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Interactions between Yeast Lees and Wine Polyphenols during Simulation of Wine Aging: I. Analysis of Remnant Polyphenolic Compounds in the Resulting Wines

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Wine aging on yeast lees is a traditional enological practice used during the manufacture of wines. This technique has increased in popularity in recent years for the aging of red wines. Although wine polyphenols interact with yeast lees to a limited extent, such interactions have a large effect on the reactivity toward oxygen of wine polyphenolic compounds and yeast lees. Various domains of the yeast cell wall are protected by wine polyphenols from the action of extracellular hydrolytic enzymatic activities. Polysaccharides released during autolysis are thought to exert a significant effect on the sensory qualities of wine. We studied the chemical composition of polyphenolic compounds remaining in solution or adsorbed on yeast lees after various contact times during the simulation of wine aging. The analysis of the remnant polyphenols in the wine indicated that wine polyphenols adsorption 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. Only very few monomeric phenolic compounds remained adsorbed on yeast lees, and no preferential adsorption of low or high polymeric size tannins occurred. The remnant condensed tannins in the wine contained fewer epigallocatechin units than the initial tannins, indicating that polar condensed tannins were preferentially adsorbed on yeast lees. Conversely, the efficiency of anthocyanin adsorption on yeast lees was unrelated to its polarity.

KEYWORDS: Wine aging; yeast; lees; polyphenols

INTRODUCTION

Wine lees, found at the bottom of fermentation tanks, is mainly yeast cells produced during alcoholic fermentation, together with tartaric salts, bacteria, and debris from plant cells. Wine aging on fine lees (essentially dead yeast cells) is a traditional enological practice used during the manufacture of wines (*1*). Wine can be aged in barrels or in large cooperage systems or stainless steel tanks with their yeasts and some grape solids. Most studies on aging on lees have only focused on compounds released into the wine by yeast autolysis. This involves the release of wall polysaccharides, nucleic acids, fatty acids, and nitrogen (*2*−*6*). Yeast lees exhibit high specific oxygen utilization rates from the second month to the third year of wine aging (*7*, *8*). Although "grands crus" white wines (such as Burgundy wines) are traditionally aged on lees (*3*), this technique has increased in popularity in recent years for the aging of red wines. Yeast lees interact with wine polyphenols. Although wine polyphenols and tannins interact with yeast lees to a limited extent, such interactions have a large effect on the reactivity of wine polyphenols and yeast lees toward oxygen during the simulation of wine aging (*9*). As contact time increases, the oxygen consumption capacity of polyphenols remaining in solution increases slightly, whereas the oxygen consumption capacity of yeast lees is substantially lowered. This results in a combined decrease of reactivity toward oxygen in comparison with the reactivity of each individual component. Because wine polyphenols isolated after contact with yeast lees have normal oxygen consumption capacities, the lowering of polyphenol reactivity is clearly linked to an adsorption of some polyphenol onto yeast lees. A surprising effect of the contact between polyphenols and yeast lees is the large decrease in the oxygen consumption capacity of yeast lees when separated from soluble polyphenols, although only a small fraction of the total polyphenols remain adsorbed on the lees. A strong interaction between adsorbed polyphenols and cell membrane lipids may explain such behavior. These lipids, including unsaturated fatty acids and sterols, may be the main targets of oxidation reactions in yeast lees during wine aging (*10*). A correlative collapsing of the yeast cytoplasmic cell intermembrane space may decrease the accessibility and reactivity of oxygen reactive species. We recently demonstrated that the interaction of yeast lees with wine polyphenols maintains the spherical yeast cell morphology, although the yeast cell wall is degraded during autolysis (*11*). Because it is the yeast cell wall that confers mechanical stability and dictates shape and external morphology (*12*) and because

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the release of mannoproteins in the medium remains unaffected, the maintenance of spherical yeast cell morphology after contact with polyphenols indicates that interaction of yeast lees with wine polyphenols also occurs at the cell wall level. Moreover, this revealed that certain domains of the yeast cell wall are protected by wine polyphenols toward the action of extracellular hydrolytic enzymes. Because polysaccharides released during autolysis are thought to exert a significant effect on the sensory qualities of wines (*13*), we can expect a strong effect of the presence of polyphenols on both the polysaccharides released during aging and the sensory properties of the final wines.

