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Sorption of wine volatile phenols by yeast lees

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Abstract

The capacity of *Saccharomyces cerevisiae* yeast lees to sorb 4-ethylguaiacol and 4-ethylphenol was investigated in a synthetic medium and in wine. Active dried yeast was more effective when volatile phenols were diluted in red wine. Partition coefficients between wine model solution and wine yeast lees were determined and compared with those measured for dried active yeast. They showed a larger affinity of volatile phenols for wine yeast lees than for dried active yeast. The effect of yeast lees on volatile phenol sorption was sensitive to yeast autolysis level and to physicochemical parameters, such as ethanol content, temperature and pH. These results could be applied in the technology of reduction of organoleptic defects in wine due to phenols.

Keywords: Wine; Yeast lees; 4-Ethylguaiacol; 4-Ethylphenol; Sorption

1. Introduction

Volatile phenols in wine, 4-ethylphenol and 4-ethylguaiacol, are produced by a contaminant yeast *Brettanomyces/Dekkera* from grape-derived phenolic acids (Chatonnet, Dubourdieu, Boidron, & Pons, 1992). The aroma associated with these phenols in wine has been generally described as horsy, medicinal and spicy (Chatonnet, Boidron, & Pons, 1990). Volatile phenols are present at different concentrations in wine with trace quantities upto 4500 μg/l (Pollnitz, Pardon, & Sefton, 2000). At higher concentrations, these volatiles can be the cause of organoleptic defects, particularly with 4-ethylphenol. Recently, decreases of 4-ethylphenol and 4-ethylguaiacol contents were found in red wine containing yeast lees compared to the same wine aged without lees (Guilloux-Benatier, Chassagne, Alexandre, Charpentier, & Feuillat, 2001). It was also demonstrated that yeast lees are able to sorb organic compounds found in wine, such as sulfur products (Lavigne & Dubourdieu, 1996; Palacios, Vasserot, & Maujean, 1997; Vasserot, Steinmetz, & Jeandet, 2003) and anthocyanin (Vasserot, Caillet, & Maujean, 1997). Razmkhab et al. (2002) showed that active dried yeast cells might be used as fining agents to decrease compounds responsible for browning in white wine. Thus, there is an interest in utilizing yeast as a biosorbent, for the removal of undesirable molecules from wine. Wine yeast is an inexpensive and easily available source of biomass. For phenol compounds, mainly the removal capacity of fungal biomass has been studied. Several fungi were able to reduce phenol concentration of olive mill wastewater (Garcia Garcia et al., 2000). Rao and Viraraghavan (2002) reported

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that *Aspergillus niger* might sorb upto 66% of phenol from an aqueous solution. The term biosorption, sometimes referred to as physical adsorption, describes the ability of inactive, alive or dead biomass to bind to molecules present in dilute solutions. Under dilute conditions, sorption of volatile might be characterized by using a single parameter, the mass partition coefficient, K_{mass}, defined as the ratio between the solid and liquid phase concentration at sorption equilibrium.

The main purpose of this study was to examine the volatile phenol removal capacity of wine-producing *Saccharomyces cerevisiae* biomass from a hydroalcoholic solution and wine containing 4-ethylguaiacol and 4-ethylphenol at concentrations of 500 and 1000 μg/l, respectively. Different types of yeast lees were studied for their volatile phenols sorption capacity. A screening factorial experimental design was performed to investigate the influence, on sorption, of parameters such as ethanol content, temperature and pH.

2. Materials and methods

2.1. Chemicals

4-Ethylphenol and 4-ethylguaiacol were from TCI-EP (Tokyo, Japan) and glycerol (99%) from Merck (Darmstadt, Germany). Ethanol, 99.9% purity, tartaric acid (99.5%), acetic acid (>99.8%), citric acid (99.5%), potassium sulphate (99%), trifluoroacetic acid (99.5%), glucose (99.5%), fructose (99.0%), asparagin (>99%), magnesium sulphate (99%), monopotassium phosphate (99.5%), myo-inositol, manganese sulphate (>99%), ammonium chloride (>99%), biotin (99%), thiamin (99.5%), pyridoxin (98%), panthothenic acid (98%), nicotinic acid (99.5%) and 3-aminobenzoic acid (99%) were obtained from Merck (Darmstadt, Germany). 3,4-Dimethylphenol (99.8%), D–L malic acid (>99%) and anhydrous sodium carbonate (>99%) were obtained from Sigma (St. Louis, MO, USA). Solutions were made up with ultra pure water, obtained from a Milli-Q system (Millipore, Bedford, MA). Dichloromethane (>99.5%) and diethyl ether (99.8%), obtained from Carlo Erba Reactives (Rodano, Italy), were used as solvents for extraction.

