JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Interactions between Yeast Lees and Wine Polyphenols during Simulation of Wine Aging: II. Analysis of Desorbed Polyphenol Compounds from Yeast Lees

JEAN-PAUL MAZAURIC AND JEAN-MICHEL SALMON*

Unité Mixte de Recherches, Sciences pour l'Oenologie, INRA, 2, place Viala, F-34060 Montpellier Cedex 1, France

In the first part of this work, the analysis of the polyphenolic compounds remaining in the wine after different contact times with yeast lees during simulation of red wine aging was undertaken. To achieve a more precise view of the wine polyphenols adsorbed on lees during red wine aging and to establish a clear balance between adsorbed and remnant polyphenol compounds, the specific analysis of the chemical composition of the adsorbed polyphenolic compounds (condensed tannins and anthocyanins) after their partial desorbtion from yeast lees by denaturation treatments was realized in the second part of the study. The total recovery of polyphenol compounds from yeast lees was not complete, since a rather important part of the initial wine colored polyphenols, especially those with a dominant blue color component, remained strongly adsorbed on yeast lees, as monitored by color tristimulus and reflectance spectra measurements. All anthocyanins were recovered at a rather high percentage (about 62%), and it was demonstrated that they were not adsorbed in relation with their sole polarity. Very few monomeric phenolic compounds were extracted from yeast lees. With the use of drastic denaturing treatments, the total recovery of condensed tannins reached 83%. Such tannins extracted from yeast lees exhibited very high polymeric size and a rather high percentage of galloylated residues by comparison with initial wine tannins, indicating that nonpolar tannins were preferentially desorbed from yeast lees by the extraction treatments.

KEYWORDS: Wine aging; yeast; lees; polyphenols

INTRODUCTION

Although the interactions between yeast cells and polyphenolic compounds are well-known, only very few works have dealt with the level and intensity of adsorption of such compounds on yeast cells. For example, from the existing works, it is difficult to draw a precise view of the exact localization of this adsorption. It was hypothesized that adsorbed polyphenols collapsed the yeast cell intermembrane space by reacting with cell membrane lipids (*1*) and that the maintenance of spherical yeast cell morphology after contact with polyphenols indicated that interaction of yeast lees with wine polyphenols occurred also at the cell wall level (*2*). It is well-known that polyphenols mainly interact colloidally with proteins by van der Waals bonds (*3*, *4*). Of the naturally occurring polyphenols, tannic acid precipitates or complexes with a great variety of macromolecules, including polysaccharides and proteins having H-bond acceptors (*3*). Moreover, yeast lees can modify the color of colored wines by the establishment of weak and reversible interactions between anthocyanins and yeast walls (*5*, *6*). However, it is important to note that most of the previous studies done on interactions between lees and polyphenols were always carried out on either rehydrated active dried yeasts or purified polyphenol molecules. Therefore, the interaction of complex wine polyphenol compounds with yeast lees was never taken into account.

To precisely obtain the eventual selectivity of interactions between the wine polyphenols and the external components of yeast lees, we performed a complete analysis of the chemical composition of polyphenolic compounds remaining in solution after different contact times with yeast lees during a simulation of wine aging (*7*). It was difficult at first to detect an eventual adsorption of monomeric phenolic compounds on yeast lees, due to the relative low amounts disappearing from the synthetic model wine. Precise quantification revealed that yeast lees at very high concentrations could almost deplete condensed tannins from the model wine. No preferential adsorption of low- or high-polymeric size tannins on yeast lees was observed. However, the remnant condensed tannins appeared to be more apolar than the adsorbed ones. About one-third of the total contents of free anthocyanins in wine was found to disappear after a 1 week contact with yeast lees; malvidine 3-glucoside was the most affected anthocyanin. Surprisingly and contrary to other previous works (*5*, *6*), we found that in our experimental conditions, the intensity of anthocyanin adsorption on yeast lees was not related to the polarity. It was then hypothesized that the adsorption of

* To whom correspondence should be addressed. Tel: (33)499 61 25 05. Fax: (33)499 61 28 57. E-mail: jmsalmon@ensam.inra.fr.

10.1021/jf060037o CCC: $33.50
Published on Web 05/09/2006

anthocyanins in a complex polyphenolic environment was limited by availability of adsorption sites at the cell surface. To strengthen these obtained results, we tried to present a clear balance between the adsorbed and the remnant polyphenolic compounds in the same experiment.

