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Effect of Different Yeast Strains and Their Culture Conditions on the Prevention of Wine Model Solution Browning by Yeast Lees

Trinidad Márquez,[†] Carmen Millán,[†] Jean-Marc Souquet,[§] and Jean-Michel Salmon*,[§]

[†] Departamento Microbiología, Universidad de Córdoba, Campus de Rabanales, 14014 Córdoba, Spain, and [§] INRA, UMR 1083 "Sciences Pour l'Oenologie", bât 28, 2 Place Viala, 34060 Montpellier Cedex 1, France

The purpose of this work was to examine the possible involvement of yeast membrane components in the adsorption of browning compounds from oxidized white wine. For this purpose, different yeast strains and growth conditions (aerobiosis and anaerobiosis) were tested for their ability to prevent browning of two model solutions consisting of (+)-catechin/acetaldehyde and (+)-catechin/glyoxylic acid. The obtained results showed that the effects of yeast lees are different according to the type of the studied model solution and the growth conditions that affect both the quantity and the quality of membrane sterols of the yeasts. Moreover, in vitro experiments proved that yeast membrane sterols could be likely involved in the yeast's ability to adsorb polyphenolic compounds and mainly the colorless intermediate compounds of the browning reactions.

KEYWORDS: Yeast lees; wine browning; sterols

INTRODUCTION

Browning can cause serious alterations in white wines and is considered to be one of the major causes of their quality loss. After wine conditioning and during wine storage, wine browning results in the appearance of new polymeric compounds arising from nonenzymatic reactions between different groups of wine polyphenols. Several nonenzymatic reactions studied in model solutions have been described to explain the color changes observed. The first one is shared by all types of beverages and involves the oxidation of phenols to quinones of variable levels of polymerization, increasing the color in the yellow-brown region. This pathway is catalyzed by metals such as iron and copper, which alter the reaction rate to an extent dependent on their concentrations (*1*−*7*). A second pathway involves the direct condensation of phenols with acetaldehyde produced by yeasts in fermented drinks (*8*−*11*). Depending on the degree of condensation, yellowish compounds that contribute to darkening of wine color are formed, acting as anthocyanin copigments. The greatest effect was induced by oligomers containing two or three elementary catechin units, whereas monomers were the poorest anthocyanin copigments among the compounds checked (*12*). In addition, a particular pathway involves condensation and oxidation reactions between flavans and glyoxylic acid, which arises from the oxidation of tartaric acid (*6*, *13*−*17*).

It is well-known that yeasts possess the ability to interact with wine polyphenols. Some authors have proved the prevention of white wine browning by the use of yeasts through the inhibition of brown pigments formation (*18*, *19*). This may be related to the adsorption of the intermediate products as the reaction develops (*20*−*23*). On the other hand, the yeast could exhibit a competition with polyphenols for oxygen consumption, exerting a protection effect on wine versus oxidation (*24*−*26*).

The interaction between polyphenols and yeast has been mainly attributed to cell walls (*27*, *28*). The polarity and the amphiphilic character of cell wall polymers give the yeast ability to adsorb molecules of different nature such as volatile compounds (*29*), fatty acids (*30*), and phenolic compounds (*22*, *23*). Nevertheless, an interaction between polyphenols and yeast membrane lipids during wine oxidation was also demonstrated (*31*). From the existing bibliography, it is therefore difficult to identify the exact target(s) of wine browning products at the surface of yeast. The purpose of this work was to examine the possible involvement of yeast membrane components in the adsorption of browning compounds from oxidized white wine, as previously suggested (*24*, *25*).

In the present work, we use different yeast strains and different growth conditions (aerobiosis and anaerobiosis) to affect both the quantity and the quality of membrane sterols of the resulting yeasts. In aerobiosis, yeasts consume oxygen to synthesize membrane lipids and, particularly, ergosterol, which is the main yeast membrane sterol. In anaerobic conditions, yeast growth normally requires the addition of sterols, which are essential for plasma membrane integrity (*32*), because yeasts are able to incorporate a wide variety of exogenous sterols, whereas it is not the case in aerobic conditions (for a review, see ref *33*). The obtained yeasts were then tested for their ability to prevent browning of model

*Corresponding author [telephone (33) 499 61 25 05; fax (33) 499 61 28 57; e-mail jmsalmon@supagro.inra.fr].

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solutions of (+)-catechin/acetaldehyde and (+)-catechin/glyoxylic acid. We demonstrate here that the effects of yeast lees are different according to the type of browning reaction model studied and that yeast membrane sterols could be likely involved in the yeast ability to adsorb brown polyphenolic compounds.

MATERIALS AND METHODS

Yeast Strains. Several commercial enological yeast strains belonging to different *Saccharomyces* variant races were used: *Saccharomyces cerevisiae* (K1, ICV-Lallemand, Montréal, Canada), *S. cerevisiae* var. *capensis* (D225, ICV, Lattes, France, abbreviated *S. capensis* in the figure captions), and *Saccharomyces uvarum* (S6U, Lallemand, Montréal, Canada). All of these commercial strains were commonly used for winemaking.

One strain of *Hanseniaspora valbyensis* (107, URC-Le Rheu, INRA, France) was also used. This strain of *H. valbyensis* exhibits a very interesting potentiality to ferment high-sugar musts and is able to ferment very slowly to dryness a must containing 200 g L^{-1} sugars at 28 °C.

Culture Media and Growth Conditions. All precultures were performed in a standard nutrient medium YPD containing yeast extract (Difco, Detroit, MI) 10 g L^{-1}, bactopeptone (Difco) 20 g L^{-1}, and glucose 20 g L^{-1}) for 24 h at 28 °C in Erlenmeyer flasks.

Aerobic cultures were grown in a YPD medium buffered to pH 3.3 for 72 h at 28 °C with strong agitation in Erlenmeyer flasks with a 1:10 liquid/ air ratio.