2.2. Yeast and wine

A commercial active dry yeast, *S. cerevisiae* (Levuline BRG™) was provided by Oenofrance (Rueil-Malmaison, France). Yeast cells were rehydrated according to the manufacturer's instructions. White wine (ethanol 12% (v/v), pH 3.05) and red wine (ethanol 13% (v/v), pH 3.4) were obtained from *Chardonnay* grapes and *Pinot noir* grapes, respectively, collected in 1998 at the vineyard of the experimental station of the University of Burgundy.

2.3. Fermentation and autolysis conditions

After rehydration, yeast cells were harvested by centrifugation at 4600*g* for 10 min at 10 °C and washed three times with 0.9% NaCl. Then, the washed yeast cells were suspended in a model synthetic grape juice medium to a final concentration of 4×10^6 cells/ml. The model grape juice medium was composed as follows: glucose (85 g/l), fructose (85 g/l), tartaric acid (3 g/l), D–L malic acid (6 g/l), citric acid (0.3 g/l), asparagin (2 g/l), monopotassium phosphate (2 g/l), ammonium chloride (2 g/l), magnesium sulfate heptahydrate (0.2 g/l), manganese sulfate monohydrate (0.01 g/l), myo-inositol (0.3 g/l), biotin (0.04 mg/l), thiamin (1 mg/l), pyridoxin (1 mg/l), panthothenic acid (1 mg/l), nicotinic acid (1 mg/l), 3-aminobenzoic acid (1 mg/l). The pH was adjusted to 3.5 with 5 M potassium hydroxide. After flash sterilization (105 °C for 1 min), 10 ml, containing the 10× concentrated vitamins solution, was added after filtering through a sterilized 0.2 μm membrane (Sartorius, Goettingen, Germany). The alcoholic fermentation was conducted in 2×4 l of medium in 5 l conical flasks at 20 °C. After fermentation was complete, yeast cells were harvested by centrifugation at 4600*g* for 10 min at 10 °C and washed three times with 0.9% NaCl to be available for sorption measurements or were added in model wine for autolysis.

Yeast biomass samples, either with active dried yeast or with cells from synthetic fermented medium, were suspended in a model wine buffer in 1 l bottles with 15 g of fresh yeast weight. The model wine buffer contained ethanol (12%, v/v), D–L malic acid (3 g/l), acetic acid (0.1 g/l), potassium sulphate (0.1 g/l), and magnesium sulphate heptahydrate (0.025 g/l). The pH was adjusted to 3.5 with 5 M potassium hydroxide. Autolysis was performed without stirring at 30 °C for 5 days (Guilloux-Benatier & Chassagne, 2003). At the end of autolysis, yeast cells were harvested by centrifugation at 4600*g* for 10 min at 10 °C.

Chardonnay and *Pinot noir* yeast lees were obtained from *Chardonnay* and *Pinot noir* musts, respectively, and inoculated with *S. cerevisiae* levuline BRG (Oenofrance, Rueil Malmaison, France). They were harvested at the end of alcoholic fermentation by centrifugation at 4600*g* at 10 °C for 10 min.

2.4. Dried weight determination

Cell dry weight was determined in triplicate by filtering 5 ml fermentation medium on pre-weighed filters (0.45 μm, Millipore Corporation, Bedford, USA). Fil-

ters were placed in tared aluminium pans, dried for 24 h at 100 °C.

2.5. Sorption measurements

Experiments were conducted by contacting yeast lees with wine or model wine (previously described in paragraph 2.3) in 120 ml capacity bottles. 4-Ethylphenol and 4-ethylguaiacol in mixture were added to the model wine and wine at concentrations of 1000 and 500 μg/l, respectively, before being tightly stoppered with teflon caps to prevent losses of the volatile phenols. Experimental samples and control samples were stored at 15 °C in a dark room without stirring. The sorbed amount of volatile phenols was calculated from the difference in concentration between control samples and experimental samples. The level of sorption was also expressed in percent of sorbed amount compared to initial amount. For each independent experiment, volatile phenol analysis was repeated in duplicate.

2.6. Volatile phenol analysis

Samples were centrifuged at 4600*g* at 10 °C for 10 min to remove yeast. The extraction and GC analysis of the liquid phase were carried out as described previously by Chatonnet and Boidron (1988). The chromatograph used was a Chrompack CP 9001 fitted with a split injector (1/10), a flame ionization detector and an HP-Wax fused silica capillary column (30 m×0.25 mm i.d.; 0.25 μm bonded phase, Hewlett–Packard, Palo Alto, CA, USA). The column temperature was programmed at 3 °C/min from 40 to 200 °C/min. The flow rate was 2 ml/min of N_2 for the carrier gas, and 30 ml/min of H_2 and 300 ml/min of air for the detector gases. The detector and injector temperatures were maintained at 200 °C. Data acquisition and treatment were carried out using Winilab II software (version 2.06E, Perichrom Inc., Paris, France).

