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Current and future strategies for wine yeast lees valorization

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ABSTRACT

Wine lees is a sludge material mainly composed of dead yeast precipitated at the bottom of wine tanks. Along with grape pomace and grape stalks, it is one of the main by-products of the winemaking industry. Given that wine lees are considered a soil pollutant, their disposal represents a cost for the wineries. Numerous wine lees recovery and valorization strategies have been proposed, with a particularly steep increase in published research in recent years. This attention is strictly linked to the concepts of circular economy and environmental sustainability that are attracting the interest of the scientific community. In this review, an overview on the available wine lees recovery and valorization strategies is reported. Additionally, the methods for the extraction and valorization of yeast cell walls polysaccharides (β-glucans and mannoproteins) are discussed. Finally, current and future innovative applications in different sectors of yeast β-glucans and mannoproteins are described and critically discussed.

Keywords: wine lees; yeast; extraction methods; by-product valorization; mannoproteins; β-glucans.

1. Introduction

Agricultural activities and transformation processes have a great environmental impact, because of the inputs required (e.g. energy and water consumption, fertilization, use of pesticides) and the outputs produced (e.g. waste products, polluted water, greenhouse gases), which result in high disposal costs. In addition to these costs one needs to consider also the fines and taxes that governments increasingly apply to discourage unsustainable practices. For example, a Spanish law states that fines are increased as a function of the toxicity of the waste produced (Spanish Law 10/1998), thus causing additional expenses that, for Spanish companies, can often reach 30,000–40,000 € (Devesa-Rey et al., 2011). Thus, in the future the "polluter pays" approach might no longer be economically viable for companies, thus forcing them to seek viable alternatives to avoid fines or taxes (e.g. carbon tax), and possibly also to make a profit from the valorization of their waste. This shift in mentality is already happening, as seen by the exponentially increasing number of research projects and as a consequence in scientific articles published on the subject in the past decade (Fig. 1). As a result, several products containing by-products from the agri-food sector are now commercially available.

Fig. 1 here

2. Winemaking by-products

In the case of the wine industry, several by-products, accounting for 31-40% of the total grapes harvested (Lavelli, Torri, Zeppa, Fiori, & Spigno, 2016), are not currently considered economically useful and therefore need to be disposed of with associated direct costs paid by the wineries, or indirect costs for the entire community. In the last years, indirect costs increased due to progressively more stringent environmental standards.

Typical waste by-products from a winery include grape pomace (skins and seeds. representing on average about 60% of the total winemaking by-products), grape stalks (14%), grape solids and fermentation (yeast) lees (25%), wastewaters rich in organic compounds (up to about 15L/L of wine) (Bonamente, Scrucca, Asdrubali, Cotana, & Presciutti, 2015; Lavelli et al., 2016), carbon dioxide from the fermentation process, exhausted filtration materials and fining agents. In view of a circular economy approach, some of these wastes can be successfully “recycled”, reused or recovered, thus improving both the economic and environmental sustainability of winemaking activities (Devesa-Rey et al., 2011). Grape pomace after fermentation is a clear example as it is traditionally exploited at industrial level for producing valuable products as spirits, grape seed oil, and several food additives (Bordiga, 2015). Furthermore, winemaking by-products could potentially supply different industries providing ingredients suitable for enriched food formulations, and plant-based cosmetics and drugs. To exploit this potential, efficient and innovative extraction procedures and extracts’ applications need to be developed. In this view, several authors have recently proposed new uses for pomaces with higher added value such as source of antioxidants (Jara-Palacios, 2019; Negro, Tommasi, & Miceli, 2003) (Bordiga, 2015; Lavelli et al., 2016; Nerantzis & Tataridis, 2006), antimicrobial agents (Serra et al., 2008), texturizers and colorants (Cappa, Lavelli, & Mariotti, 2015), and animal feed ingredients (Chikwanhaa, Voster, M; van E. Noltec, & Dugand, M. E. R; Mapiye, 2018).

Conversely, wine lees despite being the second largest winemaking by-product, and containing a wide range of potentially valuable compounds, have so far only received little attention for their valorization (Kopsahelis et al., 2018)

3. Wine Lees: an undervalued by-product

3.1 Wine lees composition

Wine lees are a sludge material made of intact or partially degraded yeast cells and other insoluble particles (Fia, 2016) that accumulates at the bottom of wine tanks after the alcoholic fermentation. Wine lees contain significant amounts of polysaccharides, proteins, lipids and other organic species possessing a high oxygen demand (BOD and COD). For this reason, wine lees are considered an environmental pollutant (Lafka, Sinanoglou, & Lazos, 2007). However, their composition depends on numerous parameters mainly related to the types of yeast and grapes used and to the vinification method, resulting in a wide compositional heterogeneity, as shown by data reported in the literature (Charpentier & Feuillat, 2008). An overview of the composition of wine lees is shown in Table 1 (Bordiga, 2015).

Table 1 here

After separation from the wine by racking, centrifugation or filtration, wine lees can be used for compost or animal feed production (Bacic, 2003). However, these applications are more a way to reduce disposal costs than a real by-product valorization strategy (Lavelli et al., 2016). Wine lees are extremely rich in organic matter, in water-soluble polyphenols, and in minerals (especially K) (Table 1). These characteristics, together with the low pH, make raw wine lees incompatible with direct agricultural applications (Bustamante et al., 2008). The high protein and total nitrogen concentrations combined with the good presence of essential amino acids (tyrosine, valine and aminocaproic acid) (Delgado De La Torre, Priego-Capote, & Luque De Castro, 2015b) led to the proposal to use wine lees as a source of proteins for ruminants (Molina-Alcaide, 2008). However, the high amounts of polyphenols associated with the proteins make a large part of that fraction not assimilable (Devesa-Rey et al., 2011).

Bustamante et al. (2008) reported that wine lees contain between 1.9 and 16.3 g of polyphenols/kg (Table 1) depending on the wine type and processing. More recent studies

have been focused on the characterization of this fraction identifying several phenolic compounds mainly belonging to the phenolic acids, flavonols, flavanols and anthocyanins subclasses (Delgado De La Torre, Priego-Capote, & Luque De Castro, 2015a; Giacobbo et al., 2019; Matos et al., 2019; Romero-Díez et al., 2019, 2018). A list of the phenolic compounds found in the solid fraction of wine lees, and the methods used for their identification and quantification is reported in Table 2.

Table 2 here

Polyphenols in wine lees can be found in both the liquid fraction (essentially wine), and in the solid fraction as a result of their adsorption on the yeast cell walls during winemaking (Ye, Harrison, Cheng, & Bekhit, 2016). The mechanism of adsorption depends on the type and quantity of the different phenolic compounds, and on several other variables including the grape variety, the degree of berry ripeness, the maceration method, and the temperature of fermentation. An hypothesized mechanism indicates that colloidal polyphenols interact with the proteins of the yeast cell walls by Van der Waal's forces (Haslam et al., 1992; Kawamoto & Nakatsubo, 1997; Salmon, Fornairon-Bonnefond, Mazauric, & Moutounet, 2000). Nevertheless, due to the weakness of these interactions polyphenols can be easily recovered from yeast lees and therefore have the potential to be exploited. Indeed, the recovery of polyphenols from wine lees has been performed using food grade organic solvents (Cruz, Domínguez, Domínguez, & Parajó, 2001), supercritical CO_2 (Wu et al., 2009), and membrane technology (Giacobbo, Bernardes, & de Pinho, 2017; Giacobbo, Do Prado, Meneguzzi, Bernardes, & De Pinho, 2015). These techniques are potentially suitable for large scale applications to obtain food grade phenolic extracts to be utilized in the food industry as antioxidant and antimicrobial natural additives (Jara-Palacios, 2019).

