LWT - Food Science and Technology xxx (xxxx) 112273

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Red and white wine lees as a novel source of emulsifiers and foaming agents

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ARTICLE INFO

Keywords:
Wine lees
Yeast mannoproteins
By-product valorization
Emulsion
Foam

ABSTRACT

Wine lees are an under-exploited sludge-like material mainly consisting of yeast cells that, upon fermentation, settle at the bottom of wine tanks. Lees from commercial red and white winemaking were processed to yield mannoprotein-rich extracts. An established autoclave-based extraction protocol, as well as a simplified version of it, were applied. The composition of the obtained wine lees extracts was determined. Extracts were tested as emulsifying and foaming agents in model food systems and benchmarked against analogues extracts derived from laboratory-grown yeast cultures of the same two strains used for red and white wines production. All extracts showed good functionalities as emulsifying and foaming agents. However, some differences were noted in both composition and functionality, and these were related to the purification process used, yeast strain, and to the extract's origin (red lees, white lees, lab-grown yeasts). Extracts from real wine lees, which contained also grape-derived polyphenols, performed equally or better than the corresponding extracts derived from laboratory-grown yeast cultures of the same strains. Both red and white wine lees can be a novel and effective source of emulsifiers and foaming agents representing a valid alternative to the yeast biomass produced in bioreactors to be potentially used in the food industry.

1. Introduction

Wine is one of the world's most consumed alcoholic beverages, with a global production recently estimated at 292 million hL, and its consumption reported in continuous growth (+2%) in both old and new markets (OIV, 2019). To ensure these volumes, modern winemaking makes use of advanced techniques requiring a significant amount of resources like labor, energy, water, chemicals, and microorganisms. Winemaking activities inevitably result in the production of outputs with environmental impact as polluted waters, greenhouse gases, and solid by-products, with the latter being mainly represented by grape pomace, grape stalks, and wine lees (De Iseppi et al., 2020). In the last decades, these by-products have been studied for their possible recovery and valorization (Bordiga, 2016), and some industrial applications for the recovery of polyphenols and grape seed oil from grape pomace have been proposed (Bordiga, 2016; Lavelli et al., 2016). Conversely, strategies for the valorization of wine lees have not yet received the same attention at both research and industrial levels (De Iseppi et al., 2020). This by-product can be defined as a sludge-like material largely made of dead and living yeast cells that sediment at the bottom of wine tanks at the end of the alcoholic fermentation (Pérez-Bibbins et al., 2015). Current applications and research focusing on their valorization are mostly oriented to the recovery of wine-derived valuable compounds like ethanol, tartaric acid, and polyphenols. In this context, the yeast biomass, the main wine lees component in weight, has been occasionally utilized as a media supplement for microbial growth with encouraging results (De Iseppi et al., 2020; Kopsahelis et al., 2018; Pérez-Bibbins et al., 2015; Salgado et al., 2014). Nevertheless, the high variability of wine lees' composition (e.g. influenced by the winemaking style and techniques, the use of chemicals and processing aids) could represent a major issue for the industrial exploitation of yeast lees (De Iseppi et al., 2020). Another yeast lees valorization option could be represented by the extraction of polysaccharides as mannoproteins and β-glucans (De Iseppi et al., 2021; Varelas et al., 2016), two macromolecules that are the main constituents of the yeast cell wall (Freimund et al., 2003; Magnani et al., 2009). Mannoproteins and β-glucans are currently extracted from industrially grown yeasts and commercialized for their functional and technological properties. In particular, mannoproteins, amphipathic polysaccharides constituted of ≈90% mannose chains attached to a protein backbone, are known for their emulsifying (Cameron, Cooper, & Neufeld, 1988; De Iseppi et al., 2019; Torabizadeh et al., 1996), foaming (Núñez et al., 2006), and wine-stabilizing

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https://doi.org/10.1016/j.lwt.2021.112273
Received 23 February 2021; Received in revised form 25 May 2021; Accepted 7 August 2021
0023-6438/

(Gonzalez-Ramos et al., 2008; Junior et al., 2020; Lomolino & Curioni, 2007; Maza, Delso, Álvarez, Raso, & Martínez, 2020; Moine-Ledoux & Dubourdieu, 1999, 2002) properties. In the last decade, mannoproteins' extraction from yeast lees from fermented alcoholic beverages such as beer (Silva Araújo et al., 2014), Thai traditional liquor (Dikit et al., 2012; Dikit et al 2010), and sugar palm wine (Dikit, Maneerat, et al., 2010) was proposed. Very recently, lees from white wine were tested as a mannoproteins' source achieving good extraction yields by an autoclave treatment followed by ethanol precipitation, and promising results for wine foamability and tartrate stabilization (De Iseppi et al., 2021).

This study aims at assessing the emulsifying activity and foaming properties, as tested in model food systems, of extracts obtained from red and white wine lees, and to develop a simple system for their preparation.