Therefore, after the first part of the study dedicated to the analysis of remnant polyphenolic compounds in the wine after yeast lees contact, the present part deals with the analysis of the chemical composition of the adsorbed polyphenolic compounds (condensed tannins and anthocyanins) after their partial desorption from yeast lees by protein denaturation treatments.

MATERIALS AND METHODS

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

Culture Media and Growth Conditions. The K1 strain, which is available as a commercial dry yeast, was not precultured and was thus directly inoculated into the fermentation medium after the rehydration procedure recommended by the manufacturer. The synthetic medium MS300 used in this study was a simulated standard grape juice containing 200 g L^{-1} glucose and 300 mg L^{-1} assimilable nitrogen (as ammonium chloride), strongly buffered to pH 3.3 (*8*). The yeast inoculum corresponded to the enological practice at the industrial scale (50 mg L^{-1}). Because anaerobic growth factors were not present in the MS300 fermentation medium, the medium was normally 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) fitted with occasional stirring under isothermal conditions (28 °C).

Cell Harvesting and Simulation of Wine Aging. Yeast cells were harvested by centrifugation exactly 100 h after the end of alcoholic fermentation as determined by the absence of residual sugar in the culture medium [concentration <2 g L^{-1} as measured with dinitrosalicylic acid reagent (*9*)]. This 100 h lag time after the end of fermentation was previously found to be necessary in order to get a null cell viability ($<10^{-3}$) within yeast cells (*10*). Cellular viability was obtained by plating about 1000 cells, as determined with the electronic particle counter, on YPD agar medium contained 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]. Petri dishes were then incubated for 48 h at 28 °C and examined for the presence of colonies. Such nonviable yeast cells were then further considered as yeast lees. As previously described (*1*, *2*, *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. This model wine medium was buffered to pH 3.3 and contained the following (per liter): citric acid, 6 g; DL-malic acid, 6 g; and 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.

Materials. All organic solvants were high-performance liquid chromatography (HPLC) grade (Merck, Darmstadt, Germany). (+)-Catechin and malvidin 3-O-glucoside were provided by Extrasynthèse (Genay, France), and phenylmethanethiol was purchased from Fluka (Buchs, Switzerland). Caftaric acid was purified from grape in accordance with the method previously described (*12*).

Total Wine Phenolic Pool. The total wine phenolic pool was prepared at the INRA experimental winery at Pech Rouge by treating 7.400 L of a 1996 colored and polyphenol-rich Cabernet Sauvignon wine, made at Arzens (Southern France), as previously described (*13*): 2500 L of the wine was pumped onto a styrene/divinylbenzene Diaion column (180 L) in the ascending mode. The column was then rinsed with 400 L of water and 400 L of 20% (v/v) ethanol in water in the ascending mode to remove sugars and polysaccharides, and the phenolic pool was finally eluted with 600 L of 95% (v/v) ethanol in water in the descending mode. Ethanol was then eliminated from the phenolic fraction by vacuum evaporation, and the final fraction was atomized to ensure stability of the sample. The total yield was about 300 g of atomized powder per 100 L of treated wine. HPLC and thiolysis analysis (*14*) of the main simple phenolic compounds of the atomized powder were (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 main polymeric size of 5.0. The rest of the powder consisted mainly of complex polyphenolic compounds of higher molecular weight. Reconstitution of the initial wine was achieved by suspending 2.9 g L^{-1} of this atomized phenolic pool in water, giving an absorbance at 280 nm of 26.75. Such a reconstituted wine exhibited a color intensity ($A_{420nm} + A_{520nm} + A_{620nm}$, $d = 1$ cm) of 4.74 and a color tint (A_{420nm}/A_{520nm}, $d = 1$ cm) of 0.71. The anthocyanin composition revealed 51% glycosidic and 49% acylated derivatives.

Experimental Protocol for Aging. Yeast lees (1.35×10^8 cells mL^{-1}), wine polyphenols (3 g L^{-1}), or yeast lees and wine polyphenols (at the same respective concentrations) in suspension in the model wine medium were deposited in tightly closed amber bottles (2.5 L) under an argon atmosphere. Bottles were continuously stirred without opening the bottle. Previous work has shown that the kinetics of wine polyphenols adsorption on yeast lees were clearly biphasic, with an initial, rapid fixation in less than 0.2 h, followed by a slow, constant, and saturating fixation, which reached a maximum after about 1 week of contact (*7*). The bottles were therefore held at 25 °C for 7 days (168 h) before harvesting the lees.