Wine lees also contain lipids that come from both grape seeds and yeast cell walls. According to Gómez et al. (2004), lees can contain high-value fatty acids like palmitic (C16:0, ≈33% of the lipid fraction), linoleic (C18:2, ≈21%) and stearic (C18:0, ≈10%). Nevertheless, these lipids were extracted using non-food grade solvents. The first attempt of food-grade lipid extraction was proposed by Naziri, Mantzouridou, & Tsimidou (2012) who developed a protocol to obtain squalene from wine lees using ultrasound-assisted hexane extraction. However, the high extraction costs and the possible lipid oxidation during current wine lees management (which include distillation, aerobic storage and transportation) could limit the industrial success of this application.

More studies are needed to have a complete view on the type and content of potentially valuable compounds of wine lees and also to understand how the yeast strain, the grape variety and the winemaking practices can affect their variability.

3.2 Main strategies for the valorization of wine lees

3.2.1 Recovery of Ethanol and tartaric acid

The European Council Regulation (EC) 479/2008 states that wine lees should be sent to distilleries to produce ethanol. This alcohol is rich in aromatic compounds deriving from the wine and then can be used to produce spirit liquors (Guadalupe Bustos, Moldes, Cruz, & Domínguez, 2004). The by-product of the distillation process (distilled lees or *vinasses*) contains several extractable high-value components as polyphenols, tartrates and yeast biomass that could also be exploited. Conversely, *vinasses* represent a potential ecological problem upon disposal as they are rich in organic matter, thus possessing a high oxygen demand.

The most important valuable compound that can be extracted from *vinasses* is tartaric acid, the prevalent acid found in grapes and wines, which is very uncommon in other plant

materials (Keller, 2015). Tartaric acid is an important acidifier used for different applications not only in food and beverage productions (Salgado, Rodríguez, Cortés, & Domínguez, 2010), but also in the pharmaceutical, cosmetic and chemical industries (Kassaian, 2000). The global consumption of tartaric acid has been estimated around 28.000 tons in 2010, while the market price reached an average of 3000 US$/ton in 2013 (Zorn & Czermak, 2010).

A tartaric acid recovery procedure is already applied at industrial level on wine lees. It foresees the lees drying and grinding followed by the potassium bitartrate solubilization using water at 70°C. After solid lees removal, calcium salts are added to determine the calcium tartrate precipitation. The latter is then decomposed using sulfuric acid obtaining solubilized tartaric acid and insoluble calcium sulfate salts, disposed as a waste. After purification and discoloration, the tartaric acid solution is concentrated, and solid tartaric acid crystallized (Kassaian, 2000). Following a modified method, Rivas, Torrado, Moldes, & Domínguez (2006) optimized an integral process for tartaric acid extraction from distilled wine lees (*vinasses*) by dissolution with HCl and precipitation with $CaCl_2$. The authors reported that, starting from 100 kg (12.24 kg dry weight) of post-distillation *vinasse*, 2.20 kg of tartaric acid could be recovered. Nevertheless, the $CaCl_2$ precipitation was considered costly and environmental offensive due to the calcium sulfate production as a waste (Kontogiannopoulos, Patsios, & Karabelas, 2016). Therefore, a less energy and chemicals consuming approach have been recently proposed by Kontogiannopoulos and colleagues (Kontogiannopoulos et al., 2016; Kontogiannopoulos, Patsios, Mitrouli, & Karabelas, 2017), involving the use of acidified water and cation exchange resins to bind K ions and solubilize tartaric acid at room temperature. In a following study, this solution rich in tartaric acid (also containing a significant amount of polyphenols) was nanofiltered through 1kDa membrane to obtain a tartaric acid rich solution that could be further

concentrated for a complete tartaric acid recovery without the production of calcium sulfate.

The solid residue from ethanol and tartaric acid extractions possessed only a low quantity of organic compounds leading to an important decrease in its chemical oxygen demand (Salgado, Rodríguez, et al., 2010). Thus, the ecological problems derived from the disposal of the *vinasses* after ethanol and tartaric acid extraction are significantly lower if compared with those of the original wine lees (Rivas et al., 2006). However, the extraction residues, essentially made of yeast biomass and polyphenols absorbed on it, represent both a disposal problem and an opportunity for their valorization.

3.2.2 Utilization of vinasses for the production of microbial culture media

An interesting approach to exploit *vinasses* is their use as a cost-effective culture media for lactic acid bacteria (G. Bustos, de la Torre, Moldes, Cruz, & Domínguez, 2007; Pérez-Bibbins, Torrado-Agrasar, Salgado, Oliveira, & Domínguez, 2015; Rivas et al., 2006). In particular, Rivas et al. (2006) proposed an approach in which *vinasses*, after being treated for tartaric acid recovery, were used as a source of nutrients for lactic acid production by *L. pentosus*, using hemicellulose-containing hydrolysates of vine shoot as carbon source. The comparison of the results obtained with this culture medium with those of both a conventional medium and a medium made with *vinasses* before tartaric acid extraction showed comparable levels of lactic acid production.

Similarly, Bustos et al. (2007) evaluated the potential of a *vinasses*-based culture medium for the fermentation of hemicellulosic hydrolysates using *L. pentosus* to produce both lactic acid and biosurfactants. Also in this case, similar results were obtained in terms of lactic acid and biosurfactants production for the tested media and the control (made with corn steep liquor and yeast extract), with no effects on the recovery of lactic acid.

Other biotechnological applications of *vinasses* after tartaric acid extraction concern their employment in the formulation of cost-effective culture media for xylitol production by *Debaryomyces hansenii*, the microorganism typically used for the bioconversion of xylose to xylitol. Salgado, Rodríguez, Cortés, & Domínguez (2009), considering the nutritional needs of *D. hansenii,* were able to calculate an economic efficiency parameter that identified *vinasses* as a lower cost and more effective nutrient source for xylitol and citric acid production in comparison to corn steep liquor, a widely used bulk nutrient. Later, Salgado et al. (2010) optimized the process for wine lees valorization which allowed the extraction of tartaric acid with a high degree of purity and the application of the residues as low-cost nutrients for xylitol production by *D. hansenii.* In subsequent studies, Salgado, Carballo, Max, & Domínguez (2010) evaluated *vinasses* from five different wines without observing significant compositional differences among them. Furthermore, these authors compared the effect of using the whole *vinasses* with its liquid phase, obtaining the best results in terms of xylitol production when the liquid phase was used. This unexpected outcome was attributed to the presence of an excessive amount of nutrients in the whole *vinasses* that could inhibit the bioconversion metabolism (Pérez-Bibbins et al., 2015).

Vinasse-based culture media were also applied for the growth of *Aspergillus* species. Salgado et al. (2014) produced extracellular lipases by solid-state fermentation (SSF) with *A. niger, A. ibericus*, and *A. uvarum,* using different waste-derived culture media including a combination of olive pomace with wine *vinasses*. In this case, the culture medium that included *vinasses* did not give satisfactory results compared with other waste-derived media. Conversely, a previous work (Salgado et al., 2009) used *vinasses* obtained before or after tartaric acid extraction for the formulation of a low-cost culture media for citric acid production by *A. niger*, showing results comparable with those of other synthetic media.

In summary, the production of microbial substrates from wine lees seems to be a viable possibility that deserves to be integrated with the extractions of ethanol and tartaric acid. However, the application of the whole *vinasses* in fermentation media did not always give encouraging results. On the other hand, all the strategies that start from *vinasses* do not include the extraction of polyphenols, although this step has been described from other matrices (Jordán, Martínez, Martínez, Moñino, & Sotomayor, 2009; León, Ruiz, Marcos, Antolin, & Del Álamo, 2006). The possibility of including polyphenols extraction in wine lees processing was considered by Kopsahelis et al. (2018) who developed a protocol starting with the initial separation of wine lees in their solid and liquid fractions. The liquid fraction was then distilled for ethanol production while the solid fraction underwent both effective polyphenols and tartrate extractions before being applied as substrate for the production of microbial oil from *Cryptococcus curvatus* and *Mortierella ramanniana*. Therefore, this approach attempted to extract all the valuable components of wine lees and then use the solid residues as substrate to obtain a high-value biotechnological product.

