Influence of Yeast Walls on the Behavior of Aroma Compounds in a Model Wine

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The capacity of yeast cell walls to bind volatile compounds was investigated in a model hydroalcoholic system. The interactions between five aroma substances and yeast walls in a model wine were shown using the head space technique and the equilibrium dialysis method. The effect of cell walls on the volatility of aroma depended on the physico-chemical nature of volatile compounds. Yeast walls led to a decrease in vapor phase concentration of all aroma substances. A greater degree of binding was noted for the more hydrophobic volatile compounds: ethyl octanoate and ß-ionone. Binding capacity of yeast cell walls was partly explained by lipid matter and the insolubility of walls in model wine.

KEY WORDS: yeast walls, aroma compounds, model wine, headspace, equilibrium dialysis method

Loss of aroma compounds during winemaking has several causes: the CO_2 produced, hence the entrainment of alcohols and esters; protein stabilization treatments with fining or ultrafiltration lead to significant loss of aroma compounds (13). Yeast walls were also assumed to bind aroma substances and contribute to loss.

Few studies have shown the capacity of yeast walls to bind volatile compounds in an aqueous medium. The enhancing effect of yeast walls on sluggish or stuck wine fermentation was explained by the adsorption of toxic fatty acids present in the growth medium (10,12). The binding of alcoholic esters in artificial wine in presence of yeast walls was shown by Geneix (7). In this experiment, 1 g/L of yeast walls was added to a volatile compounds solution. After a contact of 24 hours without stirring, the concentration of aroma compounds in the supernatant decreased by 78% for ethyl hexanoate and remained at zero for ethyl octanoate and ethyl decanoate. Voilley et al (22) also observed, with another technique, the binding of volatile compounds on a yeast walls-bentonite mixture in a fining experiment. The binding was higher for ß-ionone (about 30%) and lower for the three other volatile compounds (n-hexanol, ethyl hexanoate, isoamyl acetate).

The increasing interest in the use of yeast walls in winemaking (treatment of stuck fermentation, solid support effect for yeast) requires an understanding of the interactions between aroma and yeast walls in wine.

The purpose of this work was to use an artificial model wine to investigate, more theoretically, the interactions between yeast cell walls (winemaking use) and volatile compounds. A suitable method to study these interactions was the headspace technique which allows the determination of the partition coefficient (K) of aroma in the system. The equilibrium dialysis method was used to quantify the level of binding of aroma on yeast walls. These two methods are based on thermodynamic equilibria: liquid/vapor for the headspace analysis and liquid/liquid for the equilibrium dialysis method.

Materials and Methods

Model wine: The artificial wine was composed of an aqueous solution with ethanol, organic acids, and salts (Table 1). The pH of the wine was adjusted to 3.5 with 1 M NaOH.

Table 1. Composition of model wine.

Compound	Origin	Mass concentration (%)
Ethanol	Merck	10.0
Malic acid	Merck	0.3
Acetic acid	Merck	0.01
Potassium sulfate	Merck	0.01
Magnesium sulfate	Merck	0.025

Substrates: The yeast walls for winemaking use are produced by Fould Springer (F 94701 Maisons-Alfort) (Table 2). They were added at 1 and 10 g/L in artificial wine. The yeast walls free of lipids were prepared by extraction of lipids with chloroform/methanol 2:1 (v/v). One gram of walls were shaken two hours (3 times) with 30 mL of the chloroform/methanol mixture and were dried. The chloroform/methanol phase was reduced at 10 mL and was mixed with 2.5 mL of KCl 0.8 M. After 12 hours at -20°C, the aqueous phase was eliminated. The chloroform phase was reduced under a stream of nitrogen gas and the lipid extract was weighted (18).

Volatile compounds: Five aroma compounds usu-

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Table 2. Composition of yeast walls given by Fould Springer.

Compound	Concentration (g to 100 g)
Proteins (N_2 X 6.25)	18
Lipid matter	18
Mineral matter	4
No-nitrogen extract	55
soluble carbohydrates in CCl_3COOH	6
Mannane	8
Glycogen	11
Glucane	30
Dry extract	95

ally found in Burgundy wine were selected: isoamyl alcohol, octanal, ethyl hexanoate, ethyl octanoate, and ß-ionone. The physico-chemical characteristics of volatile compounds and concentrations used are given in Table 3. These substances were separately studied in water and in model wine.