Polyphenols interact colloidally with proteins via van der Waals interactions (*14*, *15*). Tannic acid, a naturally occurring polyphenol, precipitates or complexes with macromolecules such as polysaccharides and proteins having H-bond acceptors (*14*). Moreover, yeast lees can modify the color of wines either by the formation of weak and reversible interactions between anthocyanins and yeast walls (*16*, *17*) or by the cleavage of the 3-O-glucoside part of anthocyanins by a periplasmic β-glucosidase (*18*). It was also shown previously that yeast lees can specifically adsorb wine volatile phenols (*19*) and flavan 3-ol derivatives (*20*). However, all of these previous studies on interactions between lees and polyphenols have been carried out either on rehydrated active dried yeasts or on purified polyphenolic molecules. Therefore, the interaction of complex polyphenolic compounds with yeast lees has never been considered.

To determine the interaction of wine polyphenols and the external components of yeast lees, we analyzed polyphenolic compounds remaining in solution and those adsorbed on yeast lees at different times during a simulation of wine aging. Here, we report the analysis of remnant polyphenolic compounds in wine after contact with yeast lees.

MATERIALS AND METHODS

Yeast Strain. *Saccharomyces cerevisiae* strain K1 was a commercially available diploid homothallic wine yeast, obtained as a dry yeast (K1 ICV, INRA, Lallemand, Montpellier/Toulouse, France).

Culture Media and Growth Conditions. The K1 strain, available as a commercial dry yeast, was not precultured and was directly inoculated into the fermentation medium after rehydration, as recommended by the manufacturer. The synthetic medium used in this study (MS300) was a simulated standard grape juice containing 200 g L^{-1} glucose and 300 mg nitrogen L^{-1} assimilable nitrogen, strongly buffered to pH 3.3 (*21*). The yeast inoculum corresponded to equivalent industrial scale practice [50 mg (d.w.) L^{-1}]. Because anaerobic growth factors were not present in MS300, the fermentation medium was aerated prior to inoculation (initial oxygen concentration about 6 mg L^{-1}) and aerated on a regular basis (one oxygen saturation per day) throughout the fermentation. Fermentations were carried out in fermentors (10 L) under isothermal conditions (28 °C) with occasional stirring.

Cell Harvesting and Simulation of Wine Aging. Yeast cells were harvested by centrifugation 100 h after alcoholic fermentation had ended, as determined by the absence of residual sugar in the culture medium [concentration < 2 g L^{-1} as measured with dinitrosalicylic acid reagent (*22*)]. This 100 h wait was found to be necessary for yeast cell viability to be lost (<10^{-3} cfu/mL) (*7*). Cellular viability was determined by plating about 1000 cells, determined with an electronic particle counter, on YPD agar medium in Petri dishes [20 g L^{-1} agar, 10 g L^{-1} yeast extract (Difco, Irvine, CA), 20 g L^{-1} bactopeptone (Difco), and 20 g L^{-1} glucose). The Petri dishes were then incubated at 28 °C for 48 h and examined for the presence of colonies. Nonviable yeast cells were considered as yeast lees. As previously described (*9*−*11*), yeast lees was washed twice in a synthetic medium simulating a standard wine and suspended at the desired cell concentration in the same medium. The model wine medium was buffered to pH 3.3 and contained (per liter): 6 g of citric acid; 6 g of DL-malic acid; mineral salts (mg): KH_2PO_4, 750; KH_2SO_4, 500; $MgSO_4 \cdot 7H_2O$, 250; $CaCl_2 \cdot 2H_2O$, 155; and NaCl, 200; and 120 mL of ethanol.

Total Wine Phenolic Pool. The total wine phenolic pool was prepared in 1997 at the INRA experimental winery at Pech Rouge by treating 7400 L of a 1996 polyphenol-rich Cabernet sauvignon wine, made at Arzens (Southern France), as previously described (*23*). Wine (2500 L) was pumped onto a styrene/divinylbenzene Diaion column (180 L) in ascending mode. The column was then rinsed with 400 L of water and 400 L of 20% (v/v) ethanol in water in ascending mode to remove sugars and polysaccharides. The phenolic pool was finally eluted with 600 L of 95% (v/v) ethanol in water in descending mode. Ethanol was removed from the phenolic fraction by vacuum evaporation, and the final fraction was atomized to ensure sample stability. The total yield was about 300 g of atomized powder per 100 L of treated wine. High-performance liquid chromatography (HPLC) and thiolysis analysis (*24*) of the principle simple phenolic compounds of the atomized powder gave (on a dry weight basis): 4.22 mg g^{-1} flavonols, 19.11 mg g^{-1} anthocyanins, 8.88 mg g^{-1} phenolic acids, 15.41 mg g^{-1} flavanol monomers, and 146 mg g^{-1} tannins (determined by thiolysis) with a mean degree of polymerization (mDP) of 5.0. The remaining powder consisted mostly of complex polyphenolic compounds of higher molecular weight. The initial wine was reconstituted by suspending 2.9 g L^{-1} of the atomized phenolic pool in water and had an absorbance at 280 nm of 26.75, a color intensity ($A_{420nm} + A_{520nm} + A_{620nm}$, $d = 1$ mm) of 4.74, and a color tint (A_{420nm}/A_{520nm}, $d = 1$ mm) of 0.71.