3.2.3 Other wine lees applications

Several other studies looked at ways to exploit the solid fraction of wine lees, including the production of biogas, of high-quality digestates through anaerobic co-digestion by *Escherichia coli* in both thermophilic and mesophilic conditions (Da Ros, Cavinato, Pavan, & Bolzonella, 2014), and other applications in agriculture and animal nutrition, although the presence of phenolics made these applications problematic. Some authors tried to overcome these issues, and Paradelo, Moldes, & Barral (2010) were able to create optimal crop manures by composting mixtures of hydrolyzed grape marc and wine lees in the presence of $CaCO_3$. Under these conditions the pH of the mixtures increased (from 5.1–

6.7 to 7.1–8.1), the salinity and water-soluble carbon were reduced, and the initial phytotoxicity of wine lees and grape marcs disappeared in all the mixtures tested. Moreover, Molina-Alcaide (2008) studied the chemical composition, *in vitro* digestibility, ruminal degradability and intestinal digestibility of nutrients of vine shoots, grape marcs and wine lees, and found that a combination of these by-products may constitute a valuable source of energy and protein for ruminants (Devesa-Rey et al., 2011). Recently, Yao, Zhang, Wang, & Liu (2018) proposed to solid-state ferment lees from Yellow Wine (an alcoholic beverage made from cereals) with *Candida utilis* and *Bacillus subtilis*. This approach, potentially replicable on wine lees, allowed obtaining a more digestible biomass enriched in essential amino acid and antioxidants for animal-nutrition.

Other authors looked at the problem from a different perspective considering the presence of polyphenols as an added value. For instance, the solid fraction of wine lees was added to an ice-cream. The ice-cream antioxidant activity improved, but its rheological properties worsened when wine lees were used at high dosages, an occurrence that could lower the acceptability and the technological properties of the final product (Hwang, Shyu, & Hsu, 2009; Sharma, Kumar, Azad, & Adsule, 2015).

In summary, wine lees are produced in high quantities worldwide, but their commercial exploitation is still limited despite the potential described above. Generally wine lees are still considered as a waste to be managed in order to avoid the disposal into soils or free spill in water environments. Nevertheless, increasing disposal costs alongside with the introduction of fines are pushing the wine sector towards exploring new strategies for the valorization of its by-products. Among these, wine lees seem to have received less attention than others (e.g. grape marc or seeds), and as a consequence these are still

under-exploited (Ye *et* al., 2014; Pérez-Bibbins *et* al., 2015). In Fig. 2 the currently available valorization strategies (both real and potential) are shown.

Indeed, the unique commercial use of wine lees is for ethanol extraction by distillation, as required by the European Regulation. After ethanol, the extraction of tartaric acid from wine lees has been well developed and this method is occasionally applied on a large scale. However, it is clear how the combination of these steps are not sufficient to reduce the chemical oxygen demand of wine lees under levels considered safe for the environment, nor are sufficient for a complete by-product valorization. Therefore, future improvements are needed for an effective recovery of valuable compounds from wine lees, thus increasing the economic sustainability of the entire process. For example, a system to integrate the extraction of polyphenols with that of the ethanol and tartaric acid should be developed. For the remaining lees biomass, the production of fermentation media supplement seems a valid way to achieve a complete exploitation of the lees with the possibility to produce valuable compounds. One issue for the industrial uptake such systems is represented by the high variability of wine lees' composition, as this, for example, means that the production of standardized fermentation media would be problematic. However, alternative valorization strategies should be investigated for the solid phase of wine lees. In this context, the recovery of yeast cell wall's polysaccharides could represent an interesting option for the valorization of the wine lees biomass. Indeed, the literature reports on the extraction of mannoproteins (MPs) and β-glucans (β-G) with potential applications in several fields. However, most of the results available to date are obtained starting from cultivated yeast cells and in only very few cases lees resulting from fermented beverages were considered as a source of valuable polysaccharides (Silva Araújo et al., 2014; Varelas, Tataridis, Liouni, & Nerantzis, 2016). An overview on the studies describing various valorization strategies for yeast and yeast-based by-products is

shown In Table 3. The integrated recovery approaches, oriented to a better exploitation of the whole matrix valuable constituents, are also evidenced.

Table 3 here

4. Yeast cell wall polysaccharides: mannoproteins and β-glucans

Among all the compounds that can be found in yeasts, two principal components of the cell wall, MPs and β-G, have been studied for applications in food, viticulture and oenology, cosmetic and pharmaceutical industries. The cell wall of *Saccharomyces cerevisiae* represents 15–30% of the cell's dry weight, depending on growth conditions (Dimopoulou, Lonvaud-Funel, & Dols-Lafargue, 2017), and is made of different and interconnected polysaccharides layers (Fig. 3). The outer layer is made of MPs, connected to a matrix of amorphous β-1,3 glucan, while the inner layer consists of fibrous β-1,3 glucan, over a small quantity of chitin (Lipke & Ovalle, 1998).

Fig.3 here

MPs and β-G account for more than 90% of the yeast cell wall (Table 4). Therefore, the extraction of these compounds from wine lees would allow to completely exploiting the solid matrix remaining after ethanol, tartrate and polyphenol extractions.

Table 4 here

4.1. Yeast's β-Glucans

β-G, the primary constituent of the yeast cell wall (35 – 55%, see Table 4), are polymers of D-glucose linked by β-glycosidic bonds, representing 6 – 12% of the wine lees dry weight (Nerantzis & Tataridis, 2006). β-G are long chains of about 1500 β-1,3-linked D-glucose

units (85% of the total β-G), on which shorter branches of about 150 -D-glucose units (15% of the total β-G) are linked by β-1,6 bonds (Liu, Wang, Cui, & Liu, 2008) (Fig. 4).

Fig. 4 here

In the yeast cell wall, the different β-G chains interact each other through hydrogen bonds, forming a continuous, three-dimensional network. The elasticity of this network allows the yeast cells to withstand the osmotic stress. This matrix represents the principal structure to which the other cell wall polysaccharides (MPs and chitin) are linked (Klis, Boorsma, & De Groot, 2006). Therefore, the different methods applied to extract cell wall polysaccharides aim to destabilize the 1,3-β-glucan network.

4.1.1. Yeast β-glucans in food, nutraceutical and agricultural applications

Applications of β-G as functional ingredients have been studied since the 1940s (Liu et al., 2008). Indeed, β-G has a proven immunostimulatory effect that helps protecting the body against viral, bacterial and fungal infections, tumors, radiation effects, and stress-related immunosuppression (Adachi, Ohno, Yadomae, Ohsawa, & Oikawa, 1990; Bohn & BeMiller, 1995; di Luzio, Williams, McNamee, Edwards, & Kitahama, 1979; Kim & Yun, 2005). Additionally, β-G can lower the level of LDL-cholesterol, and act as antioxidant and free radical scavengers (Kim & Yun, 2005). However, *in vitro* and *in vivo* studies revealed that the immunomodulatory properties of β-G depend on their 3D structure, molecular weight and side chains (Bohn & BeMiller, 1995). In 2011, the European Commission introduced yeast β-G into the “list of novel food components” (Regulation EC No 258/97, 1997). As a consequence, yeast β-G are now used in functional foods (Bzducha-Wróbel et al., 2014).

β-G from yeast and cereals are of particular interest also because they can affect the physicochemical and rheological properties of foods including viscosity, water holding, oil binding, and emulsion stability (Laroche & Michaud, 2008; Thammakiti, Suphantharika, Phaesuwan, & Verduyn, 2004; Worrasinchai, Suphantharika, Pinjai, & Jamnong, 2006). Cereals' β -G were successfully used as gel stabilizers in pasta (Laroche & Michaud, 2008), salad dressings (Kontogiorgos, Biliaderis, Kiosseoglou, & Doxastakis, 2004) and cakes (Kalinga & Mishra, 2009). Analogously, yeast β-G were applied as fat-replacer for the preparation of low fat mayonnaise (Worrasinchai et al., 2006).