2.7. Experimental design

To better understand the effects of enological variables on volatile phenol sorption, a two level four factor design, following the Hadamard matrix, was adopted. The independent variables were autolysis, ethanol content, temperature and pH. The autolysis factor was related to two conditions in which the yeast was used, before or after autolysis, consequently alive or dead. The experimental levels selected for ethanol content, pH and temperature were based upon realistic limits for enological conditions in the cellar (Table 1). Two replicates for every level were performed. The statistical package used was NEMROD–W software (version 9901, LPRAI, Marseille, France).

Table 1
Coding of levels of independent variables used

Independent variable	−1	+1
Yeast	Not autolysed	Autolysed
Ethanol content (% v/v)	10	15
Temperature (°C)	10	15
pH	3	4

3. Results and discussion

3.1. Kinetics of the sorption

The kinetics of sorption to establish the equilibration time for the sorption of 4-ethylphenol and 4-ethylguaiacol were conducted in model wine with rehydrated active dried yeast (Fig. 1). More than 90% of the final sorption was reached within the first 2 h. Sorption studies were carried out for 62 h in order to determine the effect of time on sorption. No other significant losses were observed during the kinetics, indicating that, under our conditions, neither chemical nor biological degradation occurred. The equilibrium time required for the sorption was almost 3 h. *S. cerevisiae*, used as active dried yeast was able to decrease both volatile phenol concentrations in similar ratios. About 26% of total 4-ethylguaiacol and 33% of 4-ethylphenol were removed under these conditions. It is worth noting from this illustration that the sorption process is very fast, indicating that the main regime is probably a rapid attachment of molecules to the surface of the yeast (Fig. 1). Vasserot et al. (1997) also reported a rapid process of anthocyanin sorption by yeast lees. The second regime observed, less representative, is slower which is probably due to a diffusion-limited phenomenon at the interface. Intracellular diffusion is involved in the sorption process, but it is not the controlling step. Similar kinetics were obtained by Aksu and Dönmez (2003) for several yeasts being used as sorbents and a variety of organic compounds. This suggests that, for yeast cells, the uptake of hydrophobic solutes, such as 4-ethylguaiacol and 4-ethylphenol, occurs predominantly by surface binding and that available sites on the sorbent could be the limiting factor for sorption.

3.2. Effect of biomass concentration

Various amount of biomass were put into contact with fixed initial volatile concentration in model wine solution and in a *Pinot noir* wine. At steady state, the quantities of sorbed phenols were calculated for both volatiles and the results are depicted in Fig. 2. For the two liquid phases used, an increase in the sorbent concentration caused a rise in the sorbed amount, with similar curve shapes for both volatiles. However, the level of sorption seemed to be significantly higher when volatiles

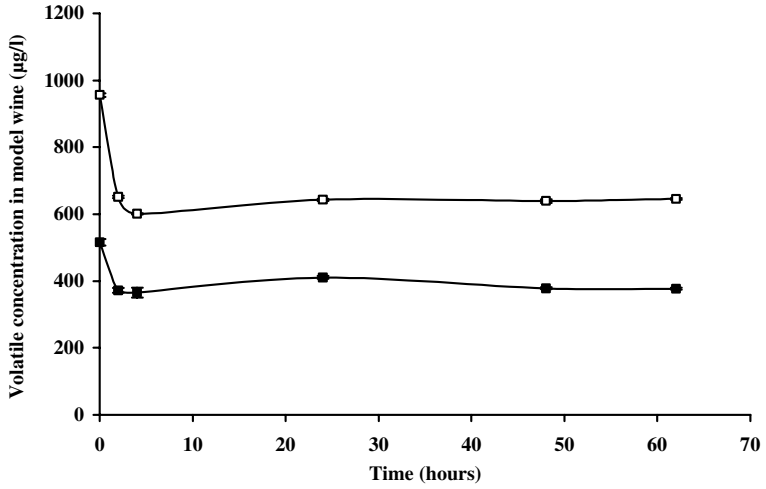

Fig. 1. Kinetics of sorption of 4-ethylguaiacol (■) and 4-ethylphenol (□) by *S. cerevisiae* active dried yeast biomass (dried weight 32 g/l) at 15 °C.