Experimental Protocol. Yeast lees (1.35×10^8 cells mL^{-1}), wine polyphenols (3, 6, or 9 g L^{-1}), or a mixture of yeast lees and wine polyphenols (at the same respective concentrations) suspended in the model wine medium were placed in tightly closed amber bottles (2.5 L) under an argon atmosphere and incubated at 25 °C. The bottles were stirred once per day without opening. Samples were withdrawn from each bottle at various times, and the bottles were sealed again after flushing the headspace with argon gas.

Analytical Methods. *Cell Counting.* Culture growth and cell numbers were monitored by using an electronic particle counter (ZBI model; Coulter-Counter Coultronics, Margency, France) linked to an analyzer (Channelyzer 254 model; Coulter-Counter Coultronics).

Colorimetric Determinations. Colorimetric determination of wine absorbency was carried out at different wavelengths (280, 320, 420, 520, and 620 nm, respectively) (*25*). Measurements were made with a SAFAS UV-MC^2 spectrophotometer (Monaco), using a 1 mm path length cell thermostated at 25 °C. All samples were first clarified by centrifugation (1865*g*, 10 min). Wine color intensity and tint were determined according to Glories (*26*). Total polyphenolic index (TPI), tannins, and hydroxycinnamic acid content were determined according to Somers and Ziemelis (*27*). The anthocyanin content was determined according to Somers and Evans (*28*).

Fractionation Procedure. Aliquots of the model wine samples were separated on a Fractogel [Toyopearl TSK gel HW40 (F), bed 12 mm × 120 mm] column. The first fraction, containing essentially monomers, was eluted with 35 mL of ethanol/water/trifluoroacetic acid (55:45:0.005; v/v). The second fraction, consisting of oligomeric and polymeric compounds (proanthocyanidins), was eluted with 35 mL of acetone/water (60:40; v/v). Both fractions were dried under vacuum and dissolved in an appropriate solvent.

HPLC/Diode Array Detection (DAD) Analyses. HPLC/DAD analyses were performed using a Waters 2690 system, a Waters 996 photodiode array detector, and the Millenium 32 chromatography manager software (Milford, MA). The elution conditions were as follows: column, Lichrospher 100-RP18 (5 μm packing, 250 mm × 2 mm i.d.) (Merck, Darmstadt, Germany); flow rate, 0.25 mL min^{-1}; oven temperature, 30 °C; solvent A, water/formic acid (95:5, v/v); solvent B, acetonitrile/solvent A (80:20, v/v); eluent profile, 2% B for 7 min, linear increase from 2 to 20% B over 15 min, from 20 to 30% B over 8 min, from 30 to 40% B over 10 min, from 40 to 50% B over 5 min, and from 50 to 80% B over 5 min, followed by washing and reequilibration of the column. Peak areas were measured at 280 and 520 nm.

Characterization and Quantification of Proanthocyanidins by Thiolysis. The thiolytic reagent was a 5% solution of phenylmethanethiol in acidified methanol (0.2 M HCl). Each fraction was dissolved in 1:1

Table 1. Color Intensity and Color Tint of Different Wine Polyphenol Suspensions and Wine Supernatants after a 1 Week of Contact of These Suspensions with Yeast Lees (1.35×10^8 Cells mL^{-1}) in a Synthetic Model Wine [pH 3.3, 12% (v/v) Ethanol] at 25 °C

wine polyphenolic powder concn (g L^{-1})		color intensity[a]	color tint[b]
3	wine polyphenols alone	4.70	0.70
3	corresponding supernatant	2.35	0.64
6	wine polyphenols alone	9.68	0.69
6	corresponding supernatant	6.02	0.64
9	wine polyphenols alone	14.62	0.66
9	corresponding supernatant	9.47	0.63

[a] $A_{420nm} + A_{520nm} + A_{620nm}$, $d = 1$ mm. [b] A_{420nm}/A_{520nm}, $d = 1$ mm. Data from ref *9*.