2. Materials and methods

2.1. Yeast material

Red and white wine lees were sourced from the cellar of the Oenology School "G.B. Cerletti" (Conegliano, Italy). Both wines were produced on a commercial scale with grapes sourced from a vineyard located in Conegliano harvested in September 2019. The red wine was produced from Merlot grapes by adopting a typical vinification protocol to produce a wine not meant for ageing. This included crushing/destemming of the grapes and a maceration step of 7 days. The fermentation was conducted by inoculating *Saccharomyces cerevisiae* Fermol Rouge (FR) strain (AEB Group, Italy). The white wine was produced using Glera grapes with a typical vinification protocol to produce a young white wine. This included whole bunch pressing, juice clarification and fermentation with a *Saccharomyces cerevisiae* Fermol Chardonnay (FC) strain (AEB Group, Italy). Fine lees were collected after the second racking (approx. 6 weeks from the end of alcoholic fermentation), concentrated by centrifugation (4225 × g, 4 °C, 15 min, Beckman Coulter Avanti J-E), and freeze-dried. Simultaneously, cultures of FR and FC *Saccharomyces cerevisiae*'s strains were produced in the laboratory using Yeast Malt broth (Sigma-Aldrich, St Louis, MO, USA) as described previously (De Iseppi et al., 2019).

2.2. Mannoproteins' extraction protocols

Two physical extractions (Process 1 and 2) were applied. Process 2 (P2) protocol is the same as the "autoclave protocol" described by De Iseppi et al. (2021), and consists in autoclaving (121 °C, 20 min) the washed wine lees suspended in a McIlvaine buffer at pH 3.4. After centrifugation (10,509 × *g*, 10 min 4 °C), the supernatant was frozen. After thawing, the sample was further centrifuged (10,509 × *g*, 10 min 4 °C), and the supernatant added with ethanol to a concentration of 70% (v/v) and placed at – 18 °C for 16 h. The formed pellet was collected *via* centrifugation (10,509 × *g*, 30 min, 4 °C) and freeze-dried. Process 1 (P1) protocol is a simplified version of P2 as it does not include any purification step after the autoclave treatment. A schematic overview of the two protocols is shown in Fig. 1. After freeze-drying, P1 and P2 extracts were stored at 4 °C until use.

For each extraction method, the starting material (wine lees/yeast biomass) and the obtained extracts were dried at 80 °C for 24 h in an oven (Jouan EB 115, Thermo Electron Corporation, USA). The extraction yields were expressed as a percentage (grams of dry extracts per 100 g of dry starting material).

2.3. Extracts' characterization

The concentration of polysaccharides and proteins was determined by high-resolution size-exclusion chromatography (HR-SEC) using an Agilent 1260 series II quaternary pump LC (Agilent Technologies, CA, USA) as previously described (De Iseppi et al., 2021; González-Royo et al., 2017). For polysaccharides, 1 mg of extracts was solubilized in 1 mL of 50 mmol/L ammonium formate (mobile phase) and filtered (0.22-μm) before being injected (10 μL). The separation was isocratically performed (0.6 mL/min for 70 min, RID temperature 35 °C) with two gel permeation columns in series (PL-Aquagel-OH 50 and 40, Agilent). Pullulans (342–805,000 Da) were used as MW standards, while polysaccharide quantification was performed using a calibration curve built with pectin and dextran (0–2 g/L). For proteins, 1 mg of extracts was dissolved in 1 mL 300 mmol/L ammonium acetate (mobile phase) and sterile filtered (0.22-μm) before being injected (100 μL) into a PL-Aquagel-OH 40 gel permeation HPLC column (Agilent Technologies, CA, USA). The elution was performed in isocratic mode at a flow rate of 0.6 mL/min for 70 min, and proteins detected with a DAD. Proteins were quantified against a calibration curve prepared with bovine serum albumin (0–10 g/L).

Mannoproteins in each extract were quantified by the Competitive Indirect Enzyme-Linked Lectin Sorbent Assay (CI-ELLSA) as proposed by Marangon et al. (2018) with the following modifications: Tris-Buffered Saline (TBS) at pH 7.4 was used instead of Phosphate-Buffered Saline (PBS), and plate wells were loaded with 100 μL of sample. The peroxidase solution contained 0.02 mg/mL of horseradish peroxidase, while the standard stock solution contained 0.2 mg/mL of *Saccharomyces cerevisiae*'s invertase.

The total polyphenols content of samples, prepared by dissolving 4 mg of each extract in 1 mL of ultrapure water, was determined by the Folin-Ciocalteau assay (Slinkard & Singleton, 1977; Waterhouse, 2003).

2.4. Emulsifying activity

Oil-in-water emulsions were prepared in a 50 mL Falcon tube added with 7.40 g of corn oil and 13.35 mL of McIlvaine buffer (pH 3.4) containing 10 mg/mL of freeze-dried mannoproteins extract. Emulsions were produced by using an Ultra-Turrax TP 18/10 (IKA-Werke GmbH & Co., Germany) set at 50% power for 1 min. Subsequently, the emulsions were stored at 4 °C and their stability monitored over time (7 days) as described previously (De Iseppi et al., 2019).

2.5. Foam stability test

The foam stability test was performed in a 50 mL Falcon tube containing 15 mL of McIlvaine buffer, pH 3.4, added with 1 mg/mL of extract. The samples were vigorously mixed for 2 min using a ZX^3 Vortex shaker (VELP Scientifica, Italy) set at maximum speed (40 Hz). The samples were kept at room temperature and the decay of the height of the produced foam was measured at 30-min intervals for 3 h.

2.6. Statistical analysis

Each measurement was performed at least in triplicate unless otherwise stated. Statistical analysis was carried out by one-way ANOVA and Tukey test at 95% confidence or, in the case of foam stability, by Student's t-test at 95% confidence. Multivariate analysis was conducted by calculating the Pearson correlation coefficient between variables significantly related at 95% confidence. The STATGRAPHICS Centurion 18.0 software (version 18.1.12; Statgraphics Technologies Inc., The Plains, Virginia, USA) was used.

