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Review

Role of lees in wine production: A review

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ABSTRACT

The sometimes contradictory role attributed by scientists to lees in wine production is discussed in this review. Studies dealing with the importance of lees in the natural removal of undesirable compounds from wine, the effect of lees–wine contact on the volatile fraction of wines, the key influence of lees on biogenic amine contents in wines, the interactions between lees and phenolic compounds, and the importance of mannoproteins and lipids released by lees have been critically reviewed. Finally, the present exploitation of lees is also outlined.

Contents

1. Introduction

The definition of wine lees given by EEC regulation No. 337/79 states that "wine lees is the residue that forms at the bottom of recipients containing wine, after fermentation, during storage or after authorized treatments, as well as the residue obtained following the filtration or centrifugation of this product".

Traditionally, only some white and sparkling wines were left in contact with their lees; at present, wine ageing on lees is more frequent in all viticultural areas (Hernández, Estrella, Carlavilla, Martín-Álvarez, & Moreno-Arribas, 2006). In this context, several studies indicate that wine ageing in the presence of lees improves its organoleptic characteristics (Doco, Vuchot, Cheynier, & Moutounet, 2003; Lubbers, Voilley, Feuillat, & Charpentier, 1994; Salmon, Fornairon-Bonnefond, & Mazauric, 2002). Fig. 1 shows a scheme of both standard white and red winemaking technology; the steps in which lees are generated have been noted.

Although the composition of lees is variable, they are mainly composed by microorganisms (mainly yeasts), and, in a minor proportion, by tartaric acid and inorganic matter. Autolysis phenomena undergone by yeast lees during wine ageing produce

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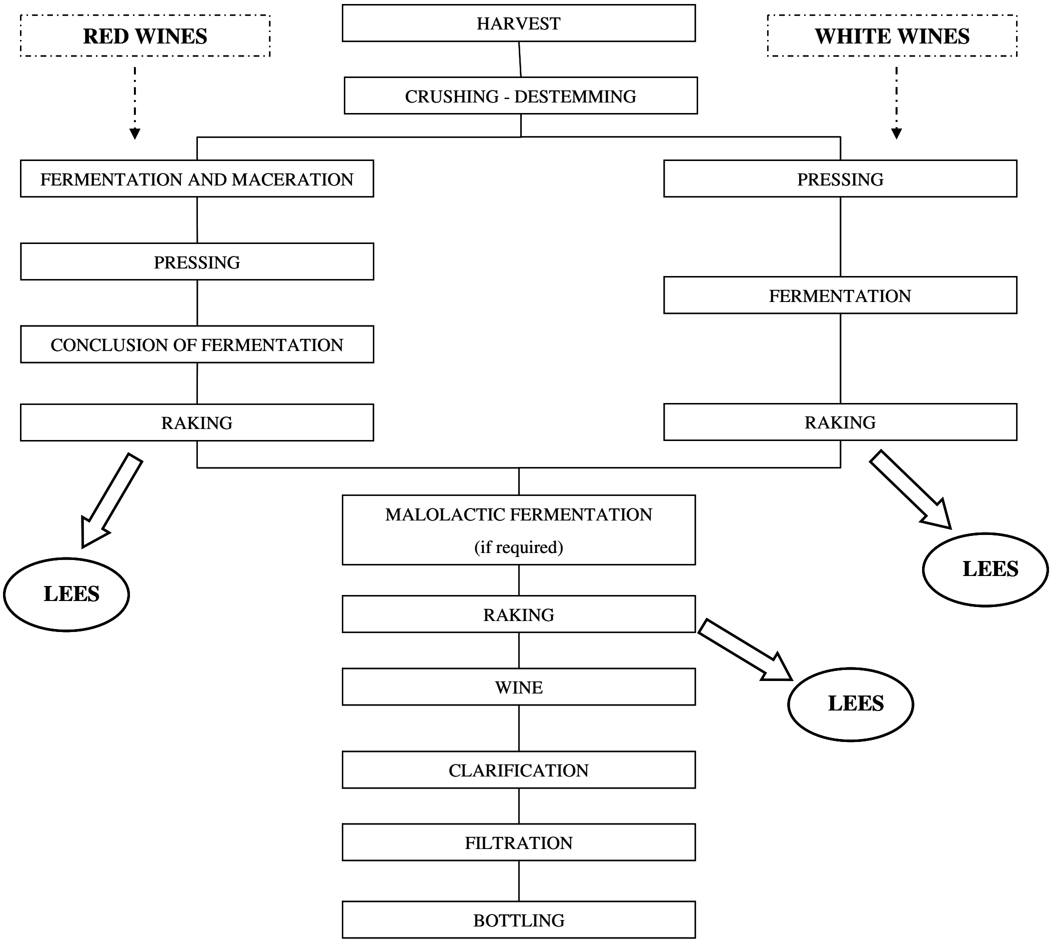

Fig. 1. Scheme of the red and white wines vinification processes.

breakdown of cell membranes, release of intracellular constituents, liberation of hydrolytic enzymes and hydrolysis of intracellular biopolymers into products of low molecular weights (Babayan & Bezrukov, 1985; Charpentier & Feuillat, 1992; Guilloux-Benatier & Chassagne, 2003; Martínez-Rodríguez & Polo, 2000); thus, yeast lees autolysis is of paramount importance in lees composition and in their influence on wine ageing.

The aim of this review is to discuss and compare the scattered present literature concerning wine lees without distinguishing lees from fermentation time and/or the ageing step. Special emphasis is given to the positive and negative consequences of the wine lees contact as well as the present and future uses of wine lees, which seem to be an undervalued byproduct of the winemaking industries. After the review from Fornairon-Bonnefond, Camarasa, Moutonet, and Salmon (2002), the new research in this field deserves to be discussed and new ways of lees valorization to be outlined.