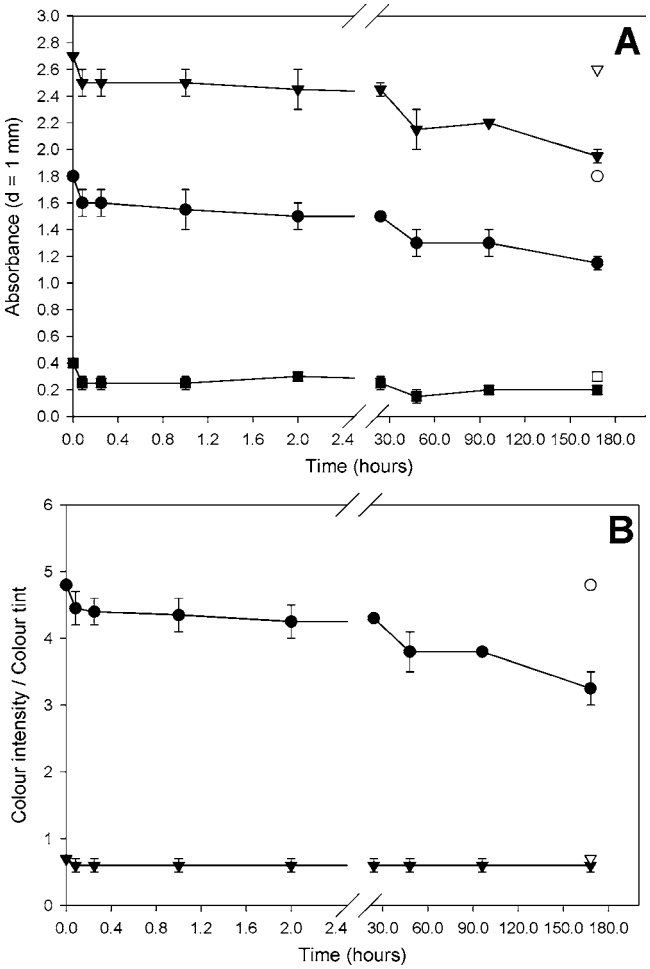

Figure 1. Adsorption kinetics of wine polyphenols (3 g L^{-1}) on yeast lees (1.35×10^8 cells mL^{-1}) during aging at 25 °C in a synthetic model wine [pH 3.3, 12% (v/v) ethanol] monitored by colorimetry ($d = 1$ mm) of wine after centrifugation (1865*g*, 10 min). (**A**) A_{520nm} (filled triangle), A_{420nm} (filled circle), A_{620nm} (filled square), and wine (open symbol) in the absence of yeast lees. (**B**) Color intensity (filled circle) ($A_{420nm} + A_{520nm} + A_{620nm}$), color tint (filled triangle) (A_{420nm}/A_{520nm}), and wine (open symbol) in the absence of yeast lees. Mean values and ranges of two experiments are given.

(v/v) methanol:thiolytic reagent and heated for 2 min at 90 °C. The breakdown products were analyzed by HPLC under conditions previously described (*29*). Peak areas for each terminal and extension unit were measured at 280 nm and calibrated with external purified standards. The mDP, the percentage of galloylated units, and the percentage of epigallocatechin units were calculated as previously described (*30*).