In 2004, Thammakiti et al. analyzed the technological properties of both commercial β-G preparations and β-G obtained from spent brewer's yeast. They found that spent brewer's yeasts are more viscous and have higher water-holding and emulsion stabilizing capacity, but similar oil-binding capacity, compared to the commercial β-G preparations. In 2006, Worrasinchai et al. studied the effect of replacing 50% of the total oil with β-G from spent brewer's yeast for the production of a low-fat mayonnaise, which, moreover, exhibited higher storage stability than the sample containing the conventional amount of oil. Moreover, β-G from exhausted yeast recovered after fermentation of apple wine have been shown to have a great potential for use as thickeners, water-holding and oil-binding agents or emulsifying stabilizers in food products such as soups, sauces, desserts and salad dressings (Rozmierska, Stecka, Kotyrba, & Piasecka-Jóźwiak, 2019).

Furthermore, given that the human digestive system cannot degrade β-G, their consumption has the double advantage of not affecting the caloric intake while increasing the dietary fiber supply (Worrasinchai et al., 2006).

All these results clearly indicate that yeast β-G is a strong candidate for being increasingly used in the food industry thanks to its functional, technological and nutraceutical

properties. Indeed, several commercially available food products (e.g. biscuits and crackers) are already prepared with yeast β-G.

The growing pressure on decreasing the use of pesticides in agriculture and the rising of alternative production systems (e.g. organic and biodynamic approaches) is boosting researchers to further study alternative methods for plant protection. In the last decade, many studies focused on the application of plant elicitors to delay or reduce the recourse to chemicals. Elicitation of the plant defense mechanisms by different polysaccharides has been described and linear β-G from algae (Laminarin from *Laminaria digitata*) have been proposed to this aim against different fungi as *Plasmopara viticola* (Portu, López, Baroja, Santamaría, & Garde-Cerdán, 2016) and *Botrytis cinerea* (Aziz et al., 2003). Recently, an increasing number of laminarin formulations are adopted to protect grapes, tobacco, apples, berries, tomatoes and other horticultural plants (Arena et al., 2017; Klarzynski et al., 2000). Also, preparations of yeast β-G and laminarin were included in some commercial formulations for downy mildew control in viticulture (Garde-Cerdán et al., 2017), although the specific effects of yeast β-G were not assessed. Therefore, studies to evaluate this point are needed before considering this as a plausible possibility for valorization of yeast lees.

4.1.2. Extraction methods

As mentioned above, some industrial preparations containing β-G extracted from yeast cell walls are already commercialized and various studies suggest their extraction from spent brewer's yeast as a mean for recovery of brewing by-products (Freimund, Sauter, Käppeli, & Dutler, 2003; Silva Araújo et al., 2014; Thammakiti et al., 2004). The first methods for β-G extraction from yeast cells have been developed using hot alkali, acid or a combination of them(Manners, Masson, & Patterson, 1973; Petravić-Tominac et al., 2011; Williams et al., 1992). These protocols are capable of solubilizing proteins and some polysaccharides

so to obtain an insoluble residue called "yeast glucan". More recently, the β-G extraction from both red and white wine lees has been carried out by combining autolysis and hot NaOH treatments (Varelas et al., 2016). The obtained extracts showed a β-G content ranging from 29% (w/w) (red wine lees) to 43 % (white wine lees), this difference being attributed to the inhibition of endogenous glucanase activities by red wine polyphenols (Varelas et al., 2016; Zinnai, Venturi, Quartacci, Andreotti, & Andrich, 2010).

However, the application of acidic and alkaline conditions can cause a strong degradation of the β-G' chains, with consequent low extraction yields, high extraction costs and limited β-G purity that could affect their bioactivity (Freimund et al., 2003; Liu et al., 2008). The limitation of these chemical extraction methods can be overcome with a physical-enzymatic approach, firstly proposed by Freimund et al. (2003) and modified by Liu et al. (2008). These milder processes involve the use of hot water to destabilize the cell wall matrix, organic solvents to extract the lipid fraction, and enzymatic treatments to solubilize the remaining proteins. Following this protocol, the β-G extract is finally collected as an insoluble material that preserves its native fibrous structure.

Extraction methods combining different approaches were also used. For example, the method by Liu et al. (2008) was modified with the introduction of a sonication step to further destabilize the cell wall matrix before lipids and proteins separation (Magnani et al. 2009). In 2014, Bzducha-Wróbel et al. compared all the published physical methods for the extraction of β-Glucan from yeast and found that bead mill extraction gave the highest yields, followed by sonication and heating in autoclave.

It is important to emphasize that all the physical methods aim to destabilize the glucan matrix to allow the release of all the water-soluble components (including MPs). Therefore, the combination of physical and enzymatic approaches could allow the extraction of both β-G and MPs with the same protocol (Freimund et al., 2003). Actually, integrated

extraction approaches seem the most promising to completely exploit the yeast lees when more than one component of these by-products must be valorized.

4.2. Yeast mannoproteins

The other major component of the yeast cell wall are MPs (see Table 4), compounds present in the external layer of the yeast cell (see Fig. 1). MPs consist of mannose polymers covalently attached to a protein backbone (Cameron, Cooper, & Neufeld, 1988). Thanks to the amphipathic structure of the MP molecule, in which the hydrophilic mannose polymers are linked to proteins, they have been tested as emulsifiers in several food applications, and in oenology as protein and tartrate stabilizer and foam and mouthfeel enhancer (de Melo, de Souza, da Silva Araujo, & Magnani, 2015; Lomolino & Curioni, 2007; Moine-ledoux & Dubourdieu, 1999; Silva Araújo et al., 2014).

4.2.1. Yeast mannoproteins in food applications

MPs emulsifying properties were first described by Cameron et al. (1988), who reported that MPs were able to stabilize oil-in-water emulsions over a broad range of conditions, (from pH 2 to 11, with up to 5% sodium chloride or up to 50% ethanol in the aqueous phase). Furthermore, this was the first article indicating that a by-product as spent brewer's yeast could be an interesting and cost-effective source of MPs. In 1996, Torabizadeh, Shojaosadati, & Tehrani compared the emulsifying performances of MPs and Sodium caseinate, showing that MPs produced more stable emulsions after 1 month at 4°C. Subsequently, Dikit, Maneerat, & H-Kittikun (2012) and Dikit, Maneerat, et al. (2010) extracted yeast MPs from a yeast biomass recovered from real industrial productions of Thai traditional liquor and sugar palm wine. In both cases, MPs showed good emulsifying properties over a broad range of pHs (5-8 and 3-12 respectively).

Moreover, the MPs extracts maintained the emulsifying activity also after heating them at 121°C, indicating that the high temperatures usually adopted in food processing (such as pasteurization or cooking), do not affect the MPs emulsifying activity (Dikit et al., 2012, 2010). In 2013, Silva Araújo et al. (2014) following the method purposed by Magnani et al. (2009) for the extraction of β-G from spent brewer's yeast, obtained a MPs fraction as intermediate product which was used as emulsifier to produce a mayonnaise. The comparison of MPs with a commercial xanthan gum showed that these two preparations had similar emulsifying and stabilizing properties. Moreover, the use of yeast MPs to replace xanthan gum in mayonnaise formulation had no negative effect on the sensory properties of the product (aroma, color, flavor and texture), even after 28 days of refrigerated storage (Silva Araújo et al., 2014). The same research group used a MPs extract (alone and in combination with soy lecithin) to produce a French salad dressing. Emulsion stability tests, color and sensory analysis indicated that the salad dressing stabilized with MPs alone presented the best results for all the considered parameters (de Melo et al., 2015).

In conclusion, from an analysis of the available data, it seems that yeast MPs can be a valid alternative to commercial emulsifiers, also suitable for vegetarian and vegan preparations. To date, the biomass collected after beer fermentation (also called “beer lees” (Pérez-Bibbins et al., 2015)) was shown to be a raw material suitable for this production and therefore possibly also yeast lees from other fermentation processes (e.g. winemaking) can be exploited to produce MPs. However, confirming this possibility needs further investigation.

