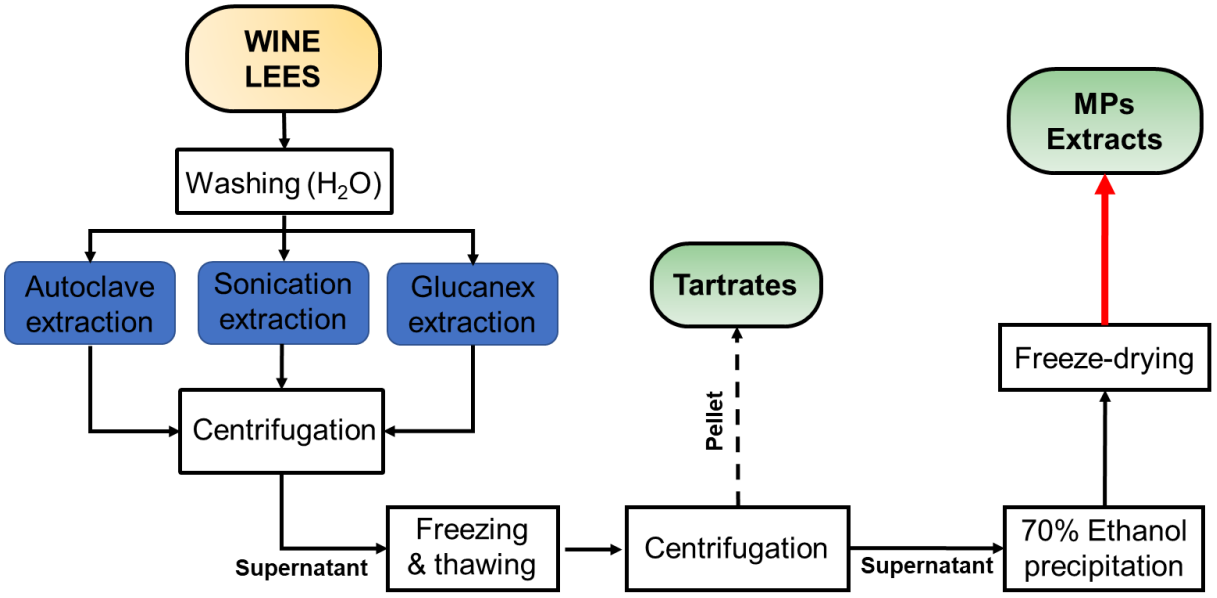


*Figure 1: Schematic representation of the different protocols for mannoproteins extraction from wine lees*

Extracts were analysed by High-Resolution Size-Exclusion Chromatography (HRSEC) for protein and polysaccharides quantification (González-Royo et al., 2017).

The impact of each extract on wine stability and foaming was assessed through the following tests.

*Tartrate stability test.* The cold stability of a tartrate-unstable wine (Pinot grigio) alone or added with 0.5 g/L of MPs extracts and a commercial MPs-preparation sold as tartrate stabiliser was analysed with the Tartarcheck apparatus (Ing. C. Bullio, Modena, Italy) as previously described (Malacarne et al., 2013).

*Protein stability test*. The protein stability of a heat-unstable wine (Sauvignon blanc) was tested in presence or absence of 0.5 g of MPs extracts. Samples were sterile filtered, and their initial turbidity measured A540 nm. Samples were then heated (80°C, 2 hours) and subsequently cooled (15°C, 20 hours) before measuring again the A540 nm turbidity. The initial turbidity value was subtracted to the final one.

*Foaming properties test*. MPs extracts were dissolved at 0.5 g/L in model wine (12% (v/v) ethanol, 2.5 g/L tartaric acid, pH 3.4), and their foamability tested by the gas sparging method (Vincenzi et al., 2014). Maximum foam height (HM), foam stability height (HS) and the time taken for the foam to collapse after gas flow interruption (TS) were measured. Model wine with no extract addition was used as the control.