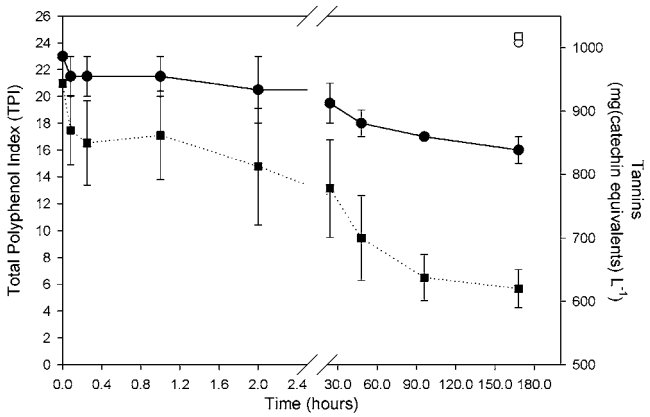

Figure 2. Estimated polyphenol content of several compounds in the final wines during adsorption of wine polyphenols (3 g L^{-1}) on yeast lees (1.35×10^8 cells mL^{-1}) during aging at 25 °C in a synthetic model wine [pH 3.3, 12% (v/v) ethanol] monitored by colorimetry ($d = 1$ mm) of wine after centrifugation (1865*g*, 10 min), as shown in **Figure 1**. TPI (filled circle) and total tannin content (filled square). Mean values and ranges of two experiments are given.

Table 2. Evolution of Wine Polyphenol Compounds from Colorimetry of the Supernatants after a 1 Week Contact of Wine Polyphenols (3 g L^{-1}) with Yeast Lees (1.35×10^8 Cells mL^{-1}) during Aging in a Synthetic Model Wine [pH 3.3, 12% (v/v) Ethanol] at 25 °C

	disappearance from the medium (% of blank experiment) during a contact with yeast lees of[a]	
	15 min	1 week
total tannins	11.3 ± 5.8	38.1 ± 3.3
anthocyanins	5.0 ± 1.5	14.7 ± 9.6
hydroxycinnamic acids	8.1 ± 6.2	27.1 ± 2.3

[a] Mean values and ranges of two experiments.

RESULTS

Analysis of Polyphenol Compounds in the Resulting Wines. Spectral analysis of the wines and of the yeast lees after contact with wine polyphenols showed that a large fraction of colored polyphenols remained adsorbed on the lees (**Table 1**). The average polyphenolic content of red wines is normally 3−9 g L^{-1}. Therefore, contact with yeast lees during wine aging may have a large effect on the color characteristics and sensory properties of the final wine. The adsorption kinetics of wine polyphenols on yeast lees were followed by colorimetric analysis of wines at different wavelengths (280, 320, 420, 520, and 620 nm, respectively, ref *25*). The absorption kinetics were clearly biphasic (**Figure 1A**). The absorbance in the visible spectrum decreased rapidly within 5 min of contact with lees, followed by a slow decrease over 1 week. This was particularly noticeable at 420 and 520 nm. Consequently, wine color intensity was strongly affected by contact with the yeast lees, whereas wine color tint was almost unaffected (**Table 1** and **Figure 1B**).

The TPI [from the absorbance at 280 nm (*27*)] decreased, following a similar biphasic curve, by about 33% after 1 week of contact with the lees (**Figure 2**). The polyphenol composition of the resulting wines was also estimated by spectral analysis (*27*, *28*). The estimated tannin content appeared to decrease following biphasic kinetics (**Figure 2**). Similar results were observed for anthocyanins and hydroxycinnamic acids (data not shown). The extent of polyphenol adsorption on yeast lees differs between the polyphenolic compounds: yeast lees interacts quickly with wine tannins, with hydroxycinnamic acids

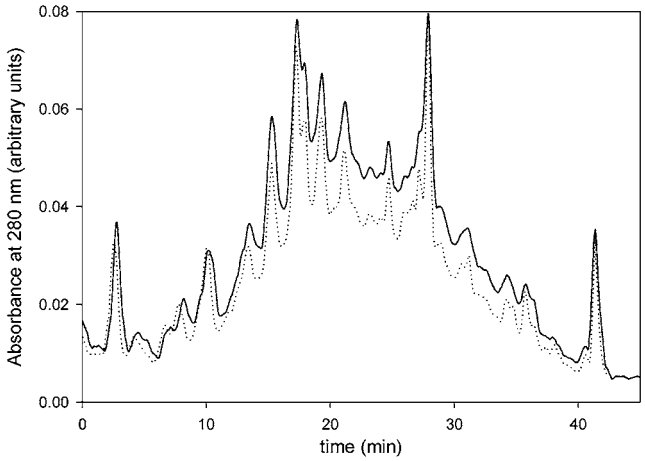

Figure 3. Comparison of HPLC chromatograms at 280 nm of wine polyphenols after adsorption of wine polyphenols (3 g L^{-1}) on yeast lees (1.35 10^8 cells mL^{-1}) during aging at 25 °C in a synthetic model wine [pH 3.3, 12% (v/v) ethanol]. Plain line, original synthetic wine containing 3 g L^{-1} wine polyphenols; dotted line, synthetic wine after a 1 week contact with yeast lees.