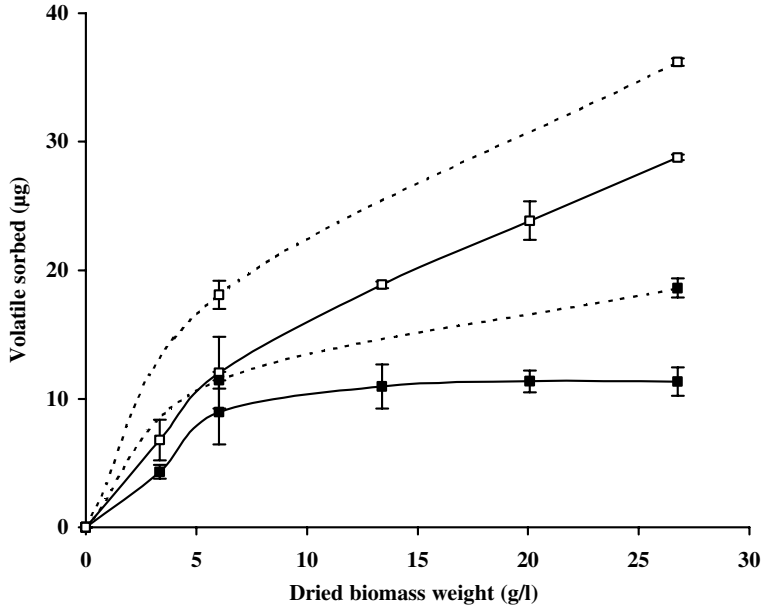

Fig. 2. Effect of active dried yeast biomass concentration on sorption of 4-ethylguaiacol (■) and 4-ethylphenol (□) from model wine solution (—) and *Pinot noir* wine (- - -) at 15 °C.

were diluted in a *Pinot noir* wine. The results obtained for the model wine were fitted by linear regression. Significant correlation ($p < 0.01$) was found only for 4-ethylphenol. The higher retention of 4-ethylphenol in the model wine at greater biomass concentration would imply a specificity of these yeast cells for this molecule. Upto 5 g/l biomass, sorbent affinity for 4-ethylguaiacol seemed to decrease in the model condition, since sorbed quantity did not change. However, this result, obtained for 4-ethylguaiacol, was not clearly found when *Pinot noir* wine was used, suggesting that physicochemical parameters of this volatile, such as solubility, might be different in this medium. According to Ramirez-Ramirez, Chassagne, Feuillat, Voilley, and Charpentier (2004), wine constituents significantly affect the partitioning of hydrophobic volatiles between liquid phase and sorbent. Overall, the residual volatile phenol concentration decreases as a function of the yeast biomass concentration. The same trend was obtained for wine anthocyanins by Vasserot et al. (1997) and for browning products in white wines by Razmkhab et al. (2002).

3.3. Effects of different yeast lees

Different types of yeast lees (from active dried yeast, synthetic medium fermented, and *Chardonnay* and *Pinot noir* yeast lees) were contacted with a fixed phenol concentration. At equilibrium, it was possible

Table 2
Partition coefficient of 4-ethylphenol and 4-ethylguaiacol for different sorbents at volatile concentrations of 1000 and 500 μg/l, respectively, in model wine at 15 °C

Type of yeast lees	Dried yeast lees weight (g/l)	K_{mass}	
		4-Ethylguaiacol	4-Ethylphenol
Active dried yeast	6.02	27.3 ± 3.5	18.9 ± 4.9
Synthetic medium fermented yeast	6.72	27.3 ± 5.6	32.7 ± 0.5
Chardonnay yeast lees	5.08	50.6 ± 8.3	45.3 ± 9.6
Pinot noir yeast lees	4.80	87 ± 27	909 ± 54

K_{mass} ± standard deviations are average of the three repetitions.

to calculate the partition coefficient of phenol between model solution or wine and yeast. On a mass fraction basis, this partition coefficient could be written: $K_{mass}=C_{yeast}/C_{liquid}$, where C_{yeast} is the concentration of volatile phenols inside the yeast, and C_{liquid} is the concentration in model solution or wine. The results are listed in Table 2. No significant differences ($p<0.05$) were found by ANOVA test for K_{mass} obtained between active dried yeast and synthetic medium fermented yeast. These results reveal that sorption capacity of yeast is not modified by the fermentation process. On the other hand, yeast lees from wine showed more affinity for volatile phenol, more particularly for 4-ethylphenol when *Pinot noir* lees were used, its distribution coefficient was 10 times greater than that of 4-ethylguaiacol. As demonstrated successively by Vasserot et al. (1997) and Razmkhab et al. (2002), red wine lees retained anthocyanins and flavan-3-ol derivatives during fermentation. Sorption, inside the cell surface, of these molecules might increase the yeast sorption capacity. Considering the low polarity of 4-ethylguaiacol and 4-ethylphenol (log $P=3.04 \pm 0.47$ and 2.55 ± 0.47, respectively, values estimated by Crippen's fragmentation), and competition effects, interactions other than hydrophobic were participating in driving the red wine constituents sorption process. In other words, the increase in volatile phenols sorption is assumed to be the result of wine compound sorption by yeast that would increase the number of sorption sites.