## Results and discussion

The yields of the three extraction methods and chemical composition of the extracts are reported in Table 1.

*Table 1: Extraction yields, concentrations of proteins and polysaccharides (g/100 g dry lees) obtained from the three different extraction methods. Polysaccharides: High MW: 1100–180 kDa; Medium MW: 180–40 kDa; Low MW: 40–7.5 kDa; Oligosaccharides: 7.5–1 kDa. Analyses were performed in triplicate. In each column, mean values followed by the same letter are not significantly different at p ≤ 0.05 by analysis of variance (ANOVA) and Tukey's test.*

| Method | Yield | Protein | Polysaccharides | | | |
|---|---|---|---|---|---|---|
| | | | *High MW* | *Medium MW* | *Low MW* | *Oligosaccharides* |
| **Autoclave** | 18.8[a] | 6.5[a] | 2.6[a] | 3.7[a] | 3.0[a] | 90.9[b] |
| **Sonication** | 20.3[a] | 2.9[b] | 1.2[b] | 1.3[b] | 2.1[a] | 95.4[a] |
| **Glucanex** | 22.7[a] | 2.9[b] | 1.2[b] | 1.7[c] | 2.4[a] | 94.7[a] |

All the extraction protocols performed equally in terms of yield, while important differences were noted for protein and polysaccharide concentrations. In particular, the Autoclave extract showed a highest protein and high-medium molecular weight (MW) polysaccharides concentration. Considering that yeast MPs are high MW polysaccharides associated with proteins, this outcome indicates the Autoclave extract as being the most performing in terms of MPs extraction, a fact confirmed by SDS-PAGE analysis (data not shown, for more information see De Iseppi et al. (2021)). This is consistent with previous studies in which the application of high temperatures allowed the extraction of high MW MPs (Costa et al., 2012; Silva Araújo et al., 2014).
The three extraction protocols allowed also the separation of a further fraction rich in tartrate salts (see Figure 1), which were obtained in the highest quantities by the Autoclave protocol (23.6%, see De Iseppi et al. (2021)). This suggests that this Autoclave approach could offer the possibility to efficiently recover also tartaric acid, a further valuable product which finds applications in many food and non-food sectors.

Tartrate stability test results of a wine treated with the yeast lees extracts are shown in Figure 2. In this test, the Autoclave extract was the only one showing a positive impact on tartrate stability acting even much better than the commercial MPs-based wine tartrate stabiliser preparation tested. This behaviour seems to be related to the high MPs content of this extract compared to the other two. Therefore, despite being not sufficient to fully stabilize the wine, the Autoclave extract shows be a promising tool to stabilise wine applications against tartrate precipitation.