Partial Extraction of Adsorbed Wine Polyphenols from Yeast Lees. To perform an extensive quantitative analysis of the wine polyphenols adsorbed on lees, lyophilized yeast lees were submitted to repetitive extraction and strong protein denaturation treatments followed by extractions of polyphenolic compounds as depicted on **Figure 1**, until no further extraction could be obtained. The first simple extraction by a methanol/water/acid system (traditionally used to extract anthocyanins) followed by extraction by acetone 60% (known to extract proanthocyanidins, *15*) was initially performed to remove the less-adsorbed polyphenolic compounds. A classical fractionation of extracted polyphenolic compounds on a Fractogel column with differential elution was performed to separate anthocyanins, flavonols, phenolic acids, and flavanol monomers (fraction F1) from oligomeric and polymeric compounds (condensed tannins) (fraction F2) (*16*). The solid residue was further submitted to several denaturation steps involving sodium dodecyl sulfate (SDS) and urea treatments (**Figure 1**). A fixation step of the released complex polyphenolic compounds on a Relite SP411 resin column (Mitsubishi Chemical) was necessary to ensure a significant removal of denaturing agents (SDS and urea) from the final fractions before lyophilization (fractions F3 and F4) (*17*). The ultimate solid residue (fraction F5) was finally submitted to a direct analysis of remnant tannins by direct thiolysis.

Analytical Methods. *Reflectance Spectra and Color Tristimulus Measurements.* Reflectance spectra of lyophilized samples were recorded on a reflectance spectrophotometer (Minolta, CM 508D model), according to Mac Laren (*18*). Color tristimulus measurements were calculated as previously described (*19*).

Fractionation Procedure. Aliquots of the different model wine samples were fractionated 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 (55:45:0.05; v/v/v). Then, a second elution with 35 mL of acetone/water (60:40; v/v) allowed us to obtain a fraction consisting of oligomeric and polymeric compounds (proanthocyanidins). Both fractions were taken to dryness under vacuum and dissolved in 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). Separation was achieved on a Lichrospher 100-RP18 column (5 μm packing, 250 mm × 2 mm i.d.) (Merck). The elution conditions were as follows: 0.25 mL min^{-1} flow rate; oven temperature, 30 °C; solvent A, water/formic acid (95:5, v/v); solvent B, acetonitrile/water/formic acid (80:15:5, v/v/v); elution began isocratically with 2% B for 7 min and was continued with linear gradients from 2 to 20% B in 15 min, from 20 to 30% B in 8 min, from 30 to 40% B in 10 min, from 40 to 50% B in 5 min, and from 50 to 80% B in 5 min, followed