4.2.2. Yeast mannoproteins in Oenological applications

In oenology, yeast MPs preparations are mostly sold for their protective activity against tartrate precipitations in both red and white wines. The precipitation of tartrate salts (mainly

potassium bitartrate) is a major cause of sediment formation in bottled wine (Guise et al., 2014) which has relevant impact on wine marketability. Tartrate instability can be prevented by adopting different treatments (i.e. cold stabilization or electrodialysis) or by adding protective colloids like carboxymethyl cellulose, polyaspartate (Bosso et al., 2015) but also yeast MPs (Lankhorst et al., 2017). MPs effect against precipitations is due to their colloid protective action that allows them to significantly reduce crystals' nucleation and growth (Gerbaud, Gabas, Blouin, & Crachereau, 2010; Guise et al., 2014; Moine-Ledoux & Dubourdieu, 2002). Despite the use of MPs is expensive and often not sufficient to fully stabilize the wine (Lasanta & Gómez, 2012), the use of yeast MPs for this purpose is widespread and authorized by the European Union and the International Organization of Wine and Vine (OIV, 2019).

The OIV recognize also the MPs' protective activity against protein haze formation in wines. Wine clarity is another important attribute for winemakers. Indeed, if a bottle shows some turbidity it is generally rejected by consumers. The most common form of haze found in white wine results from the aggregation of wine proteins during storage, an occurrence generally prevented by winemakers using bentonite, a clay that binds wine proteins that are subsequently removed from the wine together with the bentonite (e.g. by settling, filtration or centrifugation) (Van Sluyter et al., 2015). On the other hand, bentonite also removes some wine aroma components, hence lowering wine quality (Salazar et al., 2017; Vincenzi, Panighel, Gazzola, Flamini, & Curioni, 2015). Nevertheless, it was observed that aging wine on lees (*sur lies*) determines a significant reduction in the risk of protein-haze formation. This has been attributed to the release of MPs from cell walls during the autolytic process occurring during wine ageing on lees. These MPs, by interacting with wine proteins, are able to prevent their aggregation thus increasing the wine stability (Moine-ledoux & Dubourdieu, 1999). However, the exact mechanism for protein haze protection in wine remains unclear (Dupin et al., 2000). In 1999, Moine-ledoux &

Dubourdieu isolated a fragment of *S. cerevisiae*'s invertase (a highly mannosylated protein located in the periplasmic space of the yeast cell) which was shown to be responsible for improving the protein stability of white wines. Furthermore, they have developed an extraction method (using the β-glucanase enzyme Glucanex®) for the isolation of the MPs fraction from pure cultures of *Saccharomyces cerevisiae*. This process has been subsequently applied on large scale to produce a haze-protective additive that should reduce the consumption of bentonite (Moine-ledoux & Dubourdieu, 1999).

In 2007, Lomolino & Curioni tested different extraction methods (three different β-glucanases, dithiothreitol and EDTA and their combinations) for MPs isolation from pure oenological yeasts and showed different haze-protective effects in white wine, which was attributed to the presence of MPs.

Along with free peptides and amino acids, the MPs fraction is also known to have a significant impact on the wine foaming. Indeed, MPs are able to adsorb on the liquid/air interface and reduce the wine surface tension and also to increase the viscosity of the wine thus retarding the drainage of the bubbles walls, leading to an increased foam stability (Blasco, Viñas, & Villa, 2011). Furthermore a cooperation between proteins deriving from the grape and yeast MPs has been demonstrated for foam formation in Prosecco wine (Vincenzi, Crapisi, & Curioni, 2014).

All these results highlight the importance of MPs for both foam volume and stability in sparkling wines (Martínez-Lapuente, Guadalupe, Ayestarán, & Pérez-Magariño, 2015) and led to the idea to use MPs to improve these parameters.

In 2006, Núñez, Carrascosa, González, Polo, & Martínez-Rodríguez tested both thermal and enzymatic MPs extracts for improving the foaming properties of sparkling wines. The model wine supplemented with the thermal MPs extract presented the best performances in terms of foam maximum height, foam stability, and effervescence when compared to the

model wine supplemented with the enzymatic MPs extract, which had a behavior similar to that of the non-supplemented control (Núñez et al., 2006). This confirms that the method used for MPs extraction has a crucial role in determining their functionality.

The effect on viscosity of the MPs may also affect the mouthfeel attributes of the wine matrix. Wine mouthfeel encompasses the sensory attributes of viscosity, astringency and hotness (R. Gawel, Smith, & Waters, 2016). Several studies (R. Gawel et al., 2016; Li, Bindon, Bastian, Jiranek, & Wilkinson, 2017; Li, Bindon, Bastian, & Wilkinson, 2018) investigated how yeast MPs and their interaction with other wine components could modify the sensory perception of both white and red wines. In particular, high and medium molecular weight wine polysaccharides, which include MPs, showed some impact on the perceived viscosity at pH 3.6, but not at pH 3.2, and caused the reduction of palate hotness, which is due to the contact between ethanol and the oral mucosa (Gawel et al., 2016). Indeed, with their high water holding capacity, yeast MPs could disrupt the surrounding local water–water and water–ethanol structures and consequently affect the wine viscosity and the perception of hotness (Chinachoti, 1995). MPs, along with other polysaccharides, can also interact with polyphenols (mainly tannins) reducing their aggregation and precipitation which leads to a decrease in astringency. This interaction has been highly investigated, but a shared consensus regarding the explanation of the interaction mechanism has not yet been reached (Richard Gawel, Smith, Cicerale, & Keast, 2018). Some authors (Escot, Feuillat, Dulau, & Charpentier, 2001) state that interaction between polyphenols and wine polysaccharides can modify the polyphenols structure preventing their precipitation after binding to salivary proteins. Other authors (Mateus, Carvalho, Luís, & De Freitas, 2004; Ramos-Pineda, García-Estévez, Dueñas, & Escribano-Bailón, 2018) suggest that mannoprotein and other polysaccharides can contribute to the formation of polyphenols/polysaccharides/protein ternary complexes which, instead the polyphenols/protein complexes, are known to be soluble and positively

contribute to the saliva lubrication. Nevertheless, other authors report that the astringency modulating effect is primarily due to polysaccharides as rhamnogalacturonans, and only secondary to mannoproteins and arabinogalactan-proteins (Vidal, Courcoux, et al., 2004; Vidal, Francis, et al., 2004).

In conclusion, even if the technique of MPs addition cannot replace completely other stabilization methods applied in winemaking to solve the problem of protein and tartrate stability, these proteoglycans have a significant impact on wine quality attributes as foaming and mouthfeel properties. As a result, yeast MPs-based preparations are widely used in oenology since almost two decades. This is also due to the fact that, compared to other additives, MPs are already naturally present in wines and, therefore, are easily accepted, even for organic wines. Considering the actual pressure for the reduction in exogenous additives along with their wide range of applications, MPs-based preparations are expected to maintain or increase their market relevance over the years.

4.2.3. Extraction methods

Essentially two different approaches for MPs extraction are reported in the literature: one based on the use of enzymes able to degrade the yeast cell walls and the other based on physical treatments.

The enzyme-based approach involves the use of preparations containing β-glucanase activity that is able to destabilize the β-G matrix of the yeast cell wall thus allowing the release of MPs into the liquid phase. The proposed extraction methods use different types of β-glucanases, with different results. For example, Dupin et al. (2000) utilized Zymolyase, a pure β-glucanase preparation for laboratory use, while other authors employed Glucanex®, an industrial preparation of β-glucanases from *Trichoderma sp.* commonly used to facilitate filtration of wines containing glucans from *Botrytis cinerea*.

Glucanex® was used for MPs extraction from yeast by Moine-ledoux & Dubourdieu (1999) and Lomolino & Curioni (2007), who reported encouraging results for the Glucanex®-extracted MPs as protective agents against protein haze formation in white wines. Given the affordable price of Glucanex® and other commercial glucanase preparations, their use could then be considered for MPs extraction at industrial scale. However, the use of β-glucanases, although being suitable for MPs extraction, does not allow the simultaneous recovery of functional β-Gs as these are enzymatically degraded during the extraction.