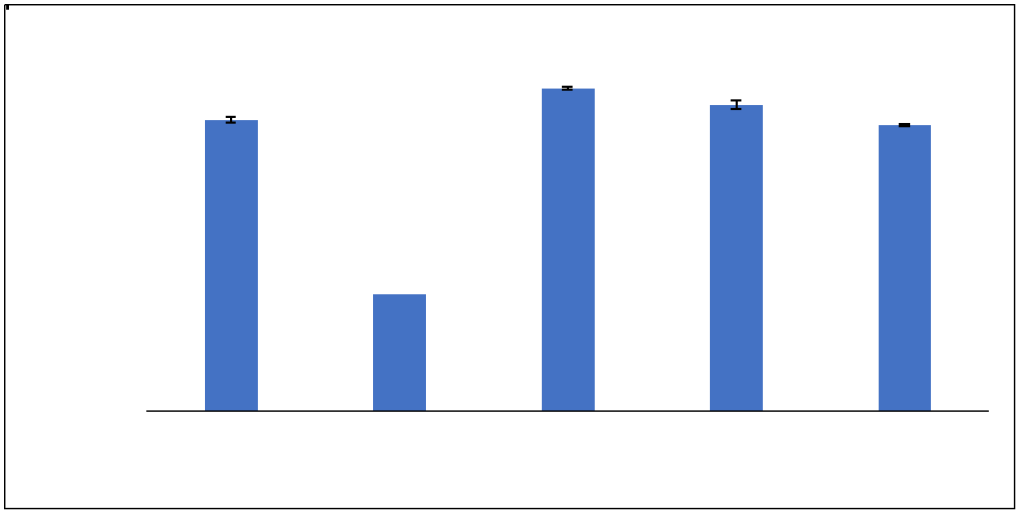

*Figure 2: Tartrate stability test performed on a Pinot grigio wine containing 0.5 g/L of the three yeast lees extracts. A commercial MPs-based protein stabiliser was tested for comparison at the same concentration. Data are expressed as the difference in the conductance values measured at the beginning and at the end of the analysis. Control: original wine.*

The results obtained for the foaming properties of the three wine lees extracts are shown in Table 2.

*Table 2: Maximum foam height (HM), foam stability (HS) and time for foam disappearance (TS) of model wine supplemented with 0.5 g/L of the three wine lees extracts. In the same column, mean values followed by the same letter are not significantly different at $P \leq 0.05$ by analysis of variance (ANOVA) and Tukey's test.*

| Method | HM (cm) | HS (cm) | TS (sec) |
|---|---|---|---|
| **Control** | $3.3^{c}$ | $0.9^{d}$ | $2^{c}$ |
| **Autoclave** | $8.8^{a}$ | $3.2^{a}$ | $58^{a}$ |
| **Sonication** | $5.7^{b}$ | $1.4^{bc}$ | $5^{c}$ |
| **Glucanex** | $5.4^{bc}$ | $1.7^{b}$ | $5^{c}$ |

As expected, all the extracts positively impacted the foam height indexes (HM and HS), thereby confirming the inclusion of foam-active compounds in all the samples. When considering the TS index, the best results were achieved with the Autoclave extract, which was the only one showing a significant effect compared to the control (Table 1). Again, this behaviour can be attributed to the high quantity of MPs and proteins in this extract. Indeed, as described by other authors, TS appears positively influenced by both proteins and MPs which have been shown to have a synergic action towards foam stability (Vincenzi et al., 2014).

Finally, the effect of the different extracts was tested on wine protein stability, as measured by the heat test. For this parameter, the Autoclave extract performed worse than the other two which, conversely, induced a statistically significant reduction in the wine turbidity which was however rather modest (Figure 3). A possible explanation for this result is the presence of a high content of proteins in the Autoclave extract which may include some heat-unstable components of yeast origin that would lower/mask the eventual stabilizing effect of MPs.