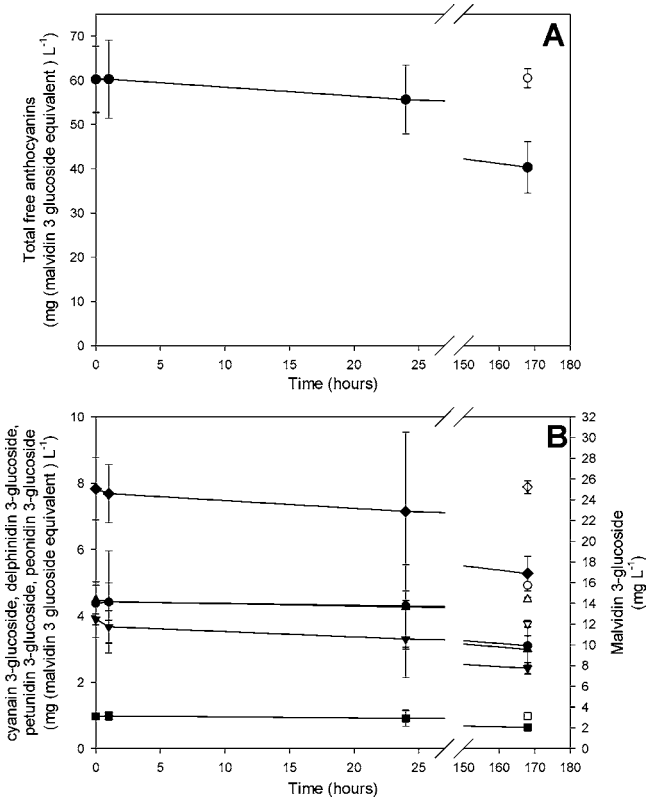

Figure 4. Evolution of free monomeric anthocyanins in synthetic model wine [pH 3.3, 12% (v/v) ethanol] containing wine polyphenols (3 g L^{-1}) in contact with yeast lees (1.35 × 10^8 cells mL^{-1}) at 25 °C. (**A**) Total free anthocyanins (filled circle). (**B**) Cyanidin 3-glucoside (filled square), delphinidin 3-glucoside (filled circle), malvidin 3-glucoside (filled diamond), petunidin 3-glucoside (filled triangle up), and peonidin 3-glucoside (filled triangle down). Wine (open symbol) in the absence of yeast lees. Mean values and ranges of two experiments are given.

to a lesser extent, and finally over the long term with all tested polyphenols to varying extents (**Table 2**).

Analysis by HPLC at 280 nm of the original wine polyphenols and of the wine polyphenols after 1 week of contact with yeast lees showed that the general form of the chromatogram was unaffected (**Figure 3**). This means that only a few monomeric