3. Results and discussion

According to previous results, autoclave treatments of laboratory-grown yeast biomasses were revealed to be suitable to obtain extracts rich in mannoproteins with the ability to form stable oil in water emulsions (De Iseppi et al., 2019). Moreover, the same extraction approach

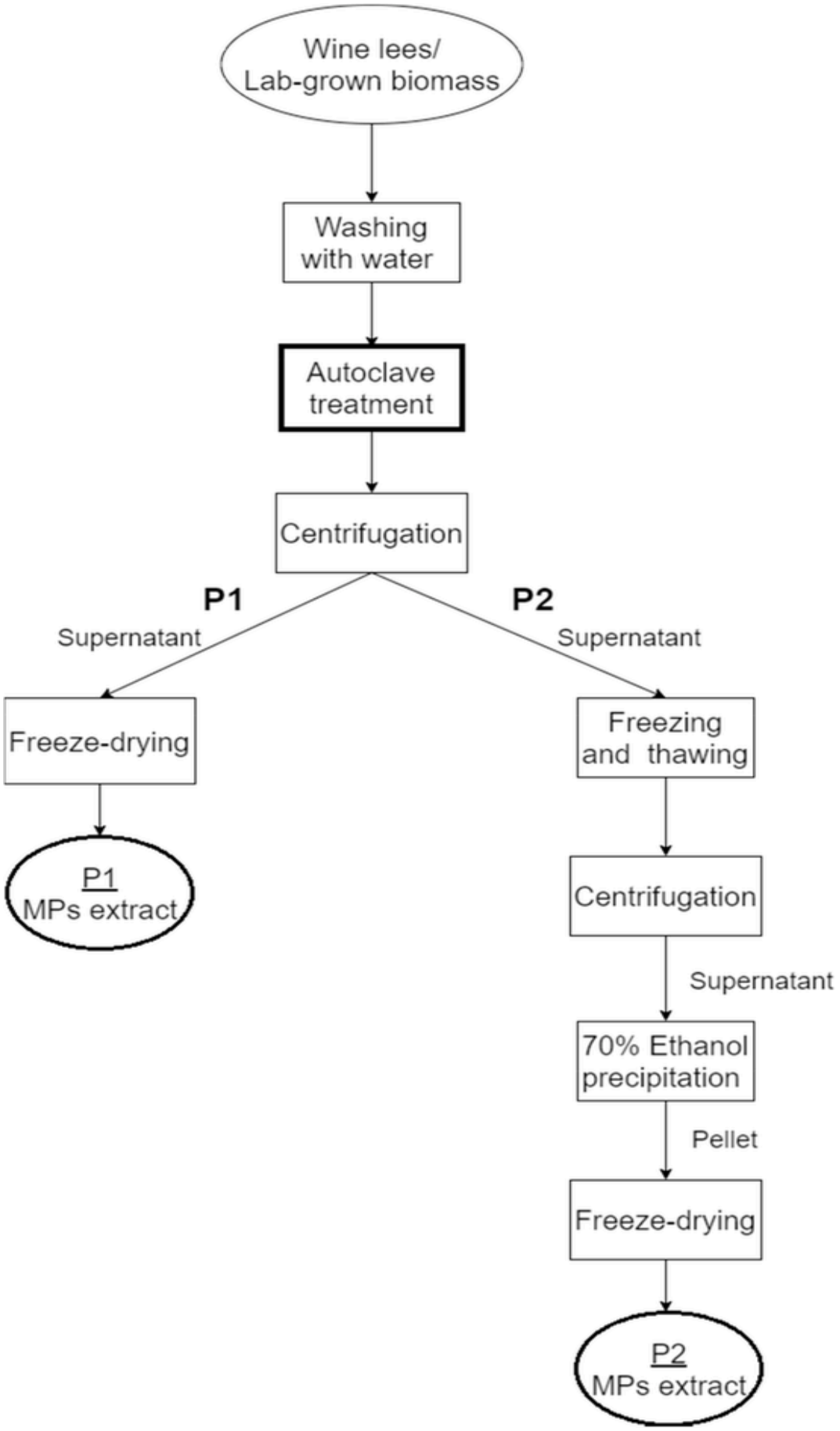
Wine lees/
Lab-grown biomass
Washing
with water
Autoclave
treatment
Centrifugation
P1
Supernatant
P2
Supernatant
Freeze-drying
Freezing
and thawing
P1
MPs extract
Centrifugation
Supernatant
70% Ethanol
precipitation
Pellet
Freeze-drying
P2
MPs extract

Fig. 1. Schematic representation of P2 and P1 mannoproteins' extraction protocols.

applied to wine lees produced extracts able to modify key wine properties including foaming (De Iseppi et al., 2021).

To assess the possibility to use wine lees as a source of surface-active agents for the food industry, the effects on emulsifying and foaming properties of extracts deriving from fine lees of commercial wines were compared with those of extracts prepared from yeasts grown in the laboratory that, most likely, are assimilable to thoseproduced in industrial bioreactors. Moreover, two extracts prepared with two methods differing in complexity (P1 and P2, see Fig. 1), were tested to assess the possibility to employ a simplified procedure to be proposed for industrial applications without affecting the final extracts' functionality.