2. Importance of lees in the natural removal of undesirable compounds from wine

Some of the studies on wine lees have been focused on the role they play in the evolution of toxic compounds such mycotoxins, volatile phenols, pesticides and defoaming agents during the vinification process.

Ochratoxin A (OTA) is a carcinogenic mycotoxin produced by several fungus species of *Aspergillus* and *Penicillium*, the presence of which in musts and wines was firstly reported by Zimmerli and Dick (1996) and is widely confirmed at present (Festas et al., 2000; Otteneder & Majerus, 2000; Siantar et al., 2003). OTA detoxification of wines has been attempted by the addition to wine of fining agents (Castellari, Versari, Fabiani, Parpinello, & Galassi, 2001; Dumeau & Trioné, 2000), bentonite (Leong, Hocking, & Scott, 2006) and active charcoal (Fernandes, Ratola, Cerdeira, Alves, & Venancio, 2007), being the most effective. The potential of yeast cells to absorb mycotoxins was first reported by Yiannikouris et al. (2003), Yiannikouris et al. (2004), and recently supported by research focused on demonstrating the utility of lees for natural removal of OTA from wines, thus avoiding the use of fining agents. García-Moruno, Sanlorenzo, Boccaccino, and Di Stefano (2005) added white and red lees to red wine samples spiked with OTA. A significant reduction of OTA was observed after only 90 min of lees–wine contact with a very small amount of wet lees (20 g/l); after contact for 7 days the OTA reduction was greater than 70% with white lees, and around 50% with red lees. Further contact times did not improve the results. The authors justified the better results obtained with white lees, as compared with red lees, by the competition between polyphenols and OTA for the same binding sites on the surface of the yeast cells (Cuinier, 1988; Feuillat, Escot, & Charpentier, 2000; Ummarino, García-Moruno, & Di Estefano, 2001). Leong et al. (2006) assumed the capability of lees for linking OTA from wine and studied if the remaining juice or wine recovered from lees after centrifugation could contain higher OTA contamination than racked juice or wine. Using Semillon and Shiraz grapes containing OTA (produced by inoculation of brunches on the wine with *Aspergillus carbonarius*), they reported that the influence on the extent of OTA removal during the early stages of vinification seems to be associated with the bulk effect of removal by precipitation of solids such as grape colloids and yeasts; and that, in certain circumstances, further compaction of these solids by centrifugation may yield additional recovery of juice or wine from the lees without increased risk of OTA contamination. In a study mainly focused on red wines produced from Vinhao grapes spiked with an *A. carbonarius* spore suspension, which produced OTA after incubation, Fernandes et al. (2007) concluded that a reduction in OTA occurs during vinification is mainly due to OTA adsorption onto suspended solids. Thus, lees recovered after alcoholic fermentation had an OTA content of 50.4% (respect to total OTA present in grapes); sediment obtained after natural settling of the wine 17.6%; and lees obtained after malolactic fermentation 3%. They also justify the lower contamination with OTA of white wines as compared with red wines by the separation of the pomace from must after crushing, step characteristic of white wine vinification; which also explains why juices are usually more contaminated than wines. From these investigations the capability of lees to remove OTA from wines can be concluded.

The presence of 4-ethylphenol and 4-ethylguaiacol in wine is due to contamination by yeast *Brettanomyces* (Chatonnet, Dubourdieu, Boidron, & Pons, 1992). Chatonnet, Boidron, and Pons (1990) described the aroma associated to the presence of these phenols in wine as horsy, medicinal and spicy. Guilloux-Benatier, Chassagne, Alexandre, Charpentier, and Feuillat (2001), and Chassagne, Guilloux-Benatier, Alexandre, and Voilley (2005), reported in their publications decreased contents of 4-ethylphenol and 4-ethylguaiacol found in wines containing yeast lees as compared to the same wines aged without lees; thus demonstrating the feasibility of lees for removing these compounds from wine. However, this behaviour is highly influenced by variables such as the autolysis state of yeast lees, pH, temperature, ethanol content and presence of other wine constituents sorbed by yeast lees.

Famoxadone is a fungicide recently applied to wines that belongs to the oxazolidinedione family. De Melo Abreu et al. (2006) applied a commercial formulation of this fungicide to wines and analysed the cake and lees obtained in the vinification process of grapes from these wines. They observed how during wine production without maceration, part of the famoxadone residues were removed with the cake, and the remained part was also removed with the lees after fermentation. In wine production with maceration process, all the famoxadone residues were separated with the cake and lees at the end of the fermentation process; thus, all residues of famoxadone were adsorbed by the solid fraction in both cases. Gennari et al. (1992) focused their research on the fate of both the fungicide chlozolinate [ethyl (*R*,*S*)-3-(3,5-dichlorophenyl)-5-methyl-2,4-dioxooxazolidine-5-carboxylate] and a compound called metabolite I [3-(3,5-dichlorophenyl)-5-methyloxazolidine-2,4-dione], which is formed as a consequence of the chlozolinate degradation during fermentation. They tested two vinification procedures, with and without maceration. In both cases racking provides a key route of residue reduction as shown by the decreased concentration of chlozolinate and metabolite I in wine and the high residue content in lees after racking. No all pesticides are equally removed by lees, as demonstrated Navarro et al. (Navarro, Barba, Oliva, Navarro, & Pardo, 1999; Navarro, García, Navarro, Oliva, & Barba, 1997) in their studies on the effect of winemaking practices on the concentration of some pesticides. So, in 1997 the authors studied the effectiveness of lees for removing feraminol and penconazole from red musts, and they found that only 1.7 and 2.7%, respectively, of these pesticides – initially added to the must – were removed by lees. Two years later, the same authors studied the evolution of residual levels of other six pesticides during the elaboration of red wines, in which chlorpyrifos, penconazole, mancozeb, vinclozolin, metalaxyl and fenarimol were removed during the racking step (adsorbed onto lees) at percentages dependent on the given pesticide (85, 43, 31, 29, 24 and 19%, respectively, in this case). From the foregoing, it can be concluded that the decrease, and in some cases almost total removal of pesticide residues from wines, depends to a great extent on their initial concentration in the harvested grapes, physical–chemical characteristics of each product, and winemaking procedure. Further research in this field is desirable in order to know the behaviour of other pesticides and test possible modifications of the winemaking procedures to improve the effectiveness of lees to remove pesticides.