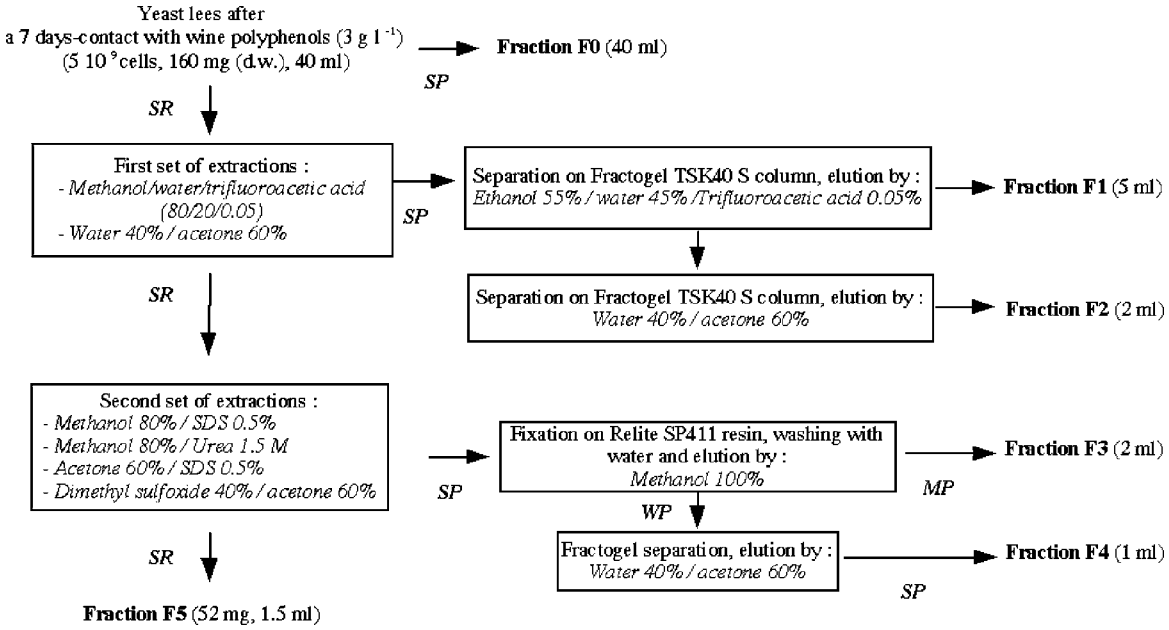

Figure 1. Extraction scheme used for the desorption of adsorbed polyphenols from lyophilized lees after 7 days of aging at 25 °C in a synthetic model wine [pH 3.3, 12% (v/v) ethanol] in the presence of wine polyphenols (3 g L^{-1}). SR, solid residue; SP, supernatant; MP, methanol phase; and WP, water phase.

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 methanol containing 0.2 M HCl. Each fraction was dissolved in methanol:thiolytic reagent (1:1, v/v) and heated for 2 min at 90 °C. The units released were analyzed by HPLC under the conditions previously described (*20*). Quantification of each terminal and extension unit was based on peak areas at 280 nm, and calibration was undertaken with external purified standards purified in our laboratory (*20*). The mean degree of polymerization (mDP), the percentage of galloylated units, and the percentage of epigallocatechin units were calculated as described elsewhere (*21*).

RESULTS

Qualitative Analysis of Yeast Lees after Partial Extraction of Adsorbed Wine Polyphenols. A complete analysis of color reflectance spectra of the corresponding lyophilized yeast lees clearly indicated that about 35−42% of the adsorbed colored wine polyphenols remained adsorbed on lees after the last extraction step (**Figure 2**). However, the overall shape of the reflectance spectra remained identical before and after complete polyphenol extraction. The determination of color tristimulus measurements on the different fractions revealed that the red (**a**) and blue (**b**) components of yeast lees color both decreased after extraction by about 45 and 20%, respectively (data not shown). This indicated that a rather important part of the initial wine colored polyphenols, especially those with a dominant blue color component, remained strongly adsorbed on yeast lees after the different extraction steps. A quantitative analysis of these extracted wine polyphenols was then performed.

Quantitative Analysis of Wine Polyphenols Desorbed from Yeast Lees. *Anthocyanins.* As shown in **Table 1**, anthocyanins were mostly recovered in fraction F1: Only about 11% of the initial adsorbed anthocyanins was recovered in this fraction. Further different extraction steps did not enhance this recovery. As a matter of fact, only fraction F5 exhibited nonquantifiable traces of anthocyanins (data not shown). All anthocyanins were recovered at a rather high percentage (about 62%). The low

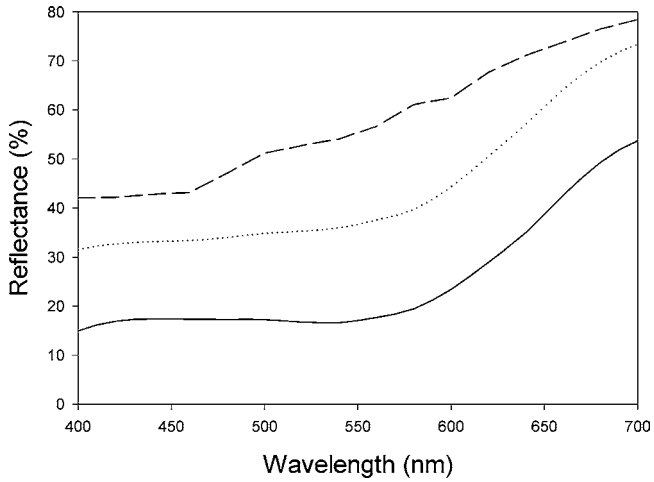

Figure 2. Reflectance spectra of different lyophilized lees during aging at 25 °C in a synthetic model wine [pH 3.3, 12% (v/v) ethanol] in the presence of wine polyphenols (3 g L^{-1}). Plain line, lyophilized initial lees in the absence of contact with wine polyphenols; long dashed line, lyophilized yeast lees after 7 days of contact with wine polyphenols; and dotted line, lyophilized fraction F5.

recovery of cyanidins (about 38%) was likely related to their very low abundance in wine (**Table 2**). More generally, acetyl derivatives remained more adsorbed than the other derivatives.

Monomeric Phenolics. No monomeric phenolics were found after polyphenolic compounds desorbtion from yeast lees (**Table 1**).

Phenolic Acids. Phenolic acids were almost totally recovered in fraction F1. No differences of adsorption were observed between coutaric and caftaric acids (data not shown).