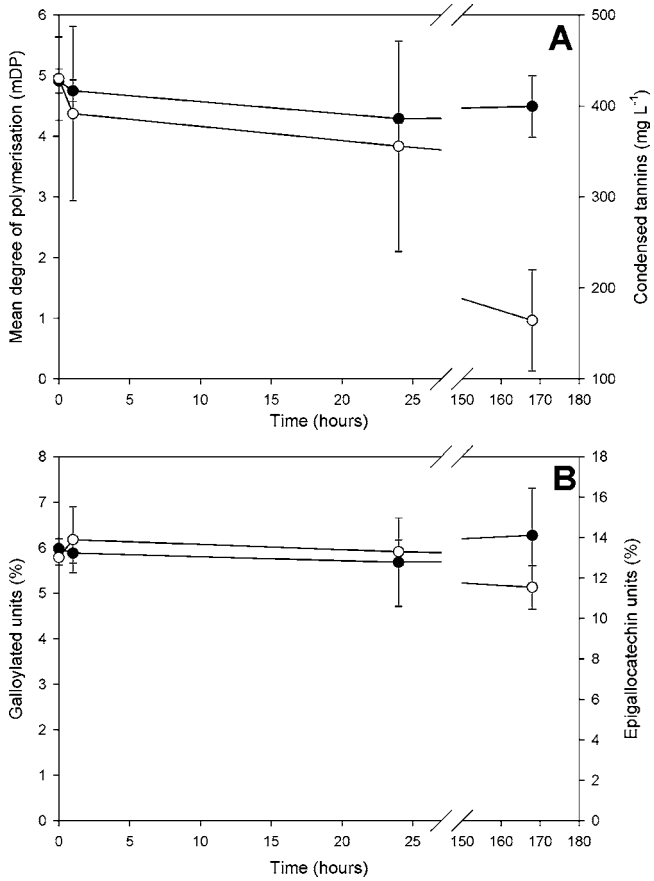

Figure 5. Evolution of wine condensed tannins and related components in synthetic model wine [pH 3.3, 12% (v/v) ethanol] containing wine polyphenols (3 g L^{-1}) in contact with yeast lees (1.35 × 10^8 cells mL^{-1}) at 25 °C. (**A**) Wine (open circle) condensed tannin content and mDP of wine tannins (mDP) (filled circle). (**B**) Percentage of galloylated (filled circle) and epigallocatechin (open circle) units in the wine condensed tannin. Mean values and ranges of two experiments are given.

phenolic compounds remained adsorbed on yeast lees. Without prior fractionation, the polymeric pigments and tannins often eluted from the HPLC column as a broad and poorly resolved hump (*31*, *32*); the intensity of this broad hump was the only difference between the two chromatograms. Similar results were obtained for all three different polyphenol concentrations tested (data not shown).

Quantitative Analysis of Wine Polyphenols in the Resulting Wines. The separation of monomers (flavanols, anthocyanins, flavonols, and phenolic acids) from oligomeric and polymeric compounds (condensed tannins) on a Fractogel chromatography column (*33*) allowed a quantitative analysis of the polyphenolic compounds present in the resulting wines. After a 1 week contact with yeast lees in the absence of oxygen, no loss of anthocyanins was observed in the blank experiment whereas around one-third of the total free anthocyanins was lost in the wine (**Figure 4A**). All free anthocyanins were affected, and the decrease was slight and continuous during exposure (**Figure 4B**), with malvidine 3-glucoside being the most affected glucoside. The efficiency of adsorption of the free anthocyanins on yeast lees seemed unrelated to their polarity since the relative adsorption is not proportional to the polarity of the anthocyanin (by decreasing polarity order: delphinidin, 29%; cyanidin, 34%; petunidin, 33%; peonidin, 38%; and malvidin, 32%). Similar results were obtained with acetyl- and coumaroyl-esterified anthocyanins (data not shown).

Table 3. Quantification and Characterization of Remnant Condensed Tannins from the Supernatants after a 1 Week Contact of Wine Polyphenols (3 g L^{-1}) with Yeast Lees at Different Concentrations during Aging in a Synthetic Model Wine [pH 3.3, 12% (v/v) Ethanol] at 25 °C

	yeast lees concn ($\times 10^8$ cells mL^{-1})	remnant condensed tannins (mg L^{-1})	mDP[a]	galloylated units (%)	epigallocatechin units (%)
initial wine polyphenols (3 g L^{-1})	none	442	4.9	6.0	13.0
supernatant resulting	1.35	136	4.8	6.3	10.3
from 100 h contact with	6.75	44	4.1	3.7	10.8
lees at the following concentrations	13.5	8	4.6	4.0	6.6

[a] The mDP and the percentage of galloylated and epigallocatechin units of remnant condensed tannins in the supernatants were obtained as described in the Material and Methods.

Up to 70% of condensed tannins (proanthocyanidins) in the wine were lost after a 1 week contact with yeast lees in the absence of oxygen, with the mDP of the remnant tannins decreasing slightly (**Figure 5A**). The percentage of galloylated and epigallocatechin units in these remnant condensed tannins indicated that tannins containing epigallocatechin units seemed to interact preferentially with yeast lees (**Figure 5B**). Analysis of the condensed tannins remaining in the wine after a 1 week contact at three different yeast lees concentrations (**Table 3**) showed that yeast lees at high concentrations almost completely remove condensed tannins from the wine. This correlates linearly with the amount of yeast lees in the assay (data not shown).