Although most must fermentations proceed without incidents, occasional violent foaming can occur; in this case, a defoaming agent is added to tanks. The use of a vegetable oil derivative as defoaming agent created no health concerns, but its residue in wine can be considered as an additive. In this context, Caputi, Ribeiro, and Byrne (2000) studied the natural removal of mono- and diglycerides of oleic acid (defoaming agents widely used in the US) from wine. With this aim, they added the defoaming agent (5 mg/l) and lees from a recently racked fermentation to finish wine. After swirling to disperse the lees through the wine, they were allowed to settle overnight; then, the wine was filtered and analysed, and no defoaming agent was detected (<0.01 mg/l) in the wine. Therefore, they concluded that the defoaming agent, if used during fermentation, is removed from wine by adsorption onto lees; thus explaining why in a survey of 28 Californian wines any defoaming agent could not be detected.

The widely reported importance of lees for natural removal of undesirable compounds from wine makes advisable the exploitation of this phenomenon in other fields such water detoxification, filters production, etc., in which lees could play a key role as natural and cheap decontaminant. In turn, this phenomenon must also be taken into account as a potential contamination source of products recovered from lees.

3. Effect of lees contact on the volatile fraction of wines

Once alcoholic fermentation is finished, yeast lees autolysis releases a number of substances to wine, which, together with volatiles formed during vinification, gives rise to complex balances of aromas that rebound in their quality (Bautista, Fernández, & Falqué, 2007). The contact of wine with lees can also decrease the content in volatile compounds and thus the fruity aroma of wines (Medina-Carnicer et al., 2002). The amount of lees and the time they are allowed to be in contact with wine is also of importance: large amounts of lees favour the synthesis of esters, which improve the quality of wines (Postel & Adams, 1987), but also produce long-chain alcohols and volatile fatty acids; compounds with detrimental effects on wine quality (Ferrari & Feuillat, 1988; Feuillat, Freyssinet, & Carpentier, 1989). The presence of lees is also decisive in the quality of wines aged in oak barrels (Garde-Cerdán & Ancín-Azpilicueta, 2006), as they may affect the distribution of aroma compounds between wine and wood and influence the overall aroma of these wines as a result (Ramirez-Ramirez, Chassagne, Feuillat, Voilley, & Charpentier, 2004). However, the main risk of wine ageing in the presence of lees is the appearance of sulfur odours and bad smelling compounds (Palacios, Varsserot, & Maujean, 1997).

Recent papers have reported the evolution of the most important volatile compounds in wine during ageing on lees, in terms of contribution to wine aroma. Bueno, Peinado, Medina, and Moreno (2006) studied the effect of a short contact time with lees on the volatile composition of Airen and Macabeo wines. Bautista et al. (2007) studied not only the effect of the contact time on the volatile composition, but also compared the results obtained using commercial and fermentation lees. Riu-Aumatell, Bosch-Fusté, López-Tamames, and Buxaderas (2006) used statistical tools such principal component analysis (PCA) and ANOVA to study the development of volatile compounds of sparkling wine during long ageing time in contact with lees. The results they obtained are discussed below.

The role of long-chain alcohols in wine aroma is highly dependent on their concentration: they contribute to wine aromatic complexity at low concentration, whereas a high concentration masks the wine aroma owing to their penetrating odours. Bautista et al. (2007) reported that the concentration of all alcohols – but that of 1-butanol, which decreases in wine matured with the commercial lees – increases with the storage time (either aged in the presence or absence of lees). In the study of Bueno et al. (2006) the behaviour of the alcohols is different in Airen and Macabeo wines; thus, in Airen wines the alcohols concentration increased after 20 days of contact with wine lees, meanwhile in Macabeo wines the behaviour was the opposite. 2-Butanol was not detected in the wines studied by Bautista et al., whereas Bueno et al. found concentrations ranging 1800–8000 μg/l.

Esters of long-chain alcohols and ethyl esters are volatile compounds which play a key role in wine aroma by providing fruity nuance (Etievant, 1991). The results provided by Riu-Aumatell et al. (2006), Bautista et al. (2007) and Bueno et al. (2006) in Macabeo wines revealed that the concentration of esters in wine decreases when the ageing time in the presence of lees increases. This fact can be explained by the synthesis-hydrolysis activity of esterases, which are released by lees yeast within the days following alcoholic fermentation, as observed by Mauricio, Moreno, and Valero (1993); behaviour opposite to that observed by Bueno et al. in the analysis of Airen wines, as almost all esters quantified in these wines increased significantly after contact with less.

The presence of medium-chain fatty acids in wines is considered undesirable. The studies from Bautista et al. and Bueno et al. allowed to deduce that the presence of lees increases the concentration of fatty acids in wine, a fact that Bueno et al. explain by desorption phenomena occurred after fermentation and caused by yeast autolysis. These authors also studied the evolution of terpenoid compounds and lactones, the presence of which in wines is desirable due to their floral and fruity odour, respectively. The behaviour observed after wine lees contact was similar in both families of compounds, increasing their concentration in Airen wines and decreasing in Macabeo wines.