3.1. Extraction yield and extracts' composition

The yields and the compositional data of the different extracts are shown in Table 1, where each wine lees' extract was compared with the corresponding one produced in the laboratory starting from the same yeast strain used for wine fermentation.

As expected, the purification level affected the extraction yield, which was significantly higher for the P1 samples ($p \leq 0.05$) as they contained all the soluble compounds released from the yeast biomass during the autoclave treatment. Among the P1 extracts, those derived from red and white wine lees showed significantly higher yields when compared to the P1 extracts from the lab-grown yeasts ($p \leq 0.05$). This difference can be due to the presence of some wine compounds as tartaric acid entrapped in wine lees (De Iseppi et al., 2021). Conversely, the P2 extraction protocol, which aims at purifying mannoproteins by eliminating wine compounds, resulted in no yield differences between the extracts from wine lees and those obtained from the lab-grown yeasts ($p \leq 0.05$).

The yields reported for the P2 extracts of white wine lees (26.9%) are in line with those obtained previously using the same extraction protocol (18.8%) (De Iseppi et al., 2021), but higher than yields obtained in similar studies (Costa et al., 2012; Silva Araújo et al., 2014). When looking at the P2 extracts from red winemaking, the extraction yields were higher than those from white winemaking, but this difference seems attributable to the different yeast strains rather than the winemaking style because it is found also when the lab-grown yeasts are considered (FR higher than FC).

The freeze-dried extracts contained from 8.9 to 21.4 % of proteins. The extracts from lab-grown yeasts showed, in three out of four cases, a significantly higher protein content when compared to the correspondent extracts from wine lees ($p \leq 0.05$). This occurrence seems attributable to the partial degradation that yeast cells in wine lees would have undergone during winemaking, which possibly caused the release of cytoplasmic proteins which in contrast should be present in the extracts of the lab-grown yeasts (Snyman, Mekoue Nguela, Sieczkowski, Marangon, & Divol, 2021). Another possible explanation can be related to the fact that the extracts from wine lees include larger amounts of other compounds (e.g. polyphenols, tartrates, etc.) which result in a "dilution" of the protein content.

Similarly to what observed for proteins, also the total content of polysaccharides is significantly higher in the P2 than in the P1 extracts ($p \leq 0.05$), and this seems to be valid for all the different MW classes. The opposite behavior was observed for oligosaccharides, with the P1 samples showing higher contents than the P2 ones, indicating that some of these low MW components are lost during the purification step (see Fig. 1). It has to be noted that the measured quantities of oligosaccharides were at times higher than the total weight of the extract (100 g), an occurrence likely due to the use of pectin and dextran as calibration standards (González-Royo et al., 2017).

To specifically measure the mannoproteins' content in each extract, the CI-ELLSA method was adopted as it exploits the specificity of concanavalin A towards mannose (Marangon et al., 2018). It must be noted that the method considers only the presence of mannose, and thus the reported values could correspond to a series of mannose-containing species with different MW which can be found in all the polysaccharide fractions considered in Table 1. Also in this case, the content of mannoproteins was significantly lower in the P1 than in the P2 extracts ($p \leq 0.05$), indicating that the additional purification steps of the P2 protocol allowed for a concentration of mannoproteins. This difference is reflected in all the three polysaccharide fractions, confirming that

Table 1
Extraction yields and composition of the extracts.

Purification level - Yeast strain	Origin	Extraction yield	Protein	Polysaccharides*				Oligosaccharides*	Mannoproteins**	Folin-Ciocalteau reactive compounds
			Total	Total	*High MW*	*Medium MW*	*Low MW*	Total	Total	
		(g/100g of biomass)	(g/100g of extract)							
P1 FR	Red wine lees	48.30[a]	10.36[b]	13.77[a]	3.32[a]	6.31[a]	4.14[b]	111.28[a]	10.55[a]	7.98[a]
	Lab-grown yeasts	43.50[b]	15.43[a]	16.92[a]	2.38[a]	7.34[a]	7.20[a]	118.24[a]	8.77[a]	2.04[b]
P2 FR	Red wine lees	30.40[a]	8.85[b]	31.31[a]	6.26[a]	16.11[a]	8.93[b]	86.26[a]	24.26[b]	2.35[a]
	Lab-grown yeasts	32.96[a]	21.37[a]	33.83[a]	3.98[b]	17.49[a]	12.37[a]	86.37[a]	33.08[a]	1.75[b]
P1 FC	White wine lees	47.23[a]	11.35[a]	18.04[b]	4.16[a]	7.52[b]	6.37[b]	116.98[a]	11.75[a]	2.7[a]
	Lab-grown yeasts	35.27[b]	13.49[a]	24.54[a]	3.38[a]	9.86[a]	11.30[a]	107.73[b]	10.10[a]	2.08[b]
P2 FC	White wine lees	26.90[a]	13.10[b]	45.81[b]	10.58[a]	21.31[b]	13.92[b]	75.47[a]	42.8[b]	1.51[a]
	Lab-grown yeasts	23.92[a]	21.28[a]	61.68[a]	7.40[b]	30.16[a]	24.11[a]	35.77[b]	88.8[a]	1.86[a]