Headspace method: The headspace technique measures the influence of a substrate on the concentration of the volatile compound in the vapor at equilibrium for a given temperature. An inert gas (nitrogen) passed through the liquid phase (10 g - 20 g) solution containing the diluted volatile compound, at 25°C and a constant flow rate (5 - 10 mL/min); the nitrogen gas carried the volatile compound into the vapor phase. The concentration in the liquid phase was determined by injection of the liquid into a gas-liquid chromatograph. A sample of the vapor phase was automatically injected into the gas chromatograph at regular intervals. The liquid/vapor equilibrium was considered to be reached when the concentration of volatile substance in the gas phase remained constant. The headspace system was connected to a Chrompack gas chromatograph model 9000 with flame ionization detector (F.I.D.) and a 3 m X 3 mm (i.d.) stainless steel column. The stationary phase was composed of 10% Carbowax 20M supported on Chromosorb W A-W 100-200 mesh. Analytical conditions were as follows: injector temperature 190°C, detector temperature 200°C, and column temperature 110°C for isoamyl alcohol, 120°C for ethyl esters, and 180°C for ß-ionone, nitrogen flow 20 mL/min. The chro-

matographic peaks were quantified with a Shimadzu integrator model CR3A. The relative volatility of the aroma compound can be expressed as partition Equation 1 and activity coefficient Equation 2:

$$K \text{ (partition)} = \frac{y}{x} \qquad \text{Eq. 1}$$

$$\gamma^\infty \text{ (activity coefficient)} = K\frac{Pt}{P} \qquad \text{Eq. 2}$$

where P = vapor pressure of the pure aroma compound at 25°C (mm Hg); Pt = total pressure (mm Hg); x = mole fraction of the aroma compound in the solution; and y = mole fraction of the aroma compound in the gas phase.

The results of headspace analysis were expressed by the ratio R; it was the ratio of the partition coefficient of a volatile compound in the model wine with yeast walls versus the partition coefficient of volatile compound in the model wine in absence of yeast walls:

$$R = \frac{K \text{ wine + yeast wall}}{K \text{ wine}}$$

The headspace method was used to determine the partition coefficient of isoamyl alcohol, octanal, ethyl hexanoate, and ethyl octanoate in water, in model wine, and in model wine with 1 g/L of yeast walls.

Equilibrium dialysis method: The equilibrium dialysis method is based on the diffusion of the volatile compound through a semi-permeable membrane placed between two compartments containing the model wine and the yeast walls. Acrylic cells (PTFE) of equal volume separated by a membrane (Spectrapor-1, molecular weight cut-off 6000 - 8000 daltons), clamped together, were used. In the experiment, 1 mL solution of yeast walls in model wine was placed on one side of the membrane (compartment C1) and 1 mL of model wine containing a known amount of the volatile compound (ligand) on the other side (compartment C2). The cells were shaken at 30°C for 12 hours to reach equilibrium of the free ligand between the two compartments of the cell. At equilibrium, the concentration of the ligand in the solution from each compartment was determined by gas chromatography. The same chromatographic conditions were used as in the headspace method. The difference in concentration of the ligand between C1 and C2

Table 3. Physico-chemical characteristics of aroma compounds.

Volatile compound	Origin	Purity (%)	Log P*	Concentration used (μL/L)
Isoamyl alcohol	Aldrich	99	1.21	100
Octanol	Aldrich	99	2.64	100
Ethyl hexanoate	Aldrich	99	2.76	100
Ethyl octanoate	Aldrich	99	3.80	40
ß-ionone	Merck	96	4.13	100

*Calculated by the Rekker method (16).

Table 4. Values of activity coefficients of aroma compounds in water and model wine.