Tannins. The total recovery of tannins remained low (about 83%), despite the use of drastic denaturing treatments. However, direct thiolysis on the final lyophilized residue (fraction F5) strongly minimized this recovery, since the remnant tannins present in the fraction F5 were not easily accessible to the thiolysis reagent and it was not possible to take into account the amount of monomeric tannins of this last fraction in the

Table 1. Total Balance of Polyphenolic Compounds Recovery after Repetitive Protein Denaturation Treatments Followed by Polyphenolic Compounds Extraction[a]

fractions	anthocyanins[b] (mg L^{-1})	monomeric phenolics[c] (mg L^{-1})	condensed tannins (mg L^{-1})	phenolic acids[d] (mg L^{-1})
initial wine polyphenols (3 g L^{-1})	55.4 ± 0.4	37.1 ± 5.1	418 ± 46	43 ± 6
fraction F0	31.9 ± 0.4	34.0 ± 2.1	240 ± 6	40.5 ± 2
fraction F1	2.6 ± 0.2	ND[e]	ND	1.3 ± 0.5
fraction F2	ND	ND	82.0 ± 15.0	ND
fraction F3	ND	ND	4.0 ± 1.7	ND
fraction F4	ND	ND	0.5 ± 0.5	ND
fraction F5	traces		22.2 ± 1.9	ND
total recovery	≈62.0%	≈91.6%	≈83.4%	≈97.2%

[a] Mean values and standard deviations of three different experiments are given. All values are calculated for 1 L of initial synthetic alcoholic medium. [b] As malvidin-3-O-glucoside equivalents. [c] As catechin equivalents. [d] As caftaric acid equivalents. [e] Not detectable.

final calculation. Despite the use of strong denaturing conditions, a rather important part of adsorbed tannins (up to about 12.5%) remained tightly bound to yeast lees (**Table 1**). It is important to notice that the recovered tannins after extraction exhibited a higher degree of polymerization than the initial wine tannins (**Table 3**). The fraction F3, obtained after strong protein denaturing conditions, contained condensed tannins with higher polymeric size (about 16.0 instead of about 4.0). More generally, the condensed tannins extracted from yeast lees exhibited a very high percentage of galloylated residues by comparison with the initial wine tannins, indicating that very nonpolar tannins were preferentially desorbed from yeast lees by the extraction treatments. The remnant tannins after extraction exhibited a percentage of galloylated and epigallocatechin units almost identical to the initial wine tannins.

DISCUSSION

Attempts to desorb polyphenols adsorbed on yeast lees were achieved by submitting lyophilized yeast lees to repetitive extraction and strong protein denaturation treatments. The determination of reflectance tristimulus color measurements on the final fraction (F5) indicated that a rather important part of the initial wine colored compounds, especially those with a dominant blue color component, remained strongly adsorbed on yeast lees after the different extraction steps. In wine, among the major polyphenol constituents, the anthocyanins and their derivatives were mainly responsible for wine color. At usual wine pH values, genuine grape anthocyanins occurred mostly as the colorless hydrated hemiketal form. Therefore, wine color was mainly due to the conversion of grape anthocyanins to complex pigments or to copigmentation with tannins. Blue and purple colors arised mainly from such anthocyanin modifications: (i) Among pyranoanthocyanins, which remained colored over a wide pH range, flavanylvinyl-pyranoanthocyanins (or portisins) were blue (*22*); (ii) ethyl-linked direct tannin−anthocyanin adducts were purple (*23*); and (c) anthocyanin copigmentation, which gave very intense and stable colors, was particularly important in the wine pH range, where bathochromic effect shifted the color toward purple (*24*).

Previous analysis of the polyphenolic compounds remaining in the wine showed that about one-third of the total contents of free anthocyanins in wine disappeared after 1 week of contact with yeast lees. Eleven percent of the adsorbed anthocyanins was recovered in fraction F1. Malvidin derivatives were the most abundant anthocyanins recovered in this fraction. More coumaroyl glucoside and glucoside than acetyl glucoside derivatives were released from yeast lees, confirming that the intensity of anthocyanin adsorption on yeast lees was not related to their polarity. This result was in accordance with that observed by simple quantification of anthocyanins in the resulting wine (*7*) but in opposition with several previous reports (*5*, *6*), reinforcing the need of complete balance analysis during such studies. Acetyl derivatives seemed specifically adsorbed on yeast lees in a very strong manner. Such results were thus in opposition with those obtained by two different research groups working on purified anthocyanins extracted from grape skins on fresh yeast cells and yeast lees (*5*, *6*) but in accordance with several results presented recently (*24*) on fresh yeast cells. It could then be supposed that the adsorption of anthocyanins on yeast lees in a complex polyphenol environment (as observed in wine) did not follow a simple adsorption mechanism involving hydrogen bonding as previously suggested (*5*). The observed specific anthocyanin adsorption on yeast lees explained why