Oak barrels have a crucial effect on wine aroma, color and stability; effect which is strongly affected by different factors which have been reviewed by Garde-Cerdán and Ancín-Azpilicueta (2006). Chatonnet, Dubourdieu, and Boidron (1992) found that wine ageing on lees prevents the wood taste and aroma from having an excessive predominance over other wine aromas. Recently, Jiménez-Moreno and Ancín-Azpilicueta (2007) studied the influence of the addition of lees, extracted from wine after alcoholic fermentation, on the concentration of oak compounds in aged red wine (other studies in this aspect have been developed with a model wine). The compounds studied were volatile phenols, furanic compounds, phenolic aldehydes and lactones. They concluded that the addition of lees to a wine aged in barrel produces a decrease in the concentration of the oak volatile compounds in wine due to binding to the lees; the retention increasing with increased concentration of lees in wine. However, more in depth studies are needed in order to clarify the influence of lees on the aroma of the wines aged in oak barrels.

Volatile sulfur products such as hydrogen sulfide, carbonyl sulfide, sulfur dioxide, polysulfides, thiols or thioesters – which are the cause of olfactory problems due to their low perception level and nauseous smell (Fowles, 1994) – have been found in wines (Rapp, Güntert, & Almy, 1985). Although volatile sulfurs may have numerous origins (Jinarek, Landridge, & Henschke, 1995), yeast lees are mainly responsible for the presence of volatile sulfurs in wines (Vasserot, Steinmetz, & Jeandet, 2003). In this context, ageing on lees could be the cause of the problem but also the solution, as evidences of both the release of sulfurs from lees (De Mora, Eschenbruch, Knowles, & Spedding, 1986; Jinarek et al., 1995; Tamayo, Ubeda, & Briones, 1999) and the removal of sulfurs by wine lees (Lavigne & Dubourdieu, 1996; Palacios et al., 1997; Vasserot et al., 2003) have been widely reported. The conditions under which each phenomenon occurs is a very complex matter closely related with the yeast strain and the winemaking conditions. Vasserot et al. (2003) suggested a mechanism for the removal of thiols by yeast lees involving metallic cations, supported by the fact that both thiol consumption and disulfide formation seem to be inhibited by EDTA.

From the foregoing, the dual role played by lees in wine sulfurs (which are released from lees or removed from wine by them) can be extrapolate, with exceptions, to the wide family of volatile compounds present in wines. So, more research is needed in order to exploit lees virtues concerning the final wine aroma, and minimize their negative aspects.

4. The role of lees in biogenic amine contents in wines

Decarboxylation of amino acids in wines can lead to histamine, tyramine, putrescine, cadaverine and phenylethylamine formation. This is the reason why wine has been commonly reported to be a health risk for some consumers due to biogenic amines intake. These amines can cause undesirable physiological effects (e.g. headache, nausea, hypotension or hypertension, cardiac palpitations and anaphylactic shock (Bauza, et al., 1995; Silla-Santos, 1996) in sensitive humans.

The role of lees in the presence of biogenic amines in wine is crucial, not only as responsible of amino acids release (Martínez-Rodríguez & Polo, 2000) which can be decarboxylated, but also as a microorganisms' reservoir. The most common microorganisms present in lees are yeast, which are responsible for fermentation, but bacteria may also be present in malolactic fermentation (Salmon et al., 2002). Thus, lees can be responsible for the presence in wines of amino acids, decarboxylase-positive microorganisms and decarboxylase enzymes (which can be released during yeast lees autolysis), which, under favorable environmental conditions, can lead to biogenic amines formation. Two recent studies have been focused on this subject. González-Marco and Ancín-Azpilicueta (2006) studied a restricted number of white wines, meanwhile Martín-Álvarez, Marcobal, Polo, and Moreno-Arribas (2006) used 224 red wines for their research. Both studies supported their results on statistics. The main conclusion in both cases was that the overall concentration of biogenic amines in wines matured with lees was higher than in those elaborated without lees. Both also coincide in putrescine as the biogenic amine most affected by the presence of lees, results which also agree with those of Bauza, et al. (1995) who also found a higher production of putrescine in wines inoculated with bacteria by lees addition. The increased putrescine content in wines aged in the presence of lees was explained by Marcobal, de las Rivas, Moreno-Arribas, and Muñoz (2004) on the basis of an ornithine decarboxylase gene in the putrescine-producer *Oenococcus oeni* BIFI-83 strain, which was isolated from lees from a wine with high concentration of putrescine. On the other hand, the results obtained by González-Marco and Ancín-Azpilicueta (2006) and Martín-Álvarez et al. (2006) concerning the behaviour of histamine and tyramine are contradictory: Martín-Álvarez et al., observed a decrease in these amines after wine lees contact, meanwhile González-Marco et al. found a significant increase in the concentration of these amines in wines aged in the presence of lees subjected to stirring. This physical process can partially explain the different behaviour. Martín-Álvarez et al. also reported that, apart from lees, skin maceration has also a strong influence on the final concentration of biogenic amines (which cannot be compared with the study by González-Marco et al. as they used white wines without maceration). So, this behaviour must be taken into account when wines are aged in the presence of lees.

5. Interactions between lees and phenolic compounds

Phenolic compounds are key components of wine directly related to its quality parameters (Hernández et al., 2006). These compounds not only contribute to the organoleptic characteristics of wine, but they also are the main cause of color changes in wine, as the light yellow color as well as the undesirable brown color in white wines are due to the phenolic content and its oxidation (Singleton, 1988). Nevertheless, phenolic compounds also act as antioxidants, with mechanisms involving both free-radical scavenging and metal chelation (Frankel, Waterhouse, & Kinsella, 1993). The phenolic composition of wines is affected by the winemaking procedure (Singleton & Trousdale, 1983) and the grape variety (De la Presa-Owens, Lamuela-Raventós, Buxaderas, & Torre-Boronat, 1995; Nagel & Wulf, 1979).