Most of the extractions made using β-glucanase activities were performed to produce MPs extracts for oenological applications. Conversely, the approach based on physical treatments to extract MPs was mainly used for food applications. In general, most of the physical protocols are based on the treatment of the yeast cells in autoclave. Depending on the cases, yeast cells are heated for 1-4 hours at the standard temperature of 121°C and then MPs fraction is precipitated from the liquid phase by ethanol addition. Finally, the extract is freeze- or spray-dried (de Melo et al., 2015; Dikit et al., 2012; Silva Araújo et al., 2014; Thammakiti et al., 2004). Unlike the enzymatic extractions, these works followed a protocol which could be consistent with the integrated extraction of both β-G and MPs discussed above (Freimund et al., 2003; Magnani et al., 2009). These authors applied an approach that was initially developed for β-G extraction, but the same method was demonstrated to be effective also to obtain MPs extracts with relevant technological properties (Silva Araújo et al., 2014). The autoclave treatment is a technique widely spread at industrial level, and therefore its application could be promising for a scale-up of this protocol. On the other hand, heat-extracted MPs do not seem to be suitable for all the applications discussed above. Indeed, autoclave-extracted MPs showed encouraging results in emulsion stabilization and wine foam enhancement (Núñez et al., 2006), but not for preventing protein haze formation in wine, where it has been reported that enzymatically-extracted MPs perform better (Dupin et al., 2000).

Recently, an improved method of extraction of MPs from enological yeasts to be used as emulsifiers was proposed by De Iseppi et al. (2019). In this study, the MPs precipitation with ethanol was replaced with a dialysis step which allowed the inclusion of a soluble β-G fraction in the final extracts. The presence of this β-G fraction, which is known to have a thickening action, could be responsible for the improvement of the emulsion stability that was higher than that reported in previous studies.

These results highlight the importance of developing cost-effective procedures to effectively extract the yeast MPs for food and wine applications. Moreover, as already done for beer lees, the possibility of using wine lees as MPs' source should be further investigated.

5. Potential problems deriving from wine lees to be used for the extraction of polysaccharides

Although being a potential source of MPs and β-G, the exploitation of wine lees present some issues. Firstly, wines less have a considerable amount of polyphenols adsorbed on the yeast cell wall, especially when the lees are obtained from red wines. Even if polyphenols are normally considered valuable compounds, their interactions with the cell wall polysaccharides (especially MPs) could affect the extraction yield, purity and functionality of the final extract. Indeed, polyphenols (i.e. tannins, anthocyanins and flavonols) are absorbed on the yeast cell wall during the contact of wine with lees. The kinetics of wine polyphenols (tannins, anthocyanins and flavonols) adsorption on yeast lees shows an initial rapid fixation (within 12 minutes), followed by a slow, constant, and saturating fixation, which reaches a maximum after about 1 week of contact, being dependent on the quantity of yeast lees (Mazauric & Salmon 2005). Later, the interactions of wine polyphenols with both MPs and yeast's cytoplasmic proteins were reported by

Mekoue Nguela, Poncet-Legrand, Sieczkowski, & Vernhet (2016). Thus, the presence of these interactions between polyphenols and cell wall components, and in particular those with MPs, should be considered for their effects on extraction yields and physicochemical properties of the extract. However, this would not be a problem for the β-G fraction, which have been shown to present only negligible interactions with wine polyphenols (Mekoue Nguela et al., 2016).

An additional factor which could clearly limit the application of the extracts in the food and beverage sectors relates to the possible presence of biocides residues adsorbed on the lees matrix. Indeed, the wine lees fraction, and especially its solid phase, has been reported to be the most contaminated winemaking by-product in terms of biocides residues (Cabras & Angioni, 2000; Čuš, Česnik, Bolta, & Gregorčič, 2010). Indeed, during winemaking an important decrease of the levels of pesticide residues in the wine occurs when the solid lees are separated from the liquid phase (Čuš et al., 2010). Nevertheless, there are few published data describing a specific interaction between a defined bioactive molecule and a specific component of the yeast cell wall (Yiannikouris et al., 2004). Also in this case, MPs seem to be the cell wall component with the higher ability to interact with pesticides/fungicides residues, likely due to their location in the external layer of the cell wall and their content in hydrophobic groups chemically affine to most of the active molecules present in the biocides (Chung, 2018). However, this problem should be evaluated case by case, considering also the type and number of agrochemical treatments performed as well as the effect of the extraction procedure on the removal of biocide residues.

In conclusion, the best way to avoid the above-mentioned problems should be to extract MPs and β-G from less derived from white winemaking of grapes produced with low inputs of chemicals as those derived from organic farming.

6. Summary and future perspectives

Wine lees are a by-product containing several high-value components with the potential to be exploited in different sectors as the food, winemaking, biotechnological and pharmaceutical industries. Despite this potential, and differently from grape skins and seeds, so far wine lees are one of the least studied and exploited winemaking by-products. As an alternative to lees direct disposal, which is a cost for wine producers, only ethanol and tartaric acid extraction are sometimes performed on large scale, starting from lees' liquid fraction and *vinasse,* respectively. The extraction of polyphenols and the use of the remaining lees as culture media for biotechnological applications have proven to be promising possibilities. The wine lees' solid fraction, essentially yeast biomass and polyphenols adsorbed on it, was also tested, with different results, for application in animal feeding, foods and agriculture. Additionally, wine lees could be also exploited to obtain cell wall MPs and β-G, two polysaccharides with several potential applications especially in food production, winemaking and viticulture. In particular, MPs could be applied as emulsifier in food emulsions (e.g. salad dressing), and, in wine, as foam and mouthfeel enhancer as well as for wine protein and tartrate stabilization. Furthermore, β-G could be applied as functional ingredients and fat replacers in food preparations (e.g. low-fat mayonnaise). In addition, other β-G functional properties such as its cholesterol reducing and antioxidant activities could represent a further added value for their use as additives.

An area that still needs to be developed to make these products viable on a commercial scale concerns the methods to be applied for their preparation, as these need to be food-grade, cost-effective, sustainable, and appropriate to yield extracts still possessing the expected functionality. While different methods for MPs and β-G extraction have been developed starting from pure yeast cultures or yeast by-products, only one study

considered the possibility to extract β-G from wine lees. Part of the reasons for this lack of efforts to valorize wine lees can be attributed to their characteristics and in particular to the high polyphenols content and the possible presence of pesticides residues adsorbed on them.

In conclusion, future applications should be aimed at developing an integrate approach aimed at extracting from wine lees the highest number and amounts of compounds with possible applications in different sectors. These compounds have been identified in ethanol, tartaric acid, polyphenol but also β-G and MPs present in the remaining biomass. The development of this model could contribute to improve the tools available for the valorization of wine lees and would give to wine industry a new important means to improve both the environmental and the economic sustainability.

Declaration of Competing Interest

None.

CRediT author statement

Alberto De Iseppi: Conceptualization; Data curation; Formal analysis; Visualization; Roles/Writing - original draft; Writing - review & editing. **Giovanna Lomolino:** Conceptualization; Funding acquisition; Project administration; Supervision; Writing - review & editing. **Andrea Curioni:** Conceptualization; Funding acquisition; Project administration; Supervision; Writing - review & editing. **Matteo Marangon:** Conceptualization; Project administration; Supervision; Writing - review & editing.