Table 2. Characterization of the Anthocyanins Recovered after Repetitive Protein Denaturation Treatments Followed by Polyphenolic Compounds Extraction as Described in the Material and Methods[a]

	glucosyl derivatives		acetyl derivatives		*p*-coumaroyl derivatives		total
fractions	mg L^{-1}	%	mg L^{-1}	%	mg L^{-1}	%	mg L^{-1}
initial wine polyphenols (3 g L^{-1})	28.5	51.4	16.7	30.1	10.2	18.5	55.4
fraction F0	20.1	63.0	5.9	18.6	5.9	18.6	31.9
fraction F1	0.9	35.0	0.4	15.0	1.3	50.0	2.6
total recovery	≈74.0%		≈38.0%		≈71.0%		≈62.0%

	delphinidins		cyanidins		petunidins		peonidins		malvidins		total
fractions	mg L^{-1}	%	mg L^{-1}	%	mg L^{-1}	%	mg L^{-1}	%	mg L^{-1}	%	mg L^{-1}
initial wine polyphenols (3 g L^{-1})	6.9	12.5	2.5	4.5	6.2	11.2	5.8	10.5	33.9	61.2	55.4
fraction F0	3.7	11.6	0.8	2.5	3.2	10.0	2.9	9.1	21.3	66.8	31.9
fraction F1	0.28	10.7	0.05	1.9	0.22	8.3	0.20	7.6	1.88	71.5	2.6
total recovery	≈58.0%		≈34.0%		≈55.0%		≈53.0%		≈68.0%		≈62.0%

[a] Mean values of three different experiments are given. Values are expressed in equivalent malvidin-3-glucoside.

Table 3. mDP and Percentage of Galloylated and Epigallocatechin Units of the Condensed Tannins Recovered after Repetitive Protein Denaturation Treatments Followed by Polyphenolic Compounds Extraction as Described in the Material and Methods[a]

fractions	mDP	galloylated units (%)	epigallocatechin units (%)
initial wine polyphenols (3 g L^{-1})	3.8 ± 0.2	4.5 ± 0.2	3.4 ± 0.2
fraction F0	3.3 ± 0.2	2.5 ± 0.2	2.8 ± 0.7
fraction F2	7.1 ± 0.4	8.1 ± 0.2	2.5 ± 0.4
fraction F3	16.2 ± 4.5	3.3 ± 1.5	1.3 ± 0.4
fraction F4	5.4 ± 2.0	5.1 ± 0.3	ND[b]
fraction F5	5.8 ± 0.2	3.9 ± 0.2	3.2 ± 0.2

[a] Mean values and standard deviations of three different experiments are given. [b] Not detectable.

pyranoanthocyanins, and specifically malvidin 3-glucoside adducts, formed during first (and, to a lesser extent, second) fermentations of sparkling wines manufactured with red varieties decreased during a prolonged time of aging on yeast lees (*26*). Moreover, the almost total recovery of adsorbed anthocyanins in the fraction F1 (extraction by methanol and acetone) was a good indication that the interactions developed between anthocyanins (at the exception of the acetyl derivatives) and that yeast lees were presumably weak. Further research was therefore needed to understand at the molecular level the distinctive feature of acetyl derivatives of anthocyanins of remaining tightly bound to yeast lees.

The first part of the present work (*7*) revealed that only very few monomeric phenolic compounds seemed adsorbed on yeast lees. The difficulty of analyzing such phenolics in the wine was linked to the very low amounts of disappeared compounds by comparison with their total amount of remnants in the wine. Analysis of the different fractions obtained after repetitive extraction and strong protein denaturation treatments of yeast lees revealed that about 8.4% of monomeric phenolics remained strongly tighten to yeast lees. Because other small compounds such as phenolic acids were recovered in fraction F1, it could be hypothesized that monomeric phenolics were not recovered in the obtained fractions, because they were associated with other elements such as anthocyanins.