Anaerobic cultures were grown in a synthetic medium MS300 strongly buffered to pH 3.3 (*34*). This medium contained (per liter) 200 g of glucose, 6 g of citric acid, 6 g of DL-malic acid, 750 mg of KH_2PO_4, 500 mg of K_2SO_4, 250 mg of $MgSO_4 \cdot 7H_2O$, 155 mg of $CaCl_2 \cdot 2H_2O$, 200 mg of NaCl, 4 mg of $MnSO_4 \cdot H_2O$, 4 mg of $ZnSO_4$, 1 mg of $CuSO_4 \cdot 5H_2O$, 1 mg of KI, 400 μg of $CoCl_2 \cdot 6H_2O$, 1 mg of H_3BO_3, 1 mg of $(NH_4)_6Mo_7O_{24} \cdot 2H_2O$, 20 mg of *myo*-inositol, 2 mg of nicotinic acid, 1.5 mg of calcium pantothenate, 250 μg of thiamin−HCl, 0.25 mg of pyridoxine−HCl, 3 μg of biotin, and 300 mg of NH_4Cl. Ergosterol (15 mg L^{-1}) and oleic acid (5 mg L^{-1}) dissolved in 1 mL of Tween 80/pure ethanol (50:50, v/v) were also added to the medium after sterilization. The fermentation medium was inoculated with 10^6 yeast cells mL^{-1}. Yeast cells were cultured anaerobically in handmade glass fermentors (working volume = 1.1 L) fitted with fermentation locks (CO_2 bubbling outlets filled with water). Fermentations were carried out under isothermal conditions (28 °C) with continuous magnetic stirring (500 rpm). The amount of CO_2 released was calculated from automatic measurements (taken every 20 min) of fermenter weight (*35*). Fermentation was considered to be complete when the medium contained < 2 g L^{-1} of glucose. None of the fermentations were stopped if they were $< 99\%$ complete.

Cell number was determined after sonication (30 s, 10 W) using an electronic particle counter (model ZB2, Beckman-Coulter, Margency, France) fitted with a 100 μm aperture probe.

Aerobically and anaerobically grown yeasts were harvested by centrifugation (1000*g*, 5 min), rinsed twice with water, and frozen at −20 °C. The corresponding biomass was then lyophilized (Christ Alpha I-5, Bioblock Scientific, Illkirch, France) and stored before use at ambient temperature under argon gas in order to protect biomass from oxidation.

Simulation of Wine Browning with Model Solutions. Two hydroalcoholic solutions containing 12 g L^{-1} (+)-catechin (Sigma-Aldrich Chemicals, St. Louis, MO) in 10 and 14% (v/v) ethanol were prepared. The first one was supplied with acetaldehyde (21.2 mM), adjusted to pH 3.2 with concentrated acetic acid, and incubated at 20 °C for 48 h. The second solution was supplied with glyoxylic acid (20.4 mM), adjusted to pH 3.2 with 4 N NaOH, and incubated at 20 °C for 25 days. All reagents were provided by Merck (Darmstadt, Germany). Lyophilized yeast cells were added in both model solutions at the beginning of the incubation or during the incubation at a concentration of 24 g (dw) L^{-1}.

In Vitro Evaluation of Direct Interaction of Brown Products with Solubilized Sterols. Pure ergosterol (Sigma-Aldrich Chemicals) was solubilized at a concentration of 15 mg mL^{-1} in a solution of Tween-80 (Sigma-Aldrich Chemicals) and ethanol (50:50, v/v), after gentle heating at 70 °C. Once a clear suspension had been obtained, ergosterol was added in the (+)-catechin/acetaldehyde model solution at a final concentration of 0.01 mg mL^{-1}, and incubation was performed at 20 °C for 48 h. A control experiment was performed with the solution of Tween-80 (Sigma-Aldrich Chemicals) and ethanol (50:50, v/v) without ergosterol. At the end of incubation time, the two phases were completely resolved by high-speed centrifugation (16000*g*, 5 min). Upper white and lower brownish phases were then simply recovered by sequential removal with a pipet.

Analytical Methods. *Reflectance Spectra and Color Tristimulus Measurements*. Spectra of lyophilized samples were recorded on a reflectance spectrophotometer (CM 508D model, Minolta, Ramsey, NJ), according to the method of MacLaren et al. (*36*). Color tristimulus measurements were calculated as previously described (*37*).

Spectrophotometric Measurements. UV−visible spectra of model solutions were recorded on a UV−visible spectrophotometer (Cary 100 Bio, Varian, Les Ulis, France) using a 1 mm path length cell. All samples were first clarified by centrifugation (1900*g*, 10 min) and subsequent membrane filtration (0.45 μm pore size, Millipore).

Dissolved Oxygen Measurements. The dissolved oxygen was measured in all cases during the incubation of the glyoxylic acid/(+)-catechin model solutions every 3 days with an oxymeter (model Oxy 197, WTW, Weilheim, Germany).

Measurement of Oxygen Consumption. Oxygen consumptions were measured at 28 °C by using a high-resolution oxygraphic system (Oroboros, Innsbruck, Austria). Data were recorded at sampling intervals of 1 s (Datlab Acquisition software, Oroboros). At the beginning of the incubation experiments, control oxygen consumption of yeast lees was measured by suspending 0.24 g of lyophilized lees in 4 mL of distilled water. During incubation of yeast lees in the presence of phenolic compounds, 500 μL of the reaction medium was harvested and diluted in 2 mL of distilled water for oxygen consumption measurements. Oxygen consumption was then rapidly measured under normoxic conditions (between 3.75 and 7.5 mg of O_2 L^{-1}).

HPLC-DAD Analysis. HPLC-DAD analyses were performed using a Waters 2690 system, a Waters 996 photodiode array detector, and Millenium 32 chromatography manager software (Milford, MA). Eluted compounds were quantified as (+)-catechin at 280 nm, using a C18 reversed-phase column (250 mm × 4.6 mm i.d., 5 μm particle size, Merck). The elution conditions were as follows: flow rate, 1 mL min^{-1}; solvent A, 98:2 v/v water/formic acid; solvent B, 80:18:2 acetonitrile/water/formic acid; elution from 5 to 30% B in 35 min, from 30 to 100% B in 5 min, held for 20 min, and followed by washing and re-equilibration of the column. The structure and the polymerization degrees of the identified polymerized compounds were controlled by liquid chromatography−ion spray mass spectrometry, as previously described (*8*, *11*, *13*).