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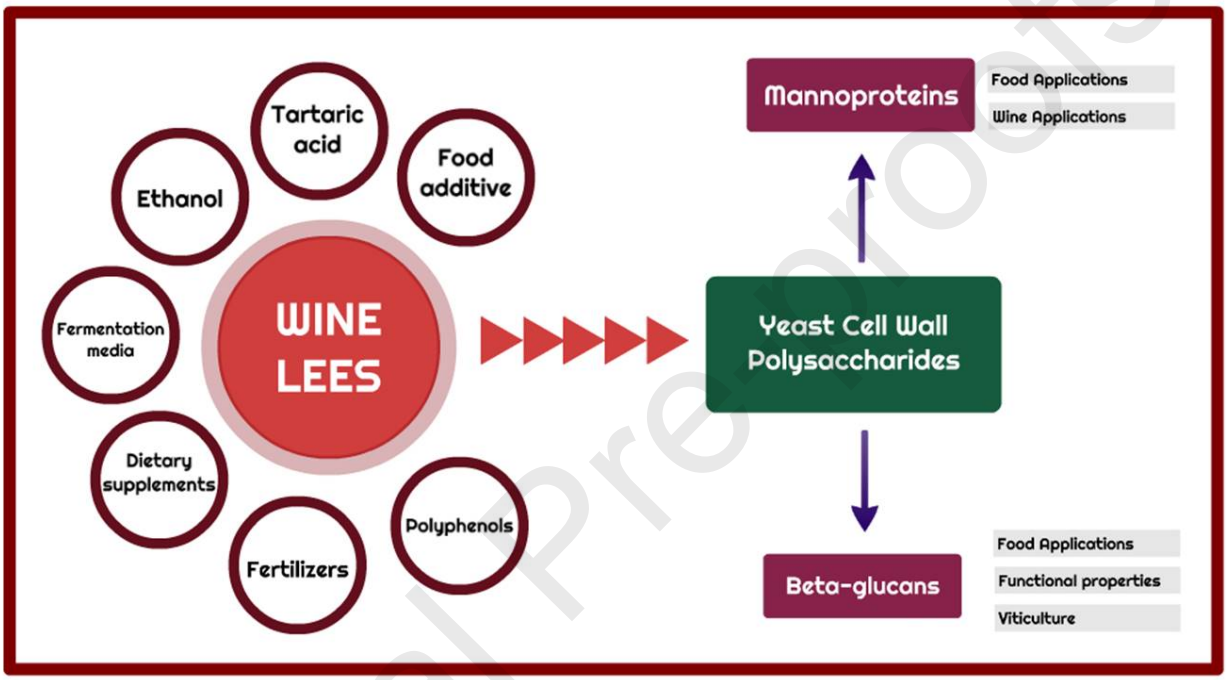
Declaration of Competing Interest

None.



Highlights

- Wine lees are soil pollutant by-products; their disposal is a cost for wineries
- Ethanol, tartaric acid and polyphenols can be extracted from wine lees
- Lees' yeast biomass can be used for the production of fermentation media
- Valuable cell wall polysaccharides can be extracted from wine lees
- Integrated extraction protocols yield different valuable components from wine lees

Table 1. Composition of wine lees (expressed on Dry Matter - DM) (Bordiga, 2015). Data sources: Bustamante et al. (2008); Gómez, Igartuburu, Pando, Rodríguez Luis, & Mourente (2004).

Parameter	Range	Parameter	Range (DM)
Organic Carbon (g/kg)	226 - 376	Ca (g/kg)	3.6 - 15.5
Water-soluble Carbon (g/kg)	44.3 - 168.9	Cu (mg/kg)	13 - 1187
Conductivity (dS/m)	4.0 - 13.8	Fe (mg/kg)	84 - 1756
Organic matter (g/kg)	598 - 936	K (g/kg)	17.6 - 158.1
pH	3.6 - 7.2	Mg (g/kg)	0.4 – 3.7
Polyphenols (g/kg)	1.9 - 16.3	Mn (mg/kg)	<0.2 – 21.0
Total Nitrogen (g/kg)	17.2 - 59.7	P (g/kg)	1.61 - 10.3
Proteins (%)	14.5 - 15.7	Zn (mg/kg)	14 - 84
Lipids (%)	5.0 - 5.9		
Sugars (%)	3.5 - 4.8		
Dietary fiber (%)	21.2 - 21.9		
Tartaric acid (%)	24.5 - 24.7		
Ash (%)	10.5 - 10.6		

Table 2. Main phenolic compounds identified in wine lees.

	Phenolic subclass	Compound	Identification method	Reference
non-Flavonoids				
	Phenolic acids	Gallic acid	LC−QTOF-MS/MS	Delgado et al., 2015
			HPLC-MS/MS	Romero-Díez et al., 2018
			HPLC–DAD–MS	Giacobbo et al., 2019
			HPLC–DAD–MS/MS	Matos et al., 2019
		2-S-glutathionylcaftaric acid	HPLC–DAD–MS/MS	Matos et al., 2019
		Caftaric acid		
		Coutaric acid		
Flavonoids				
	Flavanols	Catechin	LC−QTOF-MS/MS	Delgado et al., 2015
			HPLC-MS/MS	Romero-Díez et al., 2018
			HPLC–DAD–MS/MS	Matos et al., 2019
		Epicatechin	LC−QTOF-MS/MS	Delgado et al., 2015
			HPLC-MS/MS	Romero-Díez et al., 2018
			HPLC–DAD–MS/MS	Matos et al., 2019
		Procyanidin B2	LC−QTOF-MS/MS	Delgado et al., 2015
		Procyanidin trimer	HPLC–DAD–MS/MS	Matos et al., 2019
	Flavonols	Myricetin	LC−QTOF-MS/MS	Delgado et al., 2015
			HPLC–DAD–MS	Giacobbo et al., 2019
			HPLC-MS/MS	Romero-Díez et al., 2018
			HPLC–DAD–MS/MS	Matos et al., 2019
		Myricetin-3-O-glucoside	HPLC–DAD–MS	Giacobbo et al., 2019
			HPLC–DAD–MS/MS	Matos et al., 2019
		Quercetin	LC−QTOF-MS/MS	Delgado et al., 2015
			HPLC–DAD–MS	Giacobbo et al., 2019
			HPLC–DAD–MS/MS	Matos et al., 2019
		Quercetin 3-O-glucuronide	LC−QTOF-MS/MS	Delgado et al., 2015
		Quercetin 3-O-glucoside	HPLC–DAD–MS/MS	Matos et al., 2019
			HPLC-MS/MS	Romero-Díez et al., 2018
			HPLC–DAD–MS	Giacobbo et al., 2019
		Kaempferol	HPLC–DAD–MS/MS	Matos et al., 2019
		Kaempferol-3-O-galactoside	HPLC–DAD–MS	Giacobbo et al., 2019
		Kaempferol 3-(2′,3′-diacetylrhamnoside)-7″-rhamnoside	LC−QTOF-MS/MS	Delgado et al., 2015
		Rhamnetin	HPLC–DAD–MS/MS	Matos et al., 2019
		Syringetin-3-O-glucoside		
	Anthocyanins	Malvidin-3-O-(6-p-coumaroyl)glucoside	LC−QTOF-MS/MS	Delgado et al., 2015
			HPLC-MS/MS	Romero-Díez et al., 2019
			HPLC-MS/MS	Romero-Díez et al., 2018
			HPLC–DAD–MS	Giacobbo et al., 2019
			HPLC–DAD–MS/MS	Matos et al., 2019
		Malvidin 3-O-glucoside	HPLC-MS/MS	Romero-Díez et al., 2018