DISCUSSION

The kinetics of wine polyphenols adsorption on yeast lees was clearly biphasic, with an initial, rapid fixation in less than 0.2 h, followed by a slow, constant, and saturating fixation, which reaches a maximum after about 1 week of contact. This fixation was accompanied by a noticeable decrease of wine absorbance in the visible spectrum at 420 and 520 nm. This was previously observed and attributed to a strong decrease of pyranoanthocyanins formed during fermentation (*34*). Only very few monomeric phenolic compounds remained adsorbed on yeast lees. The tannin fraction of wine polyphenols estimated from the TPI showed that, after 1 week of contact, their adsorption on yeast lees appeared to reach an upper limit as the yeast lees concentration increased. However, precise quantification showed that wine could be almost depleted of condensed tannins by increasing the yeast lees concentration. The analysis of the mDP of the remnant condensed tannins in the wine after contact with lees indicated that there was no preferential adsorption of low or high polymeric size tannins. As the remnant condensed tannins in the wine contained less epigallocatechin units than the initial tannins, it could be suggested that polar tannins were preferentially adsorbed on yeast lees, independent of their polymeric size. From a sensory point of view, it was previously shown that trihydroxylation of the B-ring of condensed tannins led to a decrease coarseness of wines (*35*). Therefore, it could be expected from our results a correlative hypothetical increase of coarseness of red wines after aging on lees. A previous study on red wines aged on lees has shown that the amount of colloidal tannins seems to increase in stirred wines, suggesting that these tannins are less active against proteins (*36*). These results suggest a differential adsorption of tannins on lees.

The stability of anthocyanins in the blank experiment shows that the well-known reactions of anthocyanins with polyphenol compounds were not present (*37*). Because our experiments were performed in strict anaerobic conditions, such phenolic polymerization is unlikely to have involved acetaldehyde, considered the key component of oxidative polymerization (*38*). Also, the acidic rearrangement of procyanidins to xanthylium salts (*39*) and their well-known anaerobic degradation (*37*) were not observed during the experiment. However, about one-third of the total free anthocyanin in the wine disappeared after a 1 week contact with yeast lees with malvidine 3-glucoside, the most abundant anthocyanin in wine, being the most affected. The establishment of weak and reversible interactions between anthocyanins and yeast walls is well-known (*16*, *17*); however, we found that the intensity of adsorption of anthocyanins on yeast lees was not related to their polarity. This is in contrast to a previous study (*16*) with purified anthocyanins extracted from grape skins suggesting that hydrogen bonding might be involved in the adsorption mechanism. Our results suggest that the adsorption of anthocyanins in a complex polyphenolic environment is limited either by the number of adsorption sites at the cell surface or by the own reactivity of the anthocyanins. A more recent study on anthocyanin adsorption by yeast cell walls during alcoholic fermentation has shown that malvidine derivatives were slightly less adsorbed than other anthocyanin species (*17*) suggesting that adsorption of wine anthocyanins by yeast cell structures is different between live yeast cells and yeast lees. Analysis of red wines aged on lees had previously revealed that aging did not seem to have a major effect on the anthocyanin content of aged wines (*36*), whereas the same authors described that during autolysis, after stirring, the total anthocyanin content increased considerably. The decrease of free anthocyanins in the presence of yeast lees may also be attributed to the hydrolysis of the 3-O-glucoside part of anthocyanins by remnant periplasmic β-glucosidase (*18*) or to the slow complexation of anthocyanins with yeast fermentation byproducts (*40*). This leads to the formation of yellow pigments, whose reactions add to the slow condensation of anthocyanins with phenols (*41*). Moreover, it was recently shown that pyranoanthocyanins, and specifically malvidin 3-glucoside adducts, formed during first (and, to a less extent, second) fermentations of sparkling wines manufactured with red varieties decreased during a prolonged time of aging on yeast lees (*34*).

While these results appear contradictory, no previous studies have presented a clear balance between adsorbed polyphenol compounds and remnant polyphenol compounds in solution. The different physiological status of the yeasts used could account for these discrepancies. Indeed, to simulate the wine aging on lees, most of the authors used dried active yeasts directly inoculated into the wine or model wine or fresh precultures. Therefore, such yeast cells had not performed the whole fermentation process. This was not the case in the present experiments, when the yeast used was harvested after a complete alcoholic fermentation without any residual cell viability.

The present experiences were performed in model solutions that probably do not reproduce totally the phenomenon that take place in the aging of the wines. However, they represented one

approach to the knowledge of adsorption of the wine polyphenols on lees. Therefore, to better understand the adsorption of the wine polyphenols on lees, this work has been followed by a specific analysis of the chemical composition of these polyphenolic compounds (condensed tannins and anthocyanins) after their partial desorption from yeast lees by denaturation treatments. These results will be presented in a second paper.

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