Total Yeast Sterols and Squalene Determination by Gas Chromatography. The methodology used was previously described (*38*). For each extraction, cholesterol was used as standard. All organic solvents were protected from oxidation by the addition of 0.01% (w/v) butylated hydroxytoluene (BHT). Total lipids from lyophilized yeast cells (1 g) were extracted overnight with methanol/chloroform (2: 1, v/v), and the solid residue was extracted once more for 2 h with methanol/chloroform/water (2:1:0.8, v/v/v). The organic extracts were combined, dried on Na_2SO_4, and concentrated to dryness with a rotatory evaporator. After total lipid saponification, sterols were extracted with hexane, washed three times with water/ethanol (1:1, v/v) (*39*), dried on Na_2SO_4, and concentrated to dryness with a rotatory evaporator. Gravimetric determination of the total amount of the unsaponifiable material recovered was performed. Total sterols were then resuspended in 1 mL of hexane.

Sterols were silylated by incubating 0.4 mL of sterol solution and 50 μL of methylsilyltrifluoroacetamide (MSTFA, Pierce Chemicals, Perbio, Bezons, France) at 60 °C for 20 min. Fifty microliters of pure cholesterol (Sigma Chemicals) dissolved in pure pyridine (0.5 mg L^{-1}) was added as an internal standard. Sterol determination and quantification were performed using a Hewlett-Packard 5989-B gas chromatograph equipped with a DBS apolar column (J&W Scientific, Toulouse, France; 60 m × 0.32 mm × 1 μm) and coupled to a Hewlett-Packard 5890A mass spectrometer. Helium gas flow rate was set at 2.5 mL min^{-1}. Injector temperature rose from 30 to 320 °C at 180 °C min^{-1} and was kept at 320 °C for 50 min. The oven temperature rose from 250 to 320 °C at 6 °C min^{-1} and was kept at 320 °C for 40 min. A calibration curve was obtained using pure squalene, cholesterol, ergosterol, and lanosterol (Sigma Chemicals).

Statistical Analysis. The statistical KyPlot (v. 2.0 b.15) software (KyensLab, Tokyo, Japan) was used to perform ANOVA and Tukey tests (pairwise comparisons for one-way layout design) to classify the data into homogeneous groups.

RESULTS

Effect of Different Yeast Lees on the Color of Acetaldehyde/(+)-Catechin Model Solutions after 48 h of Incubation. We first used an acetaldehyde/(+)catechin model solution to mimic part of the browning reactions occurring during wine oxidation. Acetaldehyde is an oxidation product of ethanol oxidation (*40*). The reaction of condensation between (+)-catechin and acetaldehyde involves ethyl bridges and the formation of adducts as reaction intermediates without the participation of oxygen, according to the mechanism initially proposed by Timberlake and Bridle (*41*) and later confirmed by Fulcrand et al. (*8*) and Es-Safi et al. (*11*). The color in the yellow spectral region increases as the condensation degree does, because of the important role of the flavan polymerization reaction in this browning reaction. In the acetaldehyde/(+)catechin model solution tested, the absorbance at 420 nm of (+)-catechin solution increased by 40.2% at 24 h of reaction, revealing the formation of colored products absorbing in the visible region in parallel with polymerization (data not shown). To determine the potential effect of the different yeasts on the development of the polymerization reaction, lyophilized yeasts were added at the beginning of the reaction. The presence of yeasts induced a partial inhibition of browning, ranging from 40.6 to 58.6%, as measured by the percentage decrease of the absorbance at 420 nm by comparison to the control (absence of yeast).

Color tristimulus measurements (CIE-Laboratory analysis) of the final model solution samples exhibit very significant differences for the yeast strains tested between their mode of production, depending on the color stimulus tested (**Figure 1**). In general, aerobic production of the yeasts resulted in a stronger prevention of browning of the model solutions tested, especially for the protection against the appearance of yellowish compounds (parameter *b*, **Figure 1C**).

However, no significant differences in the reflectance spectra of the corresponding lyophilized lees after 2 days of incubation at 20 °C in the acetaldehyde/(+)-catechin model solution could be observed (data not shown).

Effect of Different Yeast Lees on the Formation of Polymerized Brown Compounds in Acetaldehyde/(+)-Catechin Model Solutions after 48 h of Incubation. The formation of the different polymerization reaction compounds was analyzed by HPLC with detection at 280 nm. According to previous studies (*8*), flavanols can be linked through C6