The importance of wine lees in this context comes from the fact that lees can adsorb phenolic compounds (Mazauric & Salmon, 2005; Mazauric & Salmon, 2006) and release to wine both phenolic compounds (Cheynier, Rigaud, Souquet, Duprat, & Moutounet, 1990; Schneider, 1995; Somers, Vérette, & Pocock, 1987) and enzymes (after autolysis) that can modify the phenolic fraction (Cunier, 1997; Ibern-Gómez et al., 2000). Mannoproteins released during yeast lees autolysis can interact with phenolic compounds, improving the color stability and diminishing the wine astringency (Fornairon-Bonnefond et al., 2002; Trione & Martínez, 2001; Vidal et al., 2004). Moreover, the reactivity towards oxygen of both lees and phenolic compounds can also be affected by interactions between them (Salmon et al., 2002).

Anthocyanins and their derivatives are the main responsible of wine color, which is mainly due to conversion of grape anthocyanins to complex pigments or to copigmentation with tannins (Mazauric & Salmon, 2006). This is the reason why the adsorption of anthocyanins by wine lees has been a matter of research, being generally reported anthocyanin decrease in wines after contact with lees (Mazauric & Salmon, 2006; Morata et al., 2003; Rodríguez et al., 2005; Vasserot, Caillet, & Maujean, 1997). Vasserot et al. (1997) studied sparkling wines and proposed the use of yeast lees as alternative to charcoal (used for reducing the color in colored must and wines). Although yeast lees have β-glucosidase activity, anthocyanins removal is mainly due to an adsorption mechanism involving weak and reversible interaction between anthocyanins and yeast walls. These results are in agreement with those from Morata et al. (2003) but in disagreement with those reported by Cunier (1997), who explained the decreased color of wine by anthocyanins degradation by β-glucosidase activity. Vasserot et al. (1997) reported both that hydrogen bonding could be involved in the adsorption mechanism of anthocyanins, and that the most polar anthocyanins are more adsorbed. On the contrary, Mazauric and Salmon (2006) observed that the adsorption of anthocyanins on yeast lees in a complex polyphenol environment as wine did not follow a simple adsorption mechanism involving hydrogen bonding, and they found no relationship between the efficiency of anthocyanins adsorption on yeast lees and their polarity (Mazauric & Salmon, 2005). In a recent study on the influence of lees during oak ageing of red wines, Rodriguez et al. (2005) reported that anthocyanins decrease during wines ageing can be due to: (a) the presence of oxygen (facilitated by the permeability of the oak wood), which allows the formation of ethyl bridges between anthocyanins and proanthocyanidins (Rivas-Gonzalo, Bravo-Haro, & Santos-Buelga, 1995); (b) oxidation reactions; (c) adsorption and precipitation of flavanol polymers (King, Swenny, Radford, & Iacobucci, 1980; Rivas-Gonzalo et al. 1995). The contradictory results reported concerning lees–anthocyanins interactions, and the fact that the phenomena which take place and the desired final color of wine depend on the type of wine, indicate that a more extensive research in this field is needed.

Ibern-Gómez et al. (2000) studied the color changes underwent by six sparkling wines during ageing in contact with lees, and attributed these changes to the oxidation of phenolic compounds. They detected by absorbance measurements an increase in oxidative browning since the beginning of the ageing period, a slightly decrease in browning at the beginning of the autolysis period (between the 12th and 18th ageing month) possibly due to the release of phenolic compounds adsorbed by yeast (Cheynier et al., 1990; Schneider, 1995; Somers et al., 1987), and a significant increase in browning after the 15 month of ageing which they attributed both to the oxidation of the phenolic compounds released during yeast autolysis and the action of cytoplasmatic enzymes (also released during autolysis) such as hydrolytic and isomerase enzymes that could change *trans*-coutaric acid to the *cis*-isomer acid. Rodriguez et al. (2005) concluded the key role of lees in the final color of wine based on the observation of a decay of wine color by the presence of lees, but also the statistically significant differences in color are caused by the presence of lees during oak ageing of red wine. They also reported decreased proanthocyanidin concentrations (the size of this molecule seems to be related to astringency (Cheynier, Moutounet, & Sarni-Machado, 2000). To determine the interaction between wine polyphenols and external components of yeast lees, Mazauric and Salmon (2005) reported the analysis of polyphenols in wine after contact with yeast lees. The studies were carried out by suspending yeast lees and wine polyphenols in a model wine medium, which showed that adsorption of wine polyphenols on yeast lees follows biphasic kinetics: an initial and rapid fixation is followed by a slow, constant and saturating fixation that reaches its maximum after about 1 week. On the other hand, Hernández et al. (2006) reported a wide research on the changes in 38 non-anthocyanin phenolic compounds from 47 red wines as a result of different technological processes (namely: malolactic fermentation in barrels or in stainless steel containers, ageing of wines in the presence or in the absence of lees – including or not periodic stirring of the lees – racking, clarification, cold stabilization and filtration). After applying statistical methods, they concluded that the concentration of non-anthocyanin phenolic compounds did not allow significant differentiation of wines according to the technological variables studied, since wines with the same phenolic characteristics were obtained over 14 months of ageing, concerning the storage in the presence or absence of lees; the exception of this behaviour is myricetin (with mean concentration significantly lower in wines aged without lees). These authors observed an increase in hydroxycinnamic acids during wine ageing on lees that could be due to the enzymatic activity of yeast and lactic acid bacteria from lees, although these compounds could also come from the hydroalcoholysis of oak wood (Fernández de Simón, Hernández, Cadahía, Dueñas, & Estrella, 2003; Musingo, Sims, Bates, O'Keefe, & Lamikanra, 2001) as all wines were aged in oak barrels. The variations observed in phenolics concentrations were attributed to the ageing time in oak barrel.