Aroma substances	γ^x Water	γ^x Artificial wines without substrate
Isoamyl alcohol	107.5 [102.4 - 112.5]	61.4 [58.4 - 64.2]
Octanol	12234.0 [11744 - 17127]	3117.0 [5838 - 6391]
Ethyl hexanoate	18954.0 [18214 - 19693]	9424.0 [9286 - 9661]
Ethyl octanoate	599466.0 [570691 - 628418]	300233.0 [297037 - 303429]

[-]: Standard deviation.

represented the amount of the volatile compound bound to the yeast walls. The percentage of the bound ligand can be expressed by Equation 3; it represents the mole number of the ligand bound to the yeast walls to 100 moles of the initial ligand (%Lb):

$$\%Lb = \frac{n_1 - n_2}{n_0} \times 100 \qquad \text{Eq. 3}$$

where n_0 = mole number of the ligand in C2 at the beginning; n_1 = mole number of bound ligand and free ligand in C1 at equilibrium; and n_2 = mole number of free ligand in C2 at equilibrium.

The binding of ß-ionone and ethyl hexanoate were measured in the model wine with yeast walls at 1 g/L, 10 g/L, and with lipid free yeast walls at 1 g/L, 10 g/L.

Results and Discussion

The coefficient of variation for experiments in equilibrium dialysis and in the headspace method carried out in triplicate was less than 5%. The values of partition coefficients measured with the headspace method were standardized for a temperature of 25°C and for a total pressure of 760 mm Hg.

Influence of composition of model wine on the volatility of aroma: The activity coefficients of volatile compounds obtained by the headspace method were lower in the artificial wine than in water (Table 4). The headspace responses of aroma compounds were reduced by one-half in the presence of ethanol. The volatile compounds were not very polar and were more soluble in ethanol than in water; hence, the activity coefficient decreased, as shown by Kepner et al (8) and Nawar (14) for alcoholic beverages. Salts were present at a concentration of about 1% (w/w). Voilley et al. (21) observed an increase in the concentration of acetone in the vapor phase in the presence of calcium chloride; the activity coefficient rose by 17.5% as the concentration of calcium chloride in the solution increased from 0 to 1%. De la Ossa and Galan (2) have shown two different effects:

- in presence of $CaCl_2$, the concentration of acetaldehyde, methyl acetate, ethyl acetate, methanol and ethanol in the vapor phase increase with increasing salt concentration in the liquid phase at equilibrium;

- in presence of $CaCl_2$, the concentration of propanol and 1-butanol in the vapor phase is not affected by the salts in the liquid phase.

It would appear that salts and ethanol in model wine produce opposite effects on the volatility of volatile substances.

Interactions between aroma substances and yeast walls: Interactions between aroma substances and yeast walls led to a modification of the volatility of these aroma in wine. A value of the ratio R less than 1 indicated a decrease in volatility due to binding of aroma on yeast walls. Yeast walls used at 1 g/L bound significantly at a threshold of 5% the volatile compounds (Fig. 1). Yeast walls did not bind a specific chemical class of volatile compound. The volatility of octanal, an alde-
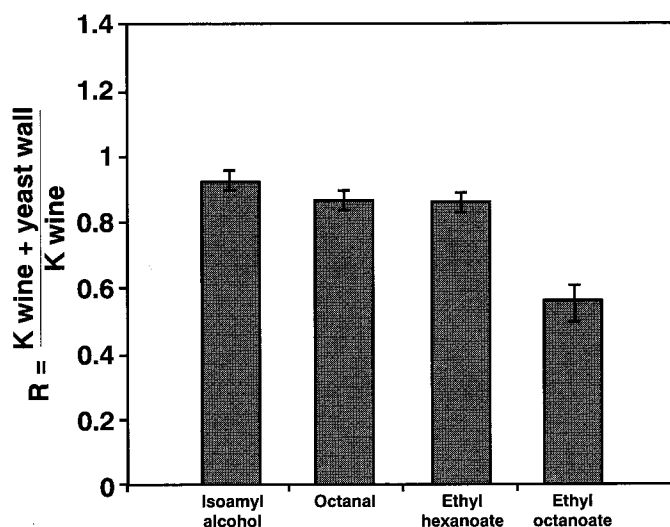

Fig 1. Effect of yeast walls at 1 g/L in model wine on the volatility of aroma compound by headspace method. R is the ratio of the partition coefficient of a volatile compound in the model wine with yeast walls on the partition coefficient of a volatile compound in the model wine in absence of yeast walls.