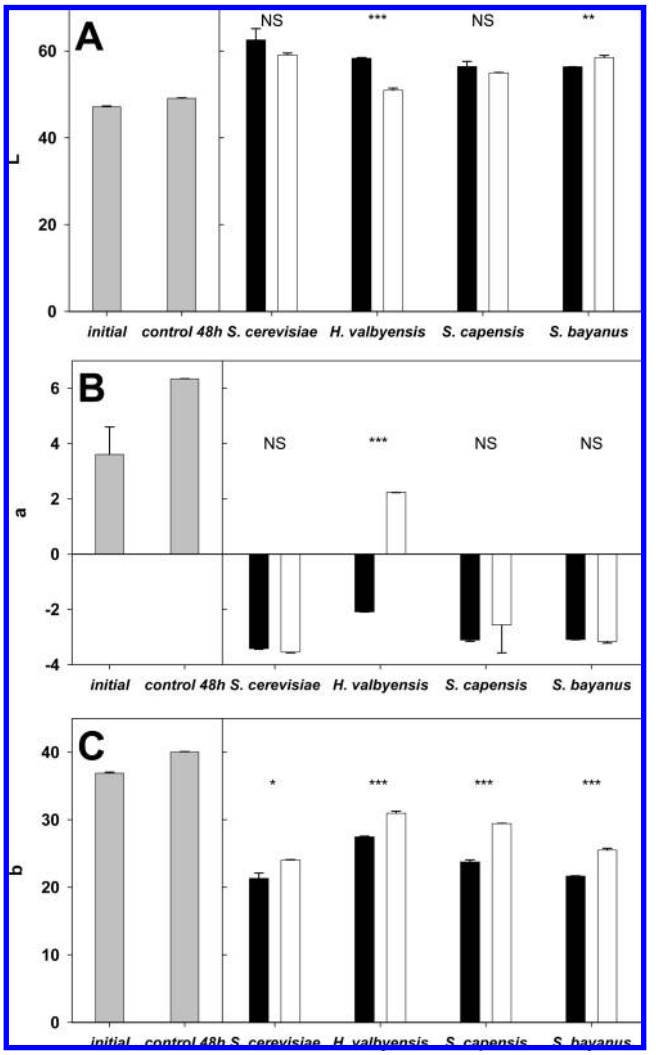

Figure 1. Effect of different yeast strains grown in aerobiosis (black columns) or anaerobiosis (white columns) at a final concentration of 24 g (dw) L^{-1} on the CIE-Laboratory tristimulus parameters (**A**, *L*; **B**, *a*; **C**, *b*) of acetaldehyde/(+)-catechin model solution [10% (v/v) ethanol, pH 3.2] after 48 h of incubation at 20 °C. Values of control solutions are given in gray columns. Mean and standard deviation of triplicates are represented. NS, nonsignificant; ∗, significant at $P \leq 0.05$; ∗∗∗, significant at $P \leq 0.001$, as tested by nonparametric Tukey statistical test.

or C8, yielding four products with C6−C6, C8−C8, and C6−C8 (*R* and *S*) bonds. Over these four different dimers, only three were clearly resolved (D1, D2, and D3), as previously published (*42*). In addition to these simple dimers, various oligomeric bridged compounds could be detected with similar retention times and not resolved. These peaks, which comprise all of the yellowish polymeric compounds (mainly oligomers containing two or three elementary catechin units) responsible for browning of this model solution, were then considered as a group and named HP (highly polymerized) materials (*12*).

As summarized in **Figure 2**, all of the yeasts tested showed a clear inhibitory effect on the formation of reaction polymers, which can thus delay the browning process. This reduction of the formation of polymerized compounds is particularly sensitive for highly polymerized oligomers and D1 and D2 dimers. As seen previously, the inhibitory effect seemed to be more important when yeasts were grown in aerobiosis. Moreover, with the exception of *H. valbyensis*, which seems to be the most efficient yeast in terms of

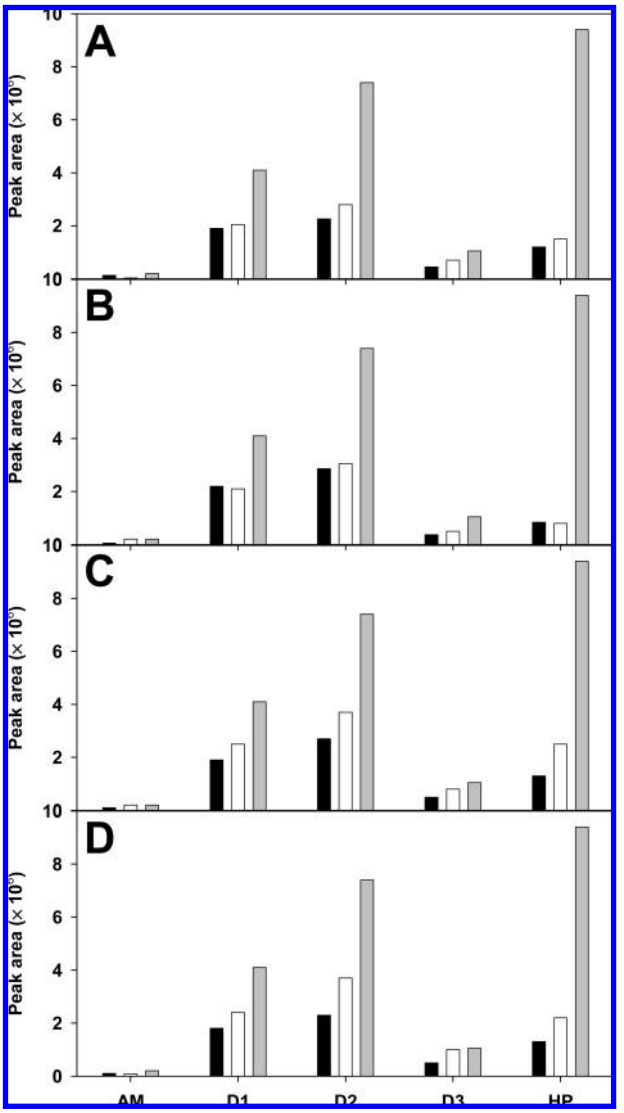

Figure 2. HPLC analysis of polymerized compounds in acetaldehyde/(+)-catechin model solutions [10% (v/v) ethanol, pH 3.2] after 48 h of incubation at 20 °C in the absence (gray columns) or in the presence of different yeast strains previously grown in aerobiosis (black columns) or anaerobiosis (white columns) at a final concentration of 24 g (dw) L^{-1}: (**A**) *S. cerevisiae* K1; (**B**) *H. valbyensis*; (**C**) *S. capensis*; (**D**) *S. uvarum*. AM, monomer adduct; D1, D2, D3, dimers; HP, highly polymerized oligomers.

browning inhibition, no differential effect could be observed between the other strains.

The effect of the addition of aerobically grown yeast (*S. cerevisiae* K1) at different times during the browning reaction was then assessed (**Figure 3**). This experiment confirms clearly that prevention of the browning reaction by yeast occurred by the preferential adsorption of D2 and D1 dimers and to a less extent of D3 dimer and monomers. On the contrary, final addition of yeast lees after 48 h of incubation led to a strong reduction of highly polymerized oligomers and D1 and D2 dimers.