Phenolic compounds are able to undergo nonenzymatic autooxidation in a favorable environment (Singleton, 1987). In this context, Salmon et al. (2002) investigated the strong interaction of yeast lees with oxygen during wine ageing in terms of impact on oxygen availability in the wine and polyphenol reactivity towards oxygen. In a wine ageing simulation they observed that as the contact time increased, oxygen consumption capacities of polyphenols remaining in solution slightly increased, while oxygen consumption by yeast lees was drastically lowered; resulting in a total decrease of reactivity towards oxygen by comparison with the reactivity of each component studied alone. They explained this behaviour by a strong interaction between adsorbed polyphenols and cell membrane lipids, which decreases the accessibility and reactivity of oxygen-reactive species towards membrane lipids, such as unsaturated fatty acids and sterols, which are suspected to be the main targets of oxidation reactions in yeast lees during wine ageing.

In general, ageing in the presence of lees produces less astringent wines, with a slightly less intense color but less yellowish nuances.

6. Importance of mannoproteins released by lees

Polysaccharides, the main group of macromolecules present in wines, have two different origins (Doco et al., 2003): arabinans, AGPs, RG-II, and RG-I come from the cell walls of grape, meanwhile mannoproteins are released by yeast either during fermentation or by enzymatic action during ageing on yeast lees by autolysis (Babayan & Bezrukov, 1985; Leroy, Charpentier, Duteurtre, Feuillat, & Charpentier, 1990). The production and release of mannoproteins to wine depends on the yeast strain. Vidal, Williams, Doco, Moutounet, and Pellerin (2003) estimated mannoproteins to be approximately 35% of total polysaccharides in red wines. The concentration of mannoproteins in wines is approximately 100–150 mg/l. Mannoproteins size is highly variable, extending from 5000 to more than 800,000 daltons; they can be hydrolyzed by α-mannosidases and proteases, releasing small peptidomannans into wine (Doco et al., 2003). The structure of mannoproteins present in wines has been described in several papers (Saulnier, Mercereau, & Vezinhet, 1991; Villetaz, Amado, Neukom, Horinsberger, & Horman, 1980; Waters, Pellerin, & Brillouet, 1994); basically, it consists of many small chains with one-to-four D-mannose residues in α-($1 \rightarrow 2$) or ($1 \rightarrow 3$), which are linked to polypeptide chains on serine or threonine residues.

Doco et al. (2003) studied the evolution of wine polysaccharides during ageing on lees, and observed that ageing on lees resulted in the enzymatic degradation of grape polysaccharides; thus, the possibility of obtaining a significant modification of these polysaccharides, that can influence the organoleptic quality of wines, increased with wine lees contact time. They also determined that mannoproteins release during wine ageing is a progressive and linear phenomenon, and that the simple contact of wine with lees was not sufficient to release mannoproteins, but resuspension of lees by stirring ("batonnage") significantly increased the amount of macromolecules extracted into wine, which is in accordance with the data reported by Llauberes and Dubourdieu (1987).

Polysaccharides play an important role in colloidal stabilization–destabilization phenomena. Several studies (Lubbers, Léger, Charpentier, & Feuillat, 1993; Moine-Ledoux & Dubourdieu, 1999; Moine-Ledoux, Perrin, Paladu, & Dubourdieu, 1997) show that mannoproteins can inhibit the crystallization of K hydrogen tartrate (KHT); Gerbaud, Gabas, Blouin, Pellerin, and Moutounet (1997) pointed out that mannoproteins from yeast hulls inhibit KHT crystallization more than yeast mannoproteins present in wines. Mannoproteins also seem to decrease tannin aggregation; so Poncet-Legrand, Doco, Williams, and Vernhet (2000) reported that mannoproteins prevent tannin aggregation and precipitation, and Riou, Vernhet, Doco, and Moutounet (2002) established that mannoproteins did not prevent initial tannin aggregation, but they slow down particle size evolution. Mannoproteins also seem to protect wines from protein precipitation (Moine-Ledoux & Dubourdieu, 1998; Waters, Wallace, Tate, & Williams, 1993; Waters et al., 1994). In this context, Dupin et al. (2000) proposed that the mechanism of haze protection may be a competition between mannoproteins and wine proteins for unknown wine component(s), the latter being required for the formation of large insoluble aggregates of denatured proteins. So, as the presence of mannoproteins decreases the available amount of the unknown components, the particle size of the haze decreases and thus visible turbidity declines.

The widely reported capacity of lees to interact with aroma compounds, and thus potentially change the sensory properties of wine, has also been related to mannoproteins (Bautista et al., 2007; Dufour & Bayonove, 1999; Lavigne & Dubourdieu, 1996; Lubbers, Charpentier, Feuillat, & Voilley, 1994b; Ramírez-Ramírez et al., 2004). Concerning the nature of such interactions, highly dependent on the structure of the volatile compounds, Lubbers et al. (1994) attributed the retention of aroma compounds to mannoproteins having a high proportion of proteins; whereas Chalier, Angot, Delteil, Doco, and Gunata (2007) reported that the strength of these interactions depends on the yeast strain from which mannoproteins comes, and justify the difficulty to understand the interactions between aroma compounds and mannoproteins by the fact that both glycosidic and peptidic parts of these glycoproteins may interact with aroma compounds.