3.4. Effects of pH, temperature, ethanol and autolysis

Using an experimental design, we studied the effects of several factors on the uptake of volatile phenols by *S. cerevisiae* yeast. These included physicochemical parameters, such as temperature, pH and ethanol content and the physiological state of the cells, autolysed or not. It is well known that the autolytic state is another parameter that influences the sorption process (Ramirez-Ramirez et al., 2004) and the interaction between wine constituents (Lubbers, Charpentier, Feuillat, & Voilley, 1994).

A full two-level factorial design (using duplicated experiments) was applied to check the influence of the four above mentioned factors and to estimate the magnitude of their effects in the appropriate experimental domain specified in Table 1.

The results obtained are depicted in the Pareto diagram (Fig. 3). This chart shows that the autolysis state plays a positive role in the sorption process, but the pH, the temperature and the ethanol content have a negative effect when their values increase. Furthermore, it can also be observed that the effect of each factor has an absolute value larger than the confidence interval for a significance level of 0.05. This fact illustrates that the effect of each of the four experimental parameters checked is significant; thus the four experimental parameters must be taken into account for the sorption process. Autolysed yeast biomass demonstrated higher volatile phenol uptake than non-autolysed yeast. This

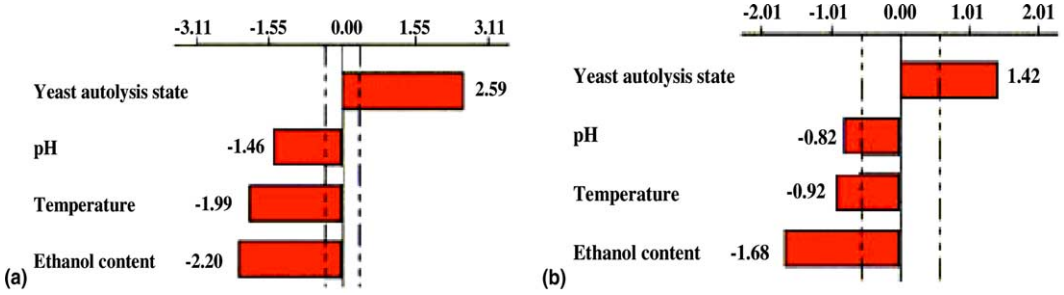

Fig. 3. Pareto diagram showing the magnitude of the effects of four factors on sorption of 4-ethylguaiacol (a) and 4-ethylphenol (b) by yeast. The *x*-axis shows the absolute magnitude of the effect of each factor determined by the statistical analysis and the *y*-axis shows the different factors. (The confidence interval is denoted by a dotted line).

could be due to the release of a large number of intracellular compounds (Guilloux-Benatier & Chassagne, 2003), especially cell wall macromolecules which have been shown to retain volatiles (Lubbers et al., 1994). Among the physicochemical factors studied, ethanol content had the highest negative effect. The increase of ethanol concentration tends to raise volatile solubility in the liquid phase (Chassagne et al., 2003), and thus could contribute to the decrease of the sorption by yeast. An explanation for the decrease in the sorption of the volatile phenols when temperature and pH increase may be found in the presence of polar bindings in addition to hydrophobic interactions between volatile phenols and yeast surface. With an increase in pH, the degree of ionization of negative groups present on the biomass would increase.

4. Conclusion

Contamination of wine by *Brettanomyces* yeast is not unusual and could lead to excessive volatile phenol levels, especially in red wine. The sorption of wine volatile phenols by yeast biomass was studied in simple and realistic systems. We have shown that yeast lees were effective in removal of 4-ethylguaiacol and 4-ethylphenol. The presence of other wine constituents sorbed by yeast influenced the sorption of both volatile phenols, with a greater effect in the case of 4-ethylphenol. Autolysis state of the yeast and low ethanol content increase the sorption level of volatile phenol by yeast lees. Thus, yeast lees provide a cost-effective and efficient approach to remove or to decrease organoleptic defects in wine due to phenols.

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