Polysaccharides: High MW: 1100–180 kDa; Medium MW: 180–40 kDa; Low MW: 40–7.5 kDa. Oligosaccharides: 7.5–1 kDa.
FR: Fermol Rouge strain; FC: Fermol Chardonnay strain.
Each analysis was performed in triplicate. Data significance was assessed by analysis of variance (ANOVA) and Tukey's test.
[a-b] Mean values followed by the same letter are not significantly different at $p \leq 0.05$.
* Measured by HR-SEC and expressed as dextran/pectin equivalents (González-Royo et al., 2017).
** Measured with CI-ELLSA method (Marangon et al., 2018).

yeast mannoproteins are present in a broad MW range (Doco et al., 2003; Li & Karboune, 2019; Rodrigues et al., 2012; Saulnier et al., 1991). Moreover, the content of mannoproteins was always significantly higher in the lab-grown yeasts than in the corresponding wine lees extracts (Table 1). This is particularly evident for the P2 FC extracts. A possible explanation of this fact is that mannoproteins would have been released into the wine during winemaking in a larger quantity than in laboratory conditions.

The extracts also showed to contain compounds that reacted with the reagent (Folin-Ciocalteau) commonly used to quantify phenolic compounds. Given that polyphenols are not present in lab-grown yeasts, the readings observed in these samples must be due to interferences with other compounds known to react with the Folin-Ciocalteau reagent, such as some amino acids (e.g. tyrosine, tryptophan, cysteine, cysteine) likely to be released from the yeast cells (Lindon, Tranter, & Koppenaal, 2016). However, the P1 extract from red wine lees showed signals 3 to 4 times higher than the other samples, clearly indicating that grape polyphenols were present, while these were eliminated by the purification steps included in the P2 protocol (Table 1). Indeed, it is known that wine lees adsorb polyphenols (Ramos-Pineda et al., 2018), but this association can be broken by the ethanol (Romero-Díez et al., 2018) used to precipitate mannoproteins during the P2 purification step (see Fig. 1). A confirmation of this was obtained by observing the reddish color of the ethanol recovered after centrifugation (data not shown).

3.2. Emulsifying activity

To assess the potential to be used as emulsifiers in food systems, the emulsifying activity (EA) of the eight extracts was investigated (Fig. 2). All tests were conducted at pH 3.4, a value representative of different food emulsions such as salad dressings (Kurtzman & James, 2006; Meyer et al., 1989). The EA was expressed as the percentage of the height of the emulsion on the total height of the three phases generated after mixing the ingredients (buffer, corn oil, and yeast extracts).

Each extract derived from wine lees was compared with that of the correspondent lab-grown yeast biomass (Fig. 2). Comparing the EA of the extracts from red wine lees and the correspondent lab-grown FR strain (Fig. 2A and B), it appears that at all times and for both levels of purification (P1 and P2), the extracts from lees showed the best EA values after 7 days. This effect is particularly visible for the P1 extracts but does not seem attributable to the mannoprotein nor to the total protein content as these seem not to be related (see Table 1). It is however probable that this difference is due to the presence of higher amounts of wine polyphenols in P1 lees' extracts (Table 1) that, in previous studies, have been shown to play a role in emulsion formation and stabilization