Apart from that already commented in this section, the presence of mannoproteins has other consequences, namely: mannoproteins can interact with phenolic compounds, thus improving the color stability and reducing the astringency of wine (Feuillat et al., 2000; Fuster & Escot, 2002; Vidal et al., 2004); stimulate malolactic fermentation and increase bacterial growth (Guilloux-Benatier, Guerreau, & Feuillat, 1995). However, the direct organoleptic effect of polysaccharides on wine does not seem to be clarified nowadays (Fornairon-Bonnefond et al. 2002).

7. Lipids from yeast lees

Clarification processes are responsible for the generally low lipids content in wines. Although during wine ageing on lees yeast autolysis releases lipids to wine, this contribution is less significant than that of other compounds (Ferrari, Meunier, & Feuillat, 1987; Fornairon-Bonnefond et al. 2002).

Lipids may affect wine flavour, as the fatty acids released may give rise to volatile components with low sensory thresholds such as esters, ketones and aldehydes (Charpentier & Feuillat, 1992). Lipids have tensioactive properties that play an essential role during alcoholic fermentation, mainly in those wines that keep in contact with their lees for a long time (Gómez, Igartiburu, Pando, Rodríguez-Luis, & Mourente, 2004). In this context, a positive relationship between the presence of some fatty acids and the foaming characteristics of sparkling wines has been reported by Pueyo, Martín-Álvarez, and Polo (1995); who also determined that foam stability and foam height were positively related with the total content of linolenic and palmitic acids, respectively.

The release of different classes of lipids during autolysis of three commercial yeast strains of *S. cerevisiae* in a model wine medium has also been reported by Pueyo, Martínez-Rodríguez, Polo, Santa-María, and Bartolomé (2000). Lipids were detected in the autolysis medium from the very first sample (after 5 h) and three different stages during the autolysis of these strains were observed: in the first stage lipids were released to the medium; in the second, lipid concentration in the autolysates decreased; and, in the third the number of viable cells increased (which can be interpreted as growth of the most resistant cells using the biomass released by the autolysed cells as nutrients) and a new cycle of lipid release was observed. Concerning lipid composition of the autolysates, neither 1-monoacylglycerols nor phospholipids were detected in any of the autolysates. The absence of phospholipids in the autolysates was attributed to degradation (Hernawan & Fleet, 1995); sterol esters and triacylglycerols were the lipids in a higher proportion (from 1 to 5 mg/l), meanwhile free fatty acids, sterols, diacylglycerols and monoacylglycerols were an order of magnitude lower (from 100 to 600 μg/l). The lipid content in autolysates was found to be dependent on the yeast strain used. Le Fur, Maune, Feuillat, and Maume (1999) studied the evolution of sterol content in *S. cerevisiae* for a 14-day period of accelerated autolysis. They observed how yeast autolysis induced a decrease of esterified sterol content, especially first intermediates in the sequence of the ergosterol biosynthesis, as zymosterol. By contrast, the yeast autolysis resulted in the release of a low quantity of sterols into the medium. At the end of the 14th day of autolysis, only 0.015% of the total sterol content in the initial biomass was found in the medium.

Gómez et al. (2004) used the Folch method to extract the lipid fraction of lees obtained from sherry wine in a study of lees without using wine samples. They estimated the relative amount of sterols esters, fatty acid methyl esters, triglycerides, free fatty acids, sterols, glycolipids, and phospholipids in lees as 15.9, 10.8, 13.0, 11.2, 25.3, 18.2 and 5.6%, respectively. The most abundant fatty acids in lees were palmitic, linoleic and stearic acids; results which are not in total agreement with those observed by Herraiz, Herraiz, Reglero, Martín-Álvarez, and Cabezudo (1990), who reported palmitic, linoleic and linolenic acids as the major fatty acids present in wines. Gómez et al. also studied the main chemical characteristics of the lipids extracted from lees by determining the acid value, saponification value, iodine value, percentage of insaponificable matter and peroxide index.

Since 1999, Salmon, Fornairon-Bonnefond and others have developed studies on oxygen consumption by wine lees. Firstly, these authors determined that yeast lees were able to exhibit oxygen consumption rates ranging from 1 to 4 μmol O_2 h^{-1} 10^{-10} cells from the second to the sixth month of wine ageing (Fornairon, Mazauric, Salmon, & Moutounet, 1999). By simulation of wine ageing in the presence of lees, they observed how some lipids in yeast lees membrane underwent a mild oxidation in contact with dissolved oxygen: the production of reactive oxygen species (ROS) leads to the formation of lipid peroxides and then to unknown end-products. These reactions explain the capacity of yeast lees to consume oxygen during wine ageing (Salmon, Fornairon-Bonnefond, Mazauric, & Moutounet, 2000). In a subsequent study (Fornairon-Bonnefond & Salmon, 2003) they tested several compounds in order to determine a relationship between oxygen consumption by yeast lees and possible changes in wine composition after ageing; no difference in the tested compounds was found but in sterol compounds: the concentration of ergosterol in lees was significantly lower in the presence than in the absence of oxygen. This may indicate that ergosterol was the main substrate of lipid oxidation. Concerning the mechanism of ergosterol decrease, the authors hypothesized a multiple attack of oxygen-derived ROS on the ergosterol molecule, which likely leads to the release of small polyenic fragments that may play an important role in the organoleptic properties of the final wine. In other study, the same authors (Fornairon-Bonnefond, Aguera, Deytieux, Sablayrolles, & Salmon, 2003) looked for a relationship between the amount of oxygen added during fermentation and the behaviour of yeast sterols. When oxygen was added during fermentation, a decrease in yeast lees reactivity towards oxygen was produced, which was concomitantly related to an increase in ergosterol synthesis and oxygen-dependent sterol degradation. Excessive oxygenation during alcoholic fermentation led to a marked increase in cell population, but also resulted in a low capacity of oxygen consumption by the corresponding lees; so oxygenation control during fermentation is mandatory.