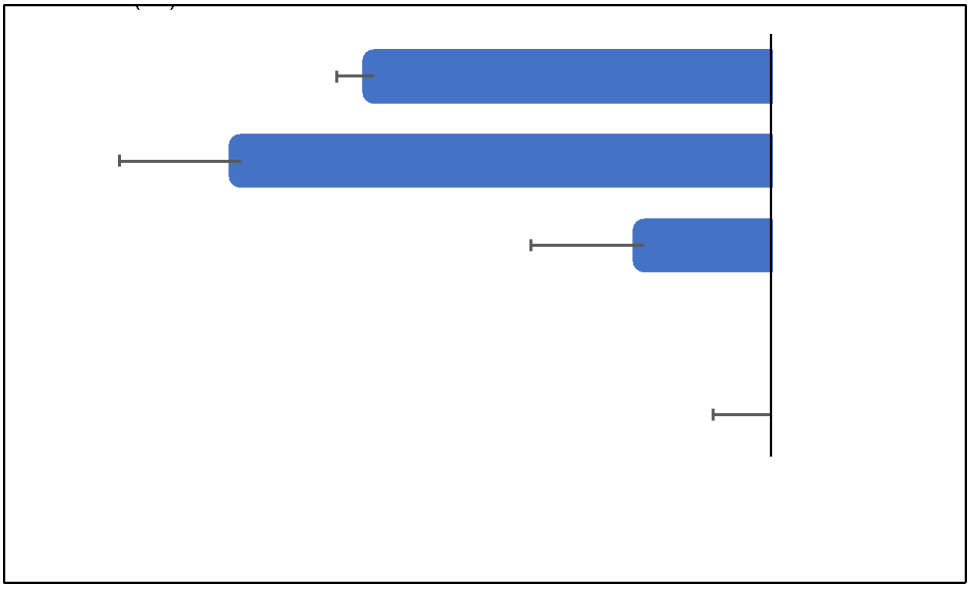

*Figure 3: Protein stability test of an unstable Sauvignon blanc wine added with 0.5 g/L of the yeast lees extracts. The reduction in absorbance of the Glucanex alone was not significant and it was subtracted from that of the Glucanex extract. Data are expressed as percent difference in $A_{540}$ from the control.*

Therefore, it seems that the extracts from wine lees are not suitable as additives for wine protein stabilisation. Actually, despite being significant, also the protein stabilising effects of the Glucanex and Sonication extracts is too low to be useful to this aim, especially if compared with what was achieved by other authors using MPs extracted from pure yeast cultures (Junior et al., 2020; Moine-Ledoux & Dubourdieu, 1999).

## Conclusions

To propose a novel approach for the valorisation of wine lees, three food-grade extraction protocols were applied for the recovery of MPs. The addition to wine of these extracts at a concentration (0.5 g/L) compatible with industrial application, showed some interesting results for tartrate stabilisation and foaming properties and did not cause any alteration of wine clarity, but was ineffective to stabilise wine against protein haze formation. Among the extraction methods, which all are compatible with food applications, the Autoclave extract (containing the highest quantity of MPs) showed the best results on wine foaming properties and tartrate stability, which was enhanced even more than using a commercial MPs-based tartrate stabiliser. Also, the autoclave protocol further yielded a tartaric acid-rich fraction as intermediate by-product, thus indicating the possibility to develop an integrated wine lees valorisation approach focussed on the recovery of both MPs and tartaric acid.
These results suggest that white wine lees could be considered a source of yeast cell wall MPs with potential applications in winemaking. In this scenario, the extraction using autoclave appears as the most promising in terms of both efficiency and extract's effectiveness. This protocol could represent the starting point for a better exploitation of wine lees through the recovery of multiple valuable compounds, thus improving the environmental and economic sustainability of the wine industry with a circular economy approach.

## Abstract

This study aims at exploiting an undervalued winemaking by-product, wine yeast lees, by developing efficient and food-grade methods for the extraction of yeast glycoproteins. These extracts were then supplemented to wine and their impact on wine properties assessed. White wine lees were produced by fermenting Sauvignon blanc grape juice with S. cerevisiae Uvaferm HPS strain. Three extraction methods were applied on lees using physical (autoclave and

sonication) or enzymatic (Glucanex®, an industrial β-glucanases) approaches. Glycoproteins extracts were characterized by SEC-HPLC and SDS-PAGE. After their addition to wine (0.5 g/L), no alteration of wine clarity was detected. The ultrasonication and enzymatic extracts, containing a relatively low amount of glycoproteins, led to a significant decrease in wine protein haze formation upon heat test (-7%). Conversely, the autoclave extract was the richest in glycoproteins and had a positive impact on wine foaming properties, inducing an increase in foam's maximum height and stability which were 2.6 and 3.6 times higher compared to a model wine. The autoclave extract improved tartrate stability as shown by a decrease in wine conductance (-11%) compared to the untreated wine. Results suggest that white wine lees could be considered a valuable source of glycosylated proteins with potential applications in winemaking. In this context, the autoclave appears as the more promising method in terms of both efficiency and extract's effectiveness. The proposed food-grade exploitation approach could represent an important tool to improve the environmental and economical sustainability of the wine supply chain.

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