Concerning tannins, the total recovery remained incomplete, despite the use of drastic denaturing treatments (**Table 1**). However, direct thiolysis performed on the final lyophilized residue (fraction F5) strongly minimized this recovery, since monomeric phenolics eventually adsorbed on tannins were not taken into account in its calculation, lowering the obtained mDP values. Moreover, it was clear that the totality of the remnant tannins present in the fraction F5 was not easily accessible to the thiolysis reagent. This was especially true for the direct purple tannin−anthocyanin adducts, as previously described (*23*). Despite the use of strong denaturing conditions, a rather important part of adsorbed tannins (about 12.5%) remained tightly bound to yeast lees (**Table 1**). At the exception of fraction F3, it is important to note that the recovered tannins in all fractions exhibited very similar mDPs, which were generally slightly higher than those of initial wine tannins (**Table 3**). The first well-known extraction by methanol and acetone extracted galloylated tannins first with a rather low mDP (**Table 3**). On the contrary, strong protein denaturing conditions extracted very polar tannins with a very high mDP. This result could be explained by either a specific adsorption of such polar tannins or a hypothetical polymerization of tannins favored by the number of different solvents used for extraction. The last tannins remaining on yeast lees seemed almost identical to the initial wine tannins, in terms of polymerization and polarity (**Table 3**). From the analysis of the main polymeric size of the remnant tannins in the wine after contact with lees, we hypothesized that polar tannins were preferentially adsorbed on yeast lees, independent of their polymeric size (*7*). From the present results, this was not completely true: As a matter of fact, about 2% of high polymeric size polar tannins (**Tables 1** and **3**) seemed specifically trapped by yeast lees by protein−tannins interactions. From a sensory point of view, this result reinforced the hypothesis that aging on lees may increase the coarseness of red wines (*7*), since trihydroxylation of the B-ring of condensed tannins led to a decrease coarseness of wines (*27*). Such a differential adsorption of tannins on lees partially explained the fact that the amount of colloidal tannins seemed to increase in stirred wines aged on lees, indicating that these tannins were less active against proteins (*28*).

ACKNOWLEDGMENT

We thank Jean-Marc Souquet and François-Xavier Sauvage for their valuable help and expertise in the polyphenols and tristimulus color analysis, respectively.

LITERATURE CITED

(1) Salmon, J. M.; Fornairon-Bonnefond, C.; Mazauric, J. P.; Moutounet, M. Oxygen consumption by wine lees: Impact on lees integrity during wine aging. *Food Chem*. **2000**, *71*, 519−528.

(2) Salmon, J. M.; Vuchot, P.; Doco, T.; Moutounet, M. Maintenance and protection of yeast morphology by contact with wine polyphenols during simulation of wine aging on lees. *J. Food Sci*. **2003**, *68*, 1782−1787.

(3) Haslam, E.; Lilley, T. H.; Warminski, E.; Liao, H.; Cai, Y.; Martin, R.; Gaffney, S. H.; Goulding, P. N.; Luck, G. Polyphenol complexation. In *Phenolic Compounds in Food and Their Effects on Health*; Ho, C. T., Lee, C. Y., Huang, M. T., Eds.; ACS Symposium Series; American Chemical Society: Washington, DC, 1992; pp 8−50.

(4) Kawamoto, H.; Nakatsubo, F. Effects of environmental factors on two-stage tannin-protein coprecipitation. *Phytochemistry* **1997**, *46*, 379−483.

(5) Vasserot, Y.; Caillet, S.; Maujean, A. Study of anthocyanin adsorption by yeast lees. Effect of some physicochemical parameters. *Am. J. Enol. Vitic.* **1997**, *48*, 433−437.

(6) Morata, A.; Gomez-Cordoves, M. C.; Suberviola, J.; Bartolome, B.; Colomo, B.; Suarez, J. A. Adsorption of anthocyanins by yeast cell walls during the fermentation of red wines. *J. Agric. Food Chem.* **2003**, *51*, 4084−4088.

(7) Mazauric, J. P.; Salmon, J. M. Interactions between yeast lees and wine polyphenols during simulation of wine aging: I. Analysis of remnant polyphenolic compounds in the resulting wines. *J. Agric. Food Chem*. **2005**, *53*, 5647−5653.

(8) Bely, M.; Sablayrolles, J. M.; Barre, P. Automatic detection of assimilable nitrogen deficiencies during alcoholic fermentation in oenological conditions. *J. Ferm. Bioeng.* **1990**, *70*, 246−252.

(9) Miller, G. L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem*. **1959**, *31*, 426−428.

(10) Fornairon, C.; Mazauric, J. P.; Salmon, J. M.; Moutounet, M. Observations on the oxygen consumption during maturation of wines on lees. *J. Int. Sci. Vigne Vin.* **1999**, *33*, 79−86.