8. Present and potential future exploitation of lees

Lees are an undervalued by product of the winemaking industry. Their main present use is the recovery of tartaric acid (Balice, 2003; Braga, Lencant e Silva, & Alves, 2002), which is reused to correct the initial pH of musts before fermentation and must distillation to obtain alcohol, which could be obtained more easily by distillation of winemaking excess (Gómez et al., 2004). In Greece, where ethyl alcohol is exclusively produced by fermentation, wine lees are used as primary raw material for this production (Vlyssides & Israilides, 1997).

Some attempts were made in the past to use lees as animal nutrient, but the yeast lees have an extremely poor nutritional value which makes them unsuitable for this purpose (Maugenet, 1973; Rivas, Torrado, Moldes, & Domínguez, 2006). Nevertheless, lees were proposed in 2000 as a complement in feeding for both human and animals due to their high content in polyunsaturated fatty acids (Gómez et al., 2004).

As yeast lees from wine are an inexpensive and easily available source of biomass, the use of lees as a biosorbent for removing unwanted compounds from wine has been proposed (Chassagne et al., 2005; Vasserot et al., 1997; *in addition to the discussion in section* "Importance of lees in the natural removal of undesirable compounds from wine").

Recently, Fonseca, Fell, Kurtzman, and Spencer-Martins (2000) have isolated an undescribed anamorphic yeast species of ascomycetous affinity from dried wine lees, for which they proposed the name *Candida tartarivorans*; thus showing the poor knowledge about wine lees so far.

Romero, Nogales and others have focused their research on the ability of the earthworm *Eisenia Andrei* to compost different winery residues, including lees. Nogales, Cifuentes, and Benítez (2005) reported that vermicomposts from lees and other winery residues can be used as organic amendments of soils, especially those with low organic-matter content. Romero, Salido, Cifuentes, Fernández, and Nogales (2006) studied the use of lees and other industrial residues as sorbents for pesticide control and the effect of vermicomposting process on these substrates; the sorption capacity of the studied residues was low for the anionic herbicides with respect to hydrophobic pesticides. Neither of these studies was really focused on lees, and in both the results obtained with lees were not the best as compared with other residues tested; however, these studies show vermicomposting process as a useful tool for other possible industrial exploitation of wine lees, although further research would be needed.

Some recent papers have reported the use of wine lees as the unique nutrient to obtain lactic acid from *Lactobacillus* strains. Bustos, Moldes, Cruz, and Domínguez (2004a) used lees from different steps of winemaking technologies (red or white wines, distilled or not, in the first or second decanting steps) as nutrients for *Lactobacillus rhamnosus*, and compared these results with those obtained using a complex medium proposed by Mercier, Yerushalmi, Rouleau, and Dochain (1992) (later noted as "MRS broth") with the same purpose. Red lees led to the worse results, which they ascribed to the presence of phenolic compounds released during red wine production, which makes difficult fermentation; so extraction with organic solvents should be required to improve the results. White lees re-collected after the second decanting step before distillation provided better results as the only nutrient than those obtained with the MRS broth, and without the disadvantages of this medium, which requires high amounts of cost nutrients. The inhibitory capacity of the phenolic compounds present in white lees was lower than that of red lees. As *Lactobacillus* has proteolytic activity, high productivities were achieved in all cases as no autolysis treatments were required to break the cell walls of yeast lees.

In a subsequent study, Bustos, Moldes, Cruz, and Domínguez (2004b) used different *Lactobacillus* strains (namely, *L. casei, L. coryniformis, L. pentosus* and *L. plantarum*). As expected, the different strains showed different behaviours: *L. casei* gave the best performance, which was similar to that achieved with *L. rhamnosus* and the MRS broth. When *L. pentosus* was used, removal of the remaining phenolic compounds by extraction with ethyl acetate was necessary in order to obtain high yields and high productivities in lactic acid. As in the previous work, better results were obtained by using white lees. In this work they reported that, in all cases, distilled lees showed better performances due to some phenolic compounds changing their composition during distillation, making these lees more appropriate for use as nutrient. This behaviour was observed for all strains, except for *L. rhamnosus* (that used in the first work). In a most recent and interesting study, Rivas et al. (2006) used lees distilled from red and white wines for tartaric acid (TA) recovery, and the residual solid as an economic nutrient for *L. pentosus*. The recovery of tartaric acid was optimized using response surface methodology. They recovered up to 92.4% of TA in lees by a sequential treatment consisting of dissolving TA and further calcium tartrate precipitation. Moreover, when lees distilled from red and white wines were used after TA extraction, slightly higher concentrations of lactic acid were obtained compared with vinification lees without TA extraction. However, the global volumetric productivities were slightly lower when TA was extracted from lees, probably because during the TA extraction some nutrients were removed. This procedure has the advantages of those proposed by Bustos et al. but also enables TA recovery.

9. Conclusion

Wine lees contact seems to be positive concerning the removal of most undesirable compounds from wine, but negative in the case of biogenic amines. There is no agreement in the effect of lees on wine aroma. On the other hand, the interactions of wine lees with phenolic compounds, lipids and mannoproteins released by them as well as the influence of all them on wine quality are not clear.

As there is no doubt that the wine lees contact during ageing contributes to the quality of wines, more research in this field is needed in order to clarify the influence of each lees component on wine ageing.

On the other hand, the scarce current applications of wine lees and their potential exploitation make them an undervalued byproduct of the winemaking industry.

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