		HPLC-MS/MS	Romero-Díez et al., 2019
		HPLC–DAD–MS/MS	Matos et al., 2019
	Malvidin 3-(6"-acetylglucoside)	HPLC-MS/MS	Romero-Díez et al., 2019
		HPLC–DAD–MS/MS	Matos et al., 2019
		HPLC–DAD–MS	Giacobbo et al., 2019
	Malvidin-3-glucoside-pyruvate	HPLC–DAD–MS	Giacobbo et al., 2019
	Malvidin 3-galactoside	LC−QTOF-MS/MS	Delgado et al., 2015
	Delphinidin-3-O-glucoside	HPLC-MS/MS	Romero-Díez et al., 2018
		HPLC-MS/MS	Romero-Díez et al., 2019
		HPLC–DAD–MS	Giacobbo et al., 2019
		HPLC–DAD–MS/MS	Matos et al., 2019
	Delphinidin-3-O-(6-p-coumaroyl)glucoside	HPLC-MS/MS	Romero-Díez et al., 2018
		HPLC–DAD–MS/MS	Matos et al., 2019
		HPLC-MS/MS	Romero-Díez et al., 2019
		HPLC–DAD–MS	Giacobbo et al., 2019
	Delphinidin 3-O-(6-p-acetylglucoside)	HPLC-MS/MS	Romero-Díez et al., 2019
		HPLC–DAD–MS/MS	Matos et al., 2019
	Petunidin-3-O-glucoside	HPLC-MS/MS	Romero-Díez et al., 2018
		HPLC-MS/MS	Romero-Díez et al., 2019
		HPLC–DAD–MS/MS	Matos et al., 2019
		HPLC–DAD–MS	Giacobbo et al., 2019
	Petunidin-3-O-(6-p-coumaroyl)glucoside	HPLC-MS/MS	Romero-Díez et al., 2018
		HPLC-MS/MS	Romero-Díez et al., 2019
		HPLC–DAD–MS/MS	Matos et al., 2019
	Cyanidin-3-O-glucoside	HPLC–DAD–MS	Giacobbo et al., 2019
		HPLC–DAD–MS/MS	Matos et al., 2019
	Cyanidin 3-O-(6"-acetylglucoside)	HPLC-MS/MS	Romero-Díez et al., 2019
		HPLC–DAD–MS/MS	Matos et al., 2019
	Cyanidin-3-(6"-p-coumaroyl)glucoside	HPLC–DAD–MS	Giacobbo et al., 2019
		HPLC–DAD–MS/MS	Matos et al., 2019
	Peonidin 3-O-glucoside	LC−QTOF-MS/MS	Delgado et al., 2015
		HPLC–DAD–MS/MS	Matos et al., 2019
		HPLC–DAD–MS	Giacobbo et al., 2019
	Peonidin-3-(6"-p-coumaroyl)glucoside	HPLC–DAD–MS	Giacobbo et al., 2019
		HPLC–DAD–MS/MS	Matos et al., 2019
	Pelargonidin 3-(6-p-coumaroyl)glucoside	LC−QTOF-MS/MS	Delgado et al., 2015
Pyranoanthocyanins	Vitisin A	HPLC-MS/MS	Romero-Díez et al., 2019
		HPLC–DAD–MS/MS	Matos et al., 2019
	10-carboxypyranomalvidin-3–6″-p-coumaroyl-glucoside	HPLC-MS/MS	Romero-Díez et al., 2019
		HPLC–DAD–MS/MS	Matos et al., 2019

Table 3. Summary of the articles that investigated valorization strategies for yeast cells and lees.

Source	Aim(s)	Method/Notes	References
Wine lees			
Targeted approaches & uses			
	Ethanol recovery	Distillation	Bordiga, 2015
	Tartaric acid recovery	Extraction using sulfuric acid	Kassaian, 2000
		Cation exchange resin	Kontogiannopoulos et al., 2016
	Polyphenols recovery	Organic solvents	Cruz et al.,1999
		Supercritical fluids	Wu et al., 2009
		Microfiltration	Giacobbo et al., 2015
		Characterization by mass spectrometry	Delgado De La Torre et al., 2015
	β-glucans recovery	Extraction using NaOH	Varelas et al., 2016
	Microbial media production	Preparation for microbial media	Pérez-Bibbins et al., 2015
	Biogas production	Anaerobic digestion	Da Ros et al., 2014
	Squalene recovery	Ultrasound Assisted Extraction	Naziri et al., 2012
	Animal feeding production	Preparation and digestibility assessment	Molina-Alcaide, 2008
	Agricultural fertilizer production	Composting	Paradelo et al., 2010
	Food ingredient (whole lees) & use	Impact on technological properties	Hwang et al., 2009; Sharma et al., 2015
Integrated approaches & uses			
	Ethanol + Tartaric acid recovery + Microbial media (from Vinasse)	Extraction and preparation for microbial media	Bustos et al., 2004 Rivas et al., 2006; Salgado et al., 2009; Salgado et al., 2010
	Ethanol recovery + Microbial media (from Vinasse)	Extraction and preparation for microbial media	Bustos et al., 2007 Salgado et al., 2010; Salgado et al., 2014
	Polyphenols + Polysaccharides recovery	Ultrafiltration and Nanofiltration	Giacobbo et al., 2017
	Tartaric acid + Polyphenols recovery	Nanofiltration	Kontogiannopoulos et al., 2017
	Ethanol + Tartaric acid + Polyphenols recovery + Microbial media	Extraction and preparation for microbial media	Kopsahelis et al., 2018
Beer Lees and other yeast by-products			
Targeted approaches & uses			
	β-glucans recovery	Chemical extraction and characterization for food applications	Thammakiti et al., 2004;L Rozmierska et al., 2019
		Chemical extraction and application for low-fat mayonnaise production	Worrasinchai et al., 2006
	Mannoproteins recovery	Enzymatic and heat extraction and characterization for food applications	Cameron et al., 1988
		Heat extraction and emulsifying activity assessment	Dikit et al., 2010; Dikit et al., 2012
		Heat extraction for mayonnaise production	de Melo et al., 2015
Integrated approaches & uses			
	β-glucans + Mannoproteins recovery	Heat and enzymatic extraction and MPs used for mayonnaise production	Silva Araújo et al., 2014
Pure yeast cultures			
Targeted approaches & uses			
	β-glucans recovery	Chemical extraction and characterisation	Jamas et al., 1984
		Extraction with dimethyl sulfoxide and functional properties assessment	Williams et al., 1992
		Comparison of different extraction methods	Bzducha-Wróbel et al., 2014

	Application for plant protection	Portu et al., 2016
Mannoproteins	Heat and enzymatic extraction and emulsifying activity assessment	Cameron et al., 1988
	Heat extraction and emulsifying activity assessment	Torabizadeh et al., 1996
	Heat and enzymatic extraction and application for wine protein stabilization	Moine-ledoux & Dubourdieu, 1999
	Application for wine tartrate stabilization	Guise et al., 2014
	Extraction with different methods and comparison for wine protein stabilization	Dupin et al., 2000
	Enzymatic extraction and impact on wine tartrate stability	Moine-Ledoux & Dubourdieu, 2002
	Heat and enzymatic and impact on wine foaming properties	Núñez et al., 2006
	Chemical and enzymatic extraction and impact on wine protein stability	Lomolino & Curioni, 2007
	Impact on wine sensory properties	Li et al., 2017; Li et al., 2018; Ramos-Pineda et al., 2018
Integrated recovery approaches & uses		
β-glucans + Mannoproteins	Heat + Enzymatic extractions	Freimund et al., 2003; Liu et al., 2008
	Heat + Chemical extractions	Petravić-Tominac et al., 2011
	Heat + Sonication + Enzymatic extractions	Magnani et al., 2009
	Extraction with different methods and emulsifying activity assessment	De Iseppi et al., 2019

Table 4. Macromolecules of *Saccharomyces cerevisiae* cell wall (Klis, Boorsma, & De Groot, 2006). Mr, molecular weight.

Macromolecule	% of wall *	Mean M_r (kDa)	Degree of polymerization
Mannoproteins	30 - 50	Highly variable	Highly variable
1,6-β-Glucan	5 - 10	24	150
1,3-β-Glucan	30 - 45	240	1500
Chitin	1.5 - 6	25	120

*by weight

Fig. 1. Number of peer-reviewed publications on “by-products valorization” in the period 1974-2019 (data from Scopus, accessed on the 14th of February 2020. Total number of articles: 1245.

Fig. 2. Comprehensive scheme of the various wine lees valorisation strategies proposed in the literature.

Fig. 3. Saccharomyces cerevisiae cell wall organization.

Fig. 4. Chemical structure of 1,3- β-D-glucan with 1,6-linked branches of glucopyranosyl units.