(11) Salmon, J. M.; Fornairon-Bonnefond, C.; Mazauric, J. P. Interactions between wine lees and polyphenols: Influence on oxygen consumption capacity during simulation of wine aging. *J. Food Sci*. **2002**, *67*, 1604−1609.

(12) Cheynier, V.; Van Hulst, M. Oxidation of *trans*-caftaric acid and 2-*S*-glutathionylcaftaric acid in model solutions. *J. Agric. Food Chem.* **1988**, *36*, 10−15.
(13) Casalini, C.; Lodovici, M.; Briani, C.; Paganelli, G.; Remy, S.; Cheynier, V.; Dolara, P. Effect of complex polyphenols and tannins from red wine (WCPT) on chemically induced oxidative DNA damage in the rat. *Eur. J. Nutr.* **1999**, *38*, 190−195.
(14) Caderni, G.; Remy, S.; Cheynier, V.; Morozzi, G.; Dolara, P. Effect of complex polyphenols on colon carcinogenesis. *Eur. J. Nutr.* **1999**, *38*, 126−132.
(15) Rohr, G. E.; Meier, B.; Sticher, O. Bioactive natural products. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, The Netherlands, 2000; pp 497−570.
(16) Derdelinckx, G.; Jerumanis, J. Separation of malt hop proanthocyanidins on Fractogel TSK HW-40 (S). *J. Chromatogr.* **1984**, *285*, 231−234.
(17) Furth, A. J.; Bolton, H.; Potter, J.; Priddle, J. D. Separating detergent from proteins. *Methods Enzymol.* **1984**, *104*, 318−328.
(18) MacLaren, E. Food colorimetry. In *Developments in Food Colours*; Walford, J., Ed.; Applied Science Publishers Ltd.: London, 1980; Tome 1, pp 27−45.
(19) Bakker, J.; Bridle, P.; Timberlake, C. F. *Tristimulus* measurements (CIELAB 76) of port wine colour. *Vitis* **1986**, *25*, 67−78.
(20) Souquet, J. M.; Cheynier, V.; Brossaud, F.; Moutounet, M. Polymeric proanthocyanidins from grape skins. *Phytochemistry* **1996**, *43*, 509−512.
(21) Rigaud, J.; Perez-Ilzarbe, X.; Ricardo-da-Silva, J. M.; Cheynier, V. Micromethod for the identification of proanthocyanidin using thiolysis monitored by high-performance liquid chromatography. *J. Chromatogr.* **1991**, *540*, 401−405.
(22) Mateus, N.; Silva, A. M. S.; Rivas-Gonzalo, J. C.; Santos-Buelga, C.; De Freitas, V. A new class of blue anthocyanin-derived pigments isolated from red wines. *J. Agric. Food Chem.* **2003**, *51*, 1919−1923.
(23) Atanasova, V.; Fulcrand, H.; Le Guernevé, C.; Cheynier, V.; Moutounet, M. Structure of a new dimeric acetaldehyde malvidin-3-glucoside condensation product. *Tetrahedron Lett.* **2002**, *43*, 6151−6153.
(24) Brouillard, R.; Mazza, G.; Saad, Z.; Albrecht-Gary, A. M.; Cheminat, A. The copigmentation reaction of anthocyanins: A microprobe for the structural study of aqueous solutions. *J. Am. Chem. Soc.* **1989**, *111*, 2604−2610.
(25) Medina, K.; Boido, F.; Dellacassa, E.; Carrau, F. Yeast interactions with anthocyanins during red wine fermentation. *Am. J. Enol. Vitic.* **2005**, *56*, 104−109.
(26) Pozo-Bayon, M. A.; Monagas, M.; Polo, M. C.; Gomez-Cordoves, C. Occurrence of pyranoanthocyanins in sparkling wines manufactured with red grape varieties. *J. Agric. Food Chem.* **2004**, *52*, 1300−1306.
(27) Vidal, S.; Francis, L.; Guyot, S.; Marnet, N.; Kwiatkowski, M.; Gawel, R.; Cheynier, V.; Waters, E. J. The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. *J. Sci. Food Agric.* **2003**, *83*, 564−573.
(28) Feuillat, M.; Escot, S.; Charpentier, C.; Dulau, L. Élevage des vins rouges sur lies fines—Intérêt des interactions entre polysaccharides de levures et polyphénols du vin. *Rev. Oenol.* **2001**, *98*, 17−18.

Received for review January 4, 2006. Revised manuscript received April 3, 2006. Accepted April 3, 2006.

JF060037O