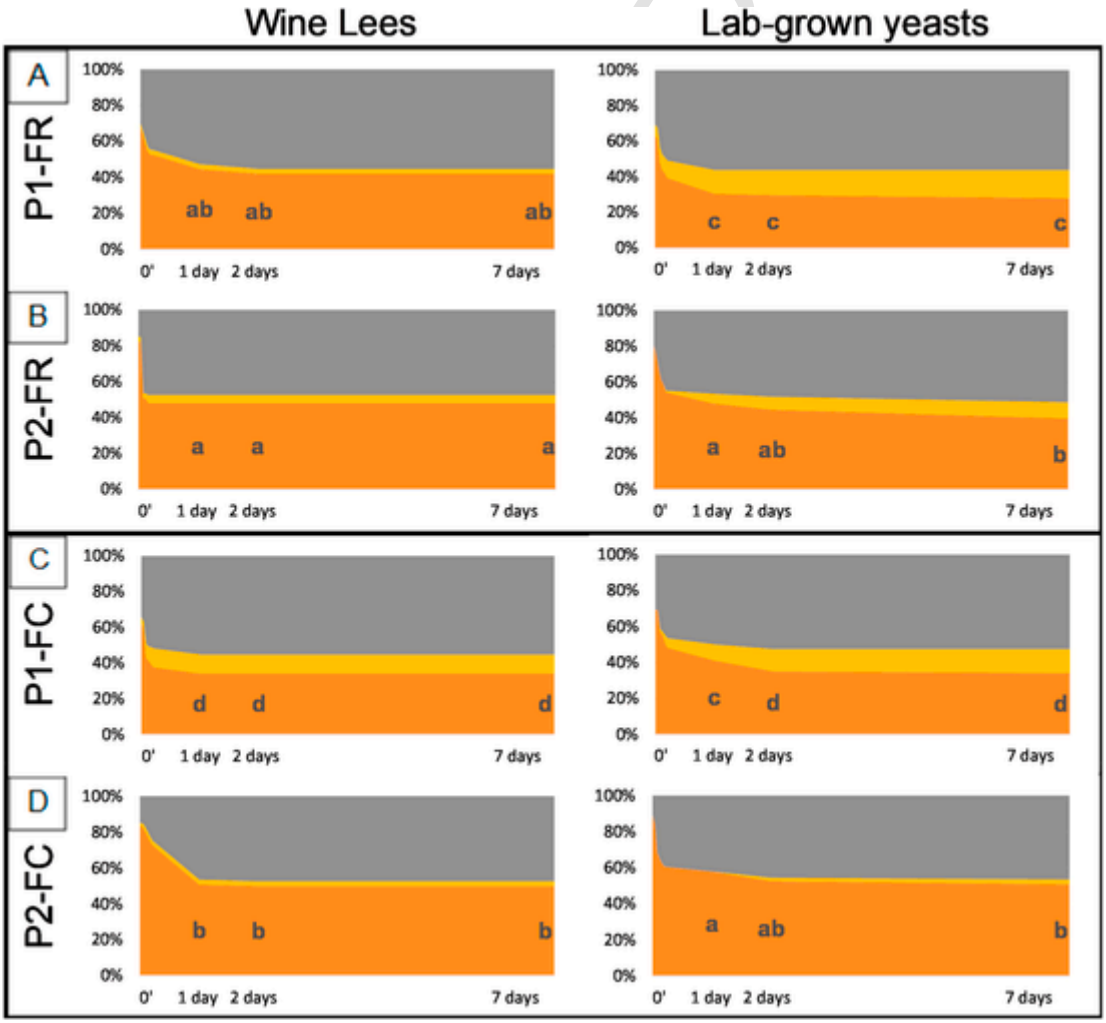

Fig. 2. Evolution over 7 days at 4 °C of emulsions prepared with the P1 and P2 yeast extracts obtained from red (A, B) and white (C, D) wine lees (right) and their correspondent lab-grown yeast strain (left). FR: Fermol Rouge strain; FC: Fermol Chardonnay strain. The percentage of the heights of the different phases (oil phase in yellow, emulsion phase in orange and watery phase in grey) on the total height of the sample during 7 days is shown. Extracts from the same yeast strain were compared by ANOVA and Tukey's test performed after 24, 48 h and 7 days. For each of the two panels (AB and CD), EA values followed by the same letter are not significantly different at $p \leq 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

(Chen et al., 2010; Zembyla et al., 2018). In particular, protein-polyphenol conjugates are widely studied for their activity in stabilizing Pickering emulsions (Shi et al., 2020). Even if in those studies this conjugation is usually induced, it was demonstrated that it can also spontaneously occur under acidic conditions (Chen et al., 2018), and even between yeast mannoproteins and grape polyphenols (Mekoue Nguela et al., 2016). For the here studied samples, such conjugation may have occurred during winemaking, giving rise to complexes with an increased hydrophobicity and surface activity which justifies the increased EA observed for the lees extracts (Fig. 2A and B). It should be noted that the quantity of polyphenols decreased greatly after purification (P2) (see Table 1). When considering differences in EA over 7 days between extracts from wine lees and lab-grown yeasts, these are larger in the P1 extracts where polyphenols are present in higher quantities, thus confirming their emulsion stabilizing effect (Fig. 2A to be compared with 2B).

Moreover, in the case of the P2 red wine lees extract, a significantly higher presence of the high MW polysaccharides was detected. Given that the ethanol precipitation step used to prepare the P2 extracts selects the yeast polysaccharides with the higher MW (Freimund et al., 2003), and that mannoproteins are the major constituents of this fraction, it is likely that high MW mannoproteins played a major role in the long-term emulsion stabilization. This assumption is in agreement with previous studies in which high and medium MW mannoproteins appeared as the more active fractions in stabilizing emulsions (De Iseppi et al., 2019; Silva Araújo et al., 2014).

Fewer differences were detected when comparing P1 and P2 extracts from white wine lees with the corresponding ones from lab-grown FC yeast. Considering both comparisons (Fig. 2C and D), extracts from lab-grown FC showed significantly higher EA values after 24 h. However, after 2 and 7 days, no differences were detected. Despite the very high content in mannoproteins in the FC P2 lab-grown samples, their presence did not result in emulsions with levels of stability higher than those of FC P2 wine lees extract (see Table 1 and Fig. 2D). Most of the polysaccharides in the studied extracts should be mannoproteins. It has been shown that mannoproteins with MW higher than 40 kDa are the most effective in stabilizing emulsions (Li & Karboune, 2019). Therefore, the similarities in EA observed in Fig. 2D could be attributed more to the similar high and medium MW polysaccharides content of the extracts (31.89 vs. 37.56 g/100g) rather than to the differences in mannoprotein concentration (42.8 vs. 88.8 g/100g). Therefore, the size of mannoproteins rather than their total quantity seems to be the driving factor for the emulsifying behavior. A similar observation can be done also for P2 FC samples, although less evident.

Independently on the quantity of the emulsion produced, this did not change in the period between 2 and 7 days, indicating a very high stability which could be expected to continue also for following periods. This stability was particularly evident when wine lees extracts were used (Fig. 2), confirming their potential role as emulsion stabilizers.

3.3. Foam stability

To assess the effect of the extracts on foam stabilization, samples were prepared adding them (in McIlvaine buffer, pH 3.4) at 1 mg/mL, a concentration ten-times lower than that used to prepare the emulsions. The progressive decrease of the foam volume was used as a proxy to quantify foam stability up to 3 h. As done for the EA test, also here P1 and P2 extracts from wine lees were compared with the correspondent P2 and P1 lab-grown yeast extracts (Fig. 3).