Effect of Different Yeast Lees on the Color of Glyoxylic Acid/(+)-Catechin Model Solutions after 25 Days of Incubation. The second model browning reaction tested consists of a mixture of (+)-catechin and glyoxylic acid (oxidation product of tartaric acid) in the presence of oxygen. In this type of browning reaction, colorless derivatives were dimeric adducts consisting of two (+)-catechin units linked by carboxymethine bridge following the mechanism demonstrated in

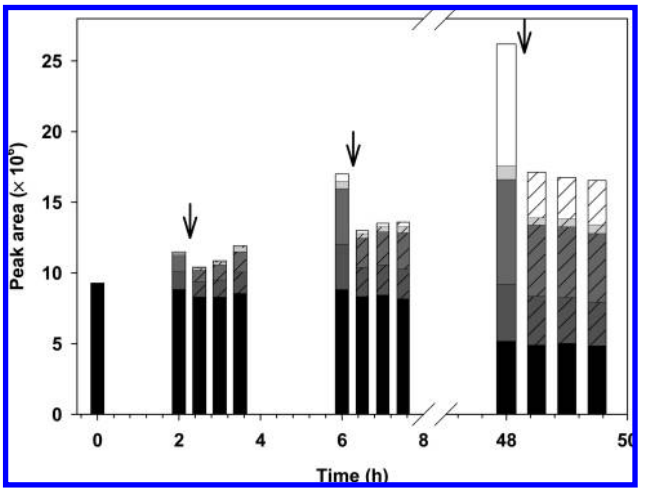

Figure 3. Effect of the addition of yeast lees (*S. cerevisiae* K1 previously grown in aerobiosis at a final concentration of 24 g (dw) L^{-1}) on the kinetics of production of polymerized compounds in acetaldehyde/(+)-catechin model solutions [10% (v/v) ethanol, pH 3.2] at 20 °C, without (plain columns) or with the addition of yeast lees (slashed columns). Yeast lees were added at 2, 6, or 48 h after the beginning of the reaction, as depicted by the black arrows. Black columns, monomers (peak area divided by 6); dark gray columns, dimer D1; gray columns, dimer D2; light gray columns, dimer D3; white columns, highly polymerized oligomers HP.

the case of acetaldehyde (*8*). In recent studies (*13*, *15*), some of the brown products were identified as esterified and nonesterified xanthylium salts arising from dehydration and oxidation of the colorless dimers. Two of the yellow pigments exhibiting absorption maximum at 440 and 460 nm were xanthylium salts obtained by evolution of the first formed colorless dimer. It was also shown that in contrast to acetaldehyde/(+)-catechin model reactions, where brown products were exclusively formed by condensation reactions, the glyoxylic acid/(+)-catechin browning reaction involves oxygen (*13*, *15*). In our reaction conditions, total oxygen consumption during the 25 days of browning reaction in the absence of added yeast was indeed about 5.08 mg L^{-1}. The protective effect of the different yeast strains was more marked, as revealed by the simple observation of the final samples (**Figure 4**). With the exception of *H. valbyensis*, all of the yeasts grown in aerobic conditions prevented browning more efficiently than yeasts grown in anaerobic conditions. This effect was very significant with aerobically grown *S. cerevisiae* K1 and *S. uvarum* strains and to a lesser extent with anaerobically grown *S. uvarum*, which all kept the glyoxylic acid/(+)-catechin model solution almost completely light throughout the studied oxidation period. The corresponding differential absorption spectra of the final model solutions (in comparison with the control oxidized model solution without yeast treatment) are given in **Figure 5A**. These spectra clearly exhibited the increase of absorbance in the 350 and 440−460 nm spectral regions in the oxidized model solutions. The reflectance spectra of the corresponding lyophilized lees after 25 days of incubation at 20 °C contact in the glyoxylic acid/(+)-catechin model solution are shown in **Figure 5B**. With the exception of anaerobically grown *H. valbyensis*, which behaves differently from all of the other yeasts tested, when the yeast is the most efficient for preventing browning reaction, yeast coloration is the lowest (**Figure 5**). Such behavior indicates a clearly preventive effect of these yeasts on the early formation of highly polymerized brown compounds. The case of anaerobically grown *H.*

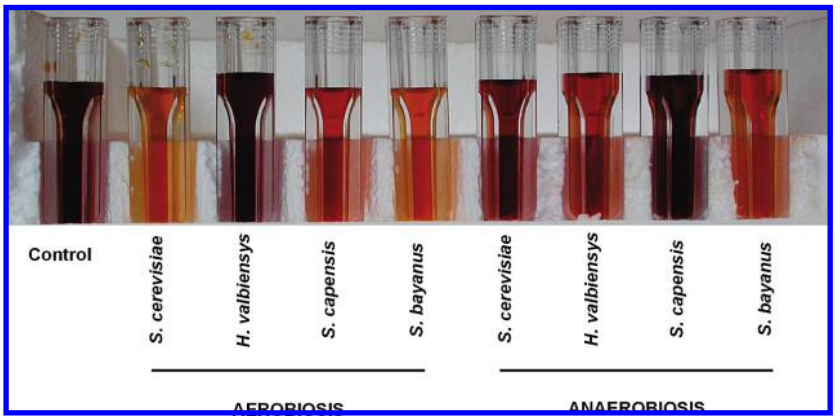

Figure 4. Color aspect of glyoxylic acid/(+)-catechin model solutions [14% (v/v) ethanol, pH 3.2] after 25 days of incubation at 20 °C in the absence (control) or in the presence of different yeasts previously grown in aerobiosis or anaerobiosis at a final concentration of 24 g (dw) L^{-1}.