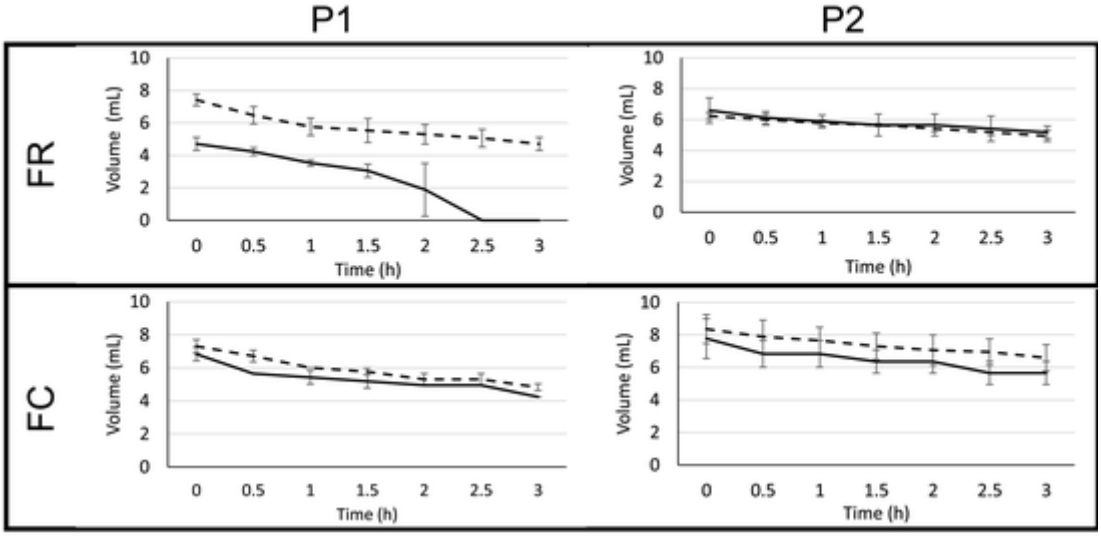

Fig. 3. Evolution over 3 h of the foam volume produced by 1 mg/mL of extracts solubilized in 15 mL McIlvaine buffer (pH 3.4). Dashed lines: lab-grown yeast extracts; Continuous lines: wine lees extracts. McIlvaine buffer alone did not produce any measurable foam.

All the extracts showed the ability to produce large foam volumes that resulted in an increase of volumes in the range 31–56%. Generally, the foam produced was very stable, with a decrease in volume over the 3 h period in the range of 21–37%, except for the extract deriving from red wine lees prepared with P1. In this case, the foam formed disappeared completely after 2.5 h, an occurrence likely attributable to the presence of large amounts of polyphenols in the extract (see Table 1). Indeed, polyphenols have been associated with reduced foam stability in model and real wines (Martínez-Lapuente et al., 2018).

3.4. Influence of extracts composition on emulsifying activity and foaming properties

The effect of the main compositional parameters of the extracts (Table 1) on their ability to stabilize emulsions and for the effects on foam volume and stability was investigated by the Pearson correlation coefficient (Fig. 4). Data obtained with the Folin-Ciocalteau methods were omitted because the obtained values cannot be clearly attributed to polyphenols only, but are likely to be affected by the presence of other interfering compounds as in section 3.1 (Lindon, Tranter, & Koppenaal, 2016).

All extracts were included in this analysis regardless of the extraction method (P1, P2) and source (wine lees, lab-grown yeast) used. For the EA test, only values at 7 days (EA7days) were considered as these are most indicative of the long-term emulsion stability and therefore those with the most practical relevance. For foam, the values corresponding to the maximum volume as measured at the beginning of the test (Foam 0h), and stability (volume measured after 3 h, Foam 3h) were selected.

The EA was positively correlated with the total mannoproteins content (0.70) and with the three polysaccharides' fractions. Particularly, the high and medium MW polysaccharides (1100–40 kDa) showed higher correlation values (0.77–0.78) with EA7days than the low MW polysaccharides (0.57). In contrast, a strong negative correlation was found between EA7days and total oligosaccharides. However, it is possible that this negative effect is due to the lower quantity of mannoproteins found in the samples containing high quantities of oligosaccharides as demonstrated by the strong negative correlation (−0.97) between these two compounds. The total protein concentration did not correlate with the EA, and this could be due to the specific nature of the yeast proteins contained in the extracts that do not seem to possess emulsifying properties.

Results differ greatly when foaming characteristics are considered. Indeed, the maximum foam volume (Foam 0h) was positively influenced by the low and medium MW polysaccharides, but not by the high MW ones. Additionally, also the proteins did not show any correlation with foam volume, an unexpected result given that proteins are generally well known for being foaming agents (Condé et al., 2017; Schramm, 2005). As noticeable by the positive correlations with Foam 3h parameter, high MW polysaccharides and proteins played a significant role in stabilizing foams. It is well known that a molecule with low effects on foam volume can be a good foam stabilizer (Martínez-Lapuente et al., 2015, 2018), and this can be the case of the high MW polysaccharides and proteins here studied. The foam stabilizing effect of these two classes of yeast compounds is in agreement with previous studies in which the synergistic impact of mannoproteins and proteins on foam stability was demonstrated (De Iseppi et al., 2021; Vincenzi et al., 2014). Nevertheless, the results of Fig. 4 indicate that the low and medium MW polysaccharides (180–7.5 kDa) are the compounds with the largest positive correlation with both foam's height and stability. The here reported findings that low MW polysaccharides positively impact foaming properties is consistent with a previous study (Núñez et al., 2006) in which it was observed that mannoproteins with MW between 10 and 30 kDa were the main responsible for the improvement of a model wine's foaming properties. These findings could indicate that medium and high MW polysaccharides (which are likely to comprise mostly mannoproteins) have the ability to impact to a higher extent emulsions' formation and stabilization, while medium and low MW polysaccharides, which also may contain mannoproteins, displayed a higher attitude in producing and stabilizing foams.