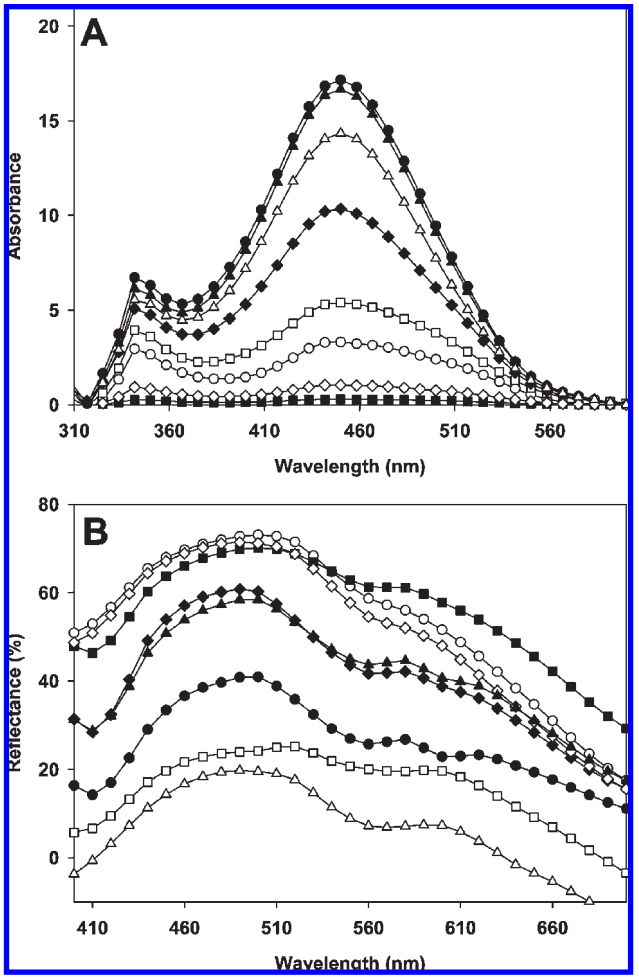

Figure 5. Differential absorbance spectra (**A**) of the resulting liquid media by comparison with the control (absence of yeast) and differential reflectance spectra (**B**) of the corresponding lyophilized lees by comparison with the control (absence of phenolic compounds) after 25 days of incubation at 20 °C in glyoxylic acid/(+)-catechin model solutions [14% (v/v) ethanol, pH 3.2] in the presence of different yeasts previously grown in aerobiosis (solid symbols) or anaerobiosis (open symbols) at a final concentration of 24 g (dw) L^{-1}: (●) *S. cerevisiae* K1; (■) *H. valbyensis*; (◆) *S. capensis*; (▲) *S. uvarum*.

valbyensis is rather different, because the poor prevention of the browning reaction is not accompanied by a coloration of the corresponding lees.

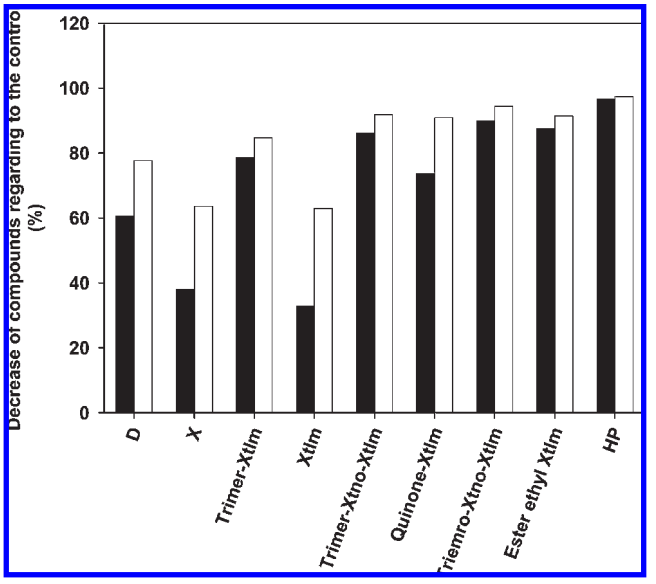

Figure 6. Decrease of polymerized compounds in glyoxylic/(+)-catechin model solutions [14% (v/v) ethanol, pH 3.2] as measured by HPLC analysis after 25 days of incubation at 20 °C by comparison with the control (absence of yeast) upon the treatment with *S. cerevisiae* K1 (black columns) and *S. uvarum* (white columns) previously grown in aerobiosis. D, all formed colorless dimers; Xtne, xanthen; Trimer-Xtlm, trimer-xanthylium; Xtlm, xanthylium; Trimer-Xtne-Xtlm, trimer-xanthen-xanthylium; Quinone-Xtlm, quinone-xanthylium; Ester-ethyl-Xtlm, ester ethyl xanthylium; HP, highly polymerized compounds.

Effect of Different Yeast Lees on the Formation of Polymerized Brown Compounds in Glyoxylic Acid/(+)-Catechin Model Solutions after 25 Days of Incubation. The different reaction compounds arising from oxidation were detected by HPLC. In this reaction, catechin dimer arises from a polycondensation mechanism involving glyoxylic acid and (+)-catechin, in an analogous way to that reported for acetaldehyde. The first formed colorless dimers may undergo dehydration to form xanthenes that subsequently oxidize in the presence of oxygen to form xanthylium cations (*14*−*16*,) (*41*). The xanthylium cations can lead to quinones and numerous other condensation products as well as esterified intermediates (*15*). Marked differences in the concentration of the different reaction products upon contact with yeast strains were observed (data not shown). This effect was significant with aerobically grown *S. cerevisiae* K1 and *S. uvarum* strains, where formation of highly polymerized

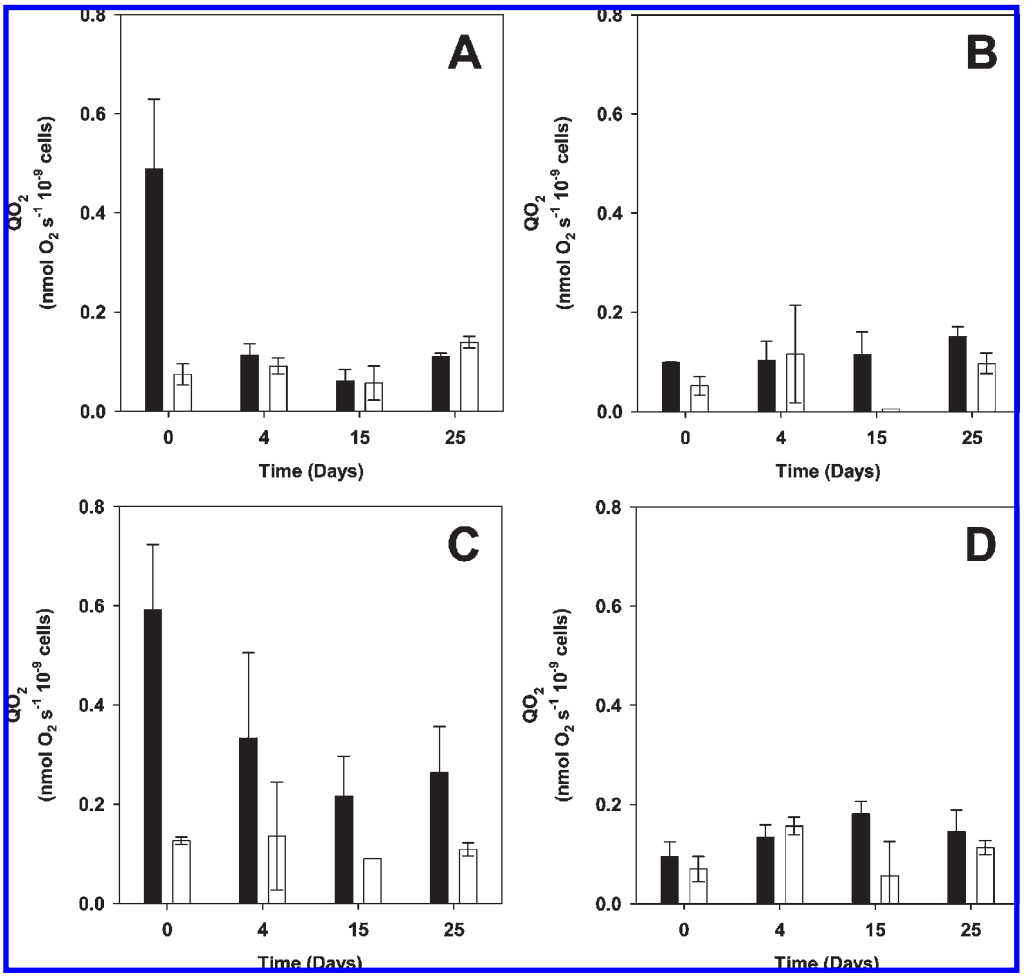

Figure 7. Specific oxygen consumption activities (nmol of O_2 s^{-1} 10^{-9} cells) of different yeast strains previously grown in aerobiosis (black columns) or anaerobiosis (white columns) at a final concentration of 24 g (dw) L^{-1} during 25 days of incubation at 20 °C in glyoxylic/(+)-catechin model solutions [14% (v/v) ethanol, pH 3.2]: (**A**) *S. cerevisiae* K1; (**B**) *H. valbyensis*; (**C**) *S. capensis*; (**D**) *S. uvarum*. Mean and standard deviation of triplicates are represented.

compounds (which are mainly responsible for color increase) was inhibited by more than 90% with regard to the control (absence of yeast) (**Figure 6**). This result confirms the previous results obtained from biomass reflectance spectra.

On the basis of the potential involvement of oxygen consumption by yeast lees as a competitor for brown polymeric compound formation, the oxygen consumption ability of the different yeast lees was measured in the reaction medium for 25 days (**Figure 7**). *S. cerevisiae* var. *capensis* grown under aerobic conditions exhibits the highest oxygen consumption ability throughout the 25 days of reaction, which can result in a delay of the oxidation process. However, this yeast did not exhibit the highest oxidation protection capacity (**Figure 5**). On the other hand, all of the other yeasts tested did not exhibit clear different oxygen consumption abilities regardless of their growth conditions, whereas they prevented browning with very different efficiencies (**Figures 5** and **6**). Therefore, it can be assumed that the capacity of yeast lees to consume oxygen is not linked to their capacity to prevent the formation of polymerized brown compounds in glyoxylic acid/(+)-catechin model solutions after 25 days of incubation.

Are the Sterol Contents of the Different Tested Yeast Lees Responsible for the Differences Observed? In the present work, we use different yeast strains and growth conditions (aerobiosis and anaerobiosis) to affect both the quantity and the quality of membrane sterols of the resulting yeasts. Analysis of the composition of the total unsaponifiable fraction of yeast lees (squalene and sterols) is given in **Table 1**. Yeast growth under aerobic condition normally led to an almost complete transformation of squalene into ergosterol, through the accumulation of some intermediate precursors such as lanosterol and various ergosta-5,7-dienols. On the other hand, yeast growth in the absence of oxygen led mainly to the accumulation of large amounts of squalene with a few sterols being detected (*43*−*45*). Such behavior was observed for all of the yeasts tested with the exception of *S. uvarum*, which exhibited a rather important transformation of squalene into ergosterol despite the absence of oxygen. It is noteworthy that the differences of color of the model reaction solutions after prolonged contact with this yeast species (**Figures 4** and **5**) grown either in aerobiosis or in aerobiosis are lower than those observed with the other yeast species. On the basis of this observation, we could hypothesize that the main differences observed between yeast grown under aerobic and anaerobic conditions with respect to their ability to adsorb polymerized phenolic compounds could result from the involvement of membrane sterols in this phenomenon.

To ascertain a direct interaction between the main yeast membrane sterol—ergosterol—and the polymerized phenolic compounds, a preliminary study was carried out. The effect of ergosterol in a solubilized form on a model solution of (+)-catechin/acetaldehyde was checked. After 90 min of

Table 1. Squalene and Sterol Contents of the Different Tested Yeast Lees as a Percentage of the Total Compounds Detected (Sterols and Squalene)[a]

	S. cerevisiae		*H. valbyensis*		*S. capensis*		*S. uvarum*	
compound	+ O_2	− O_2	+ O_2	− O_2	+ O_2	− O_2	+ O_2	− O_2
squalene	nd	88.35	nd	94.02	nd	95.56	1.06	6.48
zymosterol	4.47	0	7.14	nd	8.2	nd	1.83	nd
ergosterol	54.21	11.65	89.73	3.04	87.61	2.56	97.1	93.52
ergostratetraenol	14.22	nd	3.13	0	4.19	nd	nd	nd
ergosta-9-11-en-3-ol	2.79	nd	nd	nd	nd	nd	nd	nd
ergosterol	1.86	nd	nd	nd	nd	nd	nd	nd
methyl cholesten-3-14,24-dien-3-ol	3.86	nd	nd	nd	nd	nd	nd	nd
stigmasterol	10.36	nd	nd	nd	nd	nd	nd	nd
lanosterol	1.74	nd	nd	2.94	nd	1.88	nd	nd

[a] + O_2, aerobiosis; − O_2, anaerobiosis; nd, not detectable.