4. Conclusions

The use wine lees as a source of valuable extracts potentially applicable in the food industry as emulsifiers and foaming agents was evaluated. The obtained extracts applied in model food systems showed good emulsifying and foaming properties also when compared with the extracts obtained starting from lab-grown pure yeast biomass. It is noteworthy that extracts from red wine lees performed better as emulsifiers than those prepared from the same yeast strain grown in the laboratory. This was attributed to the presence in the extracts of wine polyphenols potentially associated with proteins and/or mannoproteins to form complexes involved in forming stable emulsions.

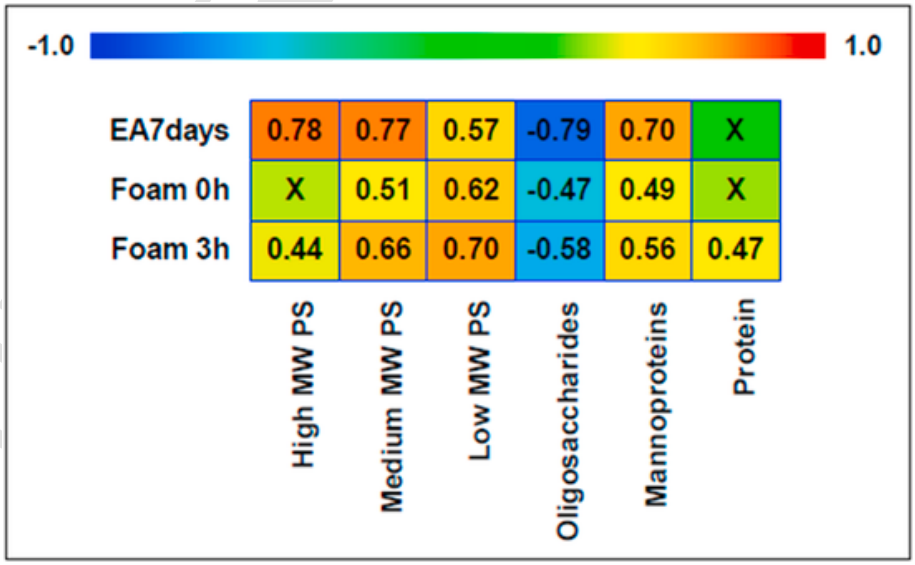

Fig. 4. Correlation between compositional data and values of the Emulsifying Activity test after 7 days (EA7days) and of foam stability tests at time 0 (Foam 0h) and after 3h (Foam 3h). Couples reporting the Pearson correlation coefficient correlate significantly ($p \leq 0.05$). X: no significant correlation.

However, the possibility to prepare such extracts starting from wine lees requires to have a simple, food grade and industrially scalable process of extraction. To this aim, an effective and simple protocol was developed, which gave extracts potentially suitable for applications in real foods, and particularly in those in which the coexistence of air-water-fat phases is required (e.g., sweet and salty dough, ice cream). Therefore, it seems possible to propose wine lees as a novel source of non-animal-based food additives with proven functionalities. However, it appears unlikely that wineries could independently implement lees extraction methods due to the intrinsic differences that lees sourced from different wines necessarily have. Conversely, a joint and large-scale collection system should be organized within wine regions to provide bio-refineries with an assembled mass of lees allowing to lower their compositional variability thus increasing the standardization needed for industrial exploitation. Additionally, the potential presence of plant protection products residues adsorbed in the lees needs to be established before proposing wine lees as a source of food additives, although the increasing application of more sustainable grape-growing practices should minimize this potential issue.

Taken all together, the results of this study indicate that wine lees may represent an equivalent or even more efficient source of yeast mannoproteins compared with the yeast biomasses currently used to this aim bythe biotechnological industry.

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.

CRediT authorship contribution statement

Alberto De Iseppi: Conceptualization, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Matteo Marangon:** Conceptualization, Project administration, Supervision, Writing – review & editing. **Giovanna Lomolino:** Conceptualization, Funding acquisition, Project administration. **Antonella Crapisi:** Funding acquisition, Project administration. **Andrea Curioni:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Acknowledgments

This study was undertaken as part of the PhD program Land, Environment, Resources, and Health (LERH) of Alberto De Iseppi. This research was partially supported by the projects "VIT-VIVE-Innovativi modelli di sviluppo, sperimentazione ed applicazione di protocolli di sostenibilità della vitivinicoltura veneta" (POR-FESR Regione Veneto), and by the University of Padova, grant number DOR1953329/19. Mara Vegro, Stefania Zannoni, and Valentino Pizzocchero (University of Padova) are thanked for their technical support; Valerio Dalmas and Carlo Montanini (AEB Group, Italy) are thanked for kindly providing the yeast strains used in this study.

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