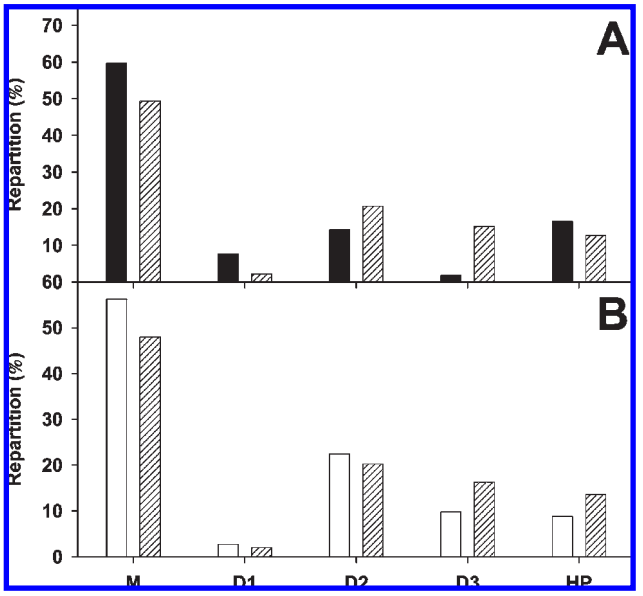

Figure 8. Repartition of the phenolic compounds (percent) as measured by HPLC found in acetaldehyde/(+)-catechin model solution [10% (v/v) ethanol, pH 3.2] after 48 h of incubation at 20 °C in the presence (**A**, slashed columns) or in the absence (**A**, black columns) of 0.01 mg mL^{-1} ergosterol solubilized in Tween-80/ethanol (50:50, v/v). Fine repartition between the white (white columns) and the brownish (slashed columns) phases are given in **B**. AM, monomer adduct; D1, D2, D3, dimers; HP, highly polymerized oligomers.

incubation, two different colored phases appeared (upper white and lower brownish, respectively). After 48 h of incubation, these two phases were completely resolved by high-speed centrifugation and analyzed separately by HPLC (**Figure 8**). In the two phases, intermediate polymerized reaction compounds were detected (**Figure 8A**). However, an unusual distribution of the reaction compounds in comparison with the control [acetaldehyde/(+)-catechin model solution after 48 h of incubation] was observed in both phases: dimers 2 and 3 were present in higher percentage (**Figure 8**). Such an observation corresponds to results previously obtained with yeast lees (**Figure 3**). A large amount of highly polymerized compounds (HP) was detected in the brownish phase. Such compounds are mainly responsible for the browning coloration in this acetaldehyde/(+)-catechin reaction (*11*) and may explain the color differences observed between the two recovered phases.

DISCUSSION

Contrary to white table wines, pale sherry wines exhibit a very special resistance to browning during biological aging, a property that disappears as soon as the flor yeasts growing on its surface are removed. The absence of browning in this type of wine has been ascribed to a protective effect of the wine against browning by these yeasts (*18*, *19*). Although flor yeasts differ notably from fermentative yeasts in their oxidative metabolism, no scientific work was dedicated to the evaluation of yeast growth conditions (aerobiosis or anaerobiosis) on their ability to prevent wine browning. In the present study, different capacities for preventing the browning of wine in simulated solutions were observed between different yeast species grown under aerobiosis or anaerobiosis.

The differences obtained between aerobically and anaerobically grown yeasts in acetaldehyde/catechin model solutions were not so marked as in the case of glyoxylic/catechin model solutions. Two hypotheses could be developed to explain such results.

A first hypothesis relies on the involvement of oxygen consumption by yeast lees as a competitor for brown polymeric compound formation in the case of glyoxylic acid/(+)-catechin model solutions, where oxygen plays an important active role in the oxidation reaction. In that way, differences in the oxygen consumption capabilities between the tested yeasts could explain the differences observed in the colors reached at the end of incubation. The obtained results show that no direct relationship could be observed between the oxygen consumption capabilities and the inhibition of browning by the tested yeast lees.

A second hypothesis relies on the fact that an observed lowering of browning could be attributed to a larger absorption of brown compounds by some yeasts. However, with the two model solutions, the colors of the model solutions are clearly not linked to the yeast biomass color after contact. As a matter of fact, an inhibition of the formation of highly polymerized compounds was observed when inhibition of browning by the tested yeast lees is observed. In the present work, we show that such an inhibition of browning by the tested yeast lees could be attributed to a focused adsorption of the colorless intermediate compounds, mainly (+)-catechin and primary dimers, in the case of acetaldehyde/catechin model solutions. Because the role of these colorless compounds as intermediates in the reaction pathways leading to brown pigments has been previously demonstrated (*11*, *14*, *15*), the observed inhibition of browning could be attributed to the strong decrease of these compounds, which are indeed necessary to develop the polymerization reactions.

However, it is also important to note that the threshold for exclusion of the *S. cerevisiae* cell wall (its "porosity") was estimated between 620 and 780 (*46*) up to 4500 (*47*). It is therefore expected that phenolic monomers, dimers, and trimers could easily enter the periplasmic space through

the cell wall, whereas this is not the case for the majority of the highly polymerized components. However, the yeast cell wall is plastic in many ways (*48*): it is different among yeast strains, it is modified during cell growth, and it is modified after cell death. For all of these reasons, it is definitely difficult to draw any conclusions on the role of the yeast cell wall as a "filter" between the different protagonists of the browning reaction.

Moreover, this work raised new questions on the nature of the component of the yeast lees responsible for the strong adsorption of the colorless intermediate compounds. In a preliminary study, we checked the potential direct interaction between ergosterol (the main yeast membrane sterol) in a solubilized form and these compounds. We proved that membrane sterols could be involved in the yeast's ability to adsorb brown phenolic compounds, as already hypothesized previously (*24*, *25*). On this basis, further studies dedicated to checking the reactivity of different sterols trapped in artificial membranes against brown phenolic compounds are already in progress.

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