hyde, and the ethyl hexanoate, an ester, decreased by 14% with yeast walls in the model wine. The effect of walls was greater on the volatility of ethyl octanoate. The partition coefficient decreased by 45% with 1 g/L of walls. The hydrophobic nature of the volatile substance seemed an important factor. The volatile compound with the highest hydrophobic constant (Log P = 3.88) (16), ethyl octanoate, was bound to a greater extent on yeast walls. On the contrary, isoamyl alcohol, with Log P = 1.21, was less fixed: the decrease in volatility was 9%. Damodaran and Kinsella (3) showed that the binding affinity of ketones in a homologous series for soy
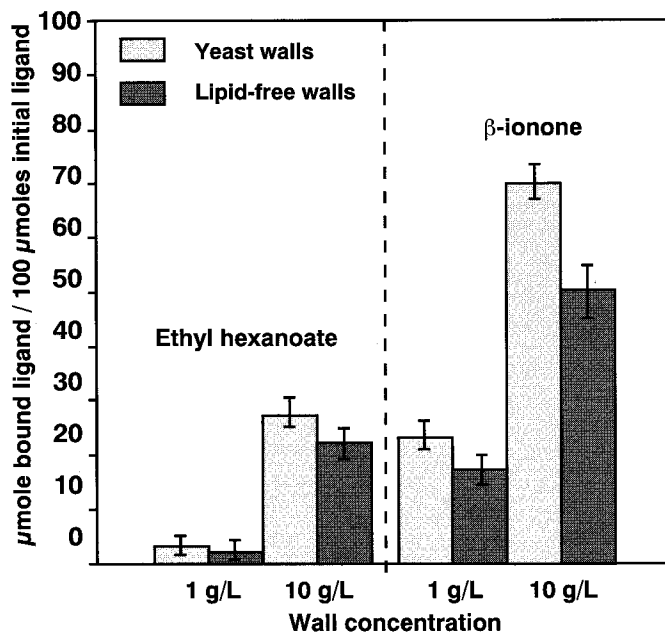

Fig 2. Percentage of binding with yeast walls and lipid-free yeast walls at 1 g/L and 10 g/L in the model wine by the equilibrium dialysis method.

proteins increases as the hydrophobic constant increases.

The effect of yeast walls on the volatility of aroma depended on the nature of the compound. Yeast walls consist mainly of polysaccharides with glucose as the main component, proteins and lipids (Table 2). Many studies on interactions with polysaccharides in model aqueous systems have been published. The sugar monomers or polysaccharides in aqueous systems produce opposite effects on the volatility of the aroma components. In some cases, the volatilities of aroma increased with sugars (17); in others cases, it decreased or remained constant (9,19). Our results showed that yeast walls led to a decrease of the volatility of all aroma compounds studied.

The equilibrium dialysis method was used to estimate the level of binding of aroma compounds on yeast walls. The experiments were carried out with ß-ionone and ethyl hexanoate in the model wine. ß-ionone was selected because the above studies showed that ß-ionone was always strongly bound on different substrates (22,23). The binding of ethyl hexanoate increased from 2.2% to 25% with increasing yeast wall concentration from 1 to 10 g/L (Fig. 2). The percentage of binding rose proportionately with the increasing of substrate concentration. It should be noted that the value of ethyl hexanoate binding obtained with a wall concentration of 1 g/L was not significant at the threshold of 5%. The binding of ß-ionone was 23% with yeast walls at 1 g/L and 70% with yeast walls at 10 g/L in the model wine. The percentage of binding rose about 3 as the concentration of walls increased by 10. The same trend was observed with lipid-free walls. This result suggested a saturation of the substrate by the volatile compound. The insolubility of walls in the model wine may explain the capacity to bind volatile substances. Some studies showed that the solubility of substrate modified the rate of binding of aroma compounds. Damodaran and Kinsella (4) showed that binding capacity was higher for an insoluble suspension of fish actomyosin than for the soluble form. It seemed that volatile compounds were fixed on yeast walls by adsorption. This may explain the interaction between ethyl hexanoate and yeast walls. However, our results with ß-ionone indicated that more specific binding sites were possible. ß-ionone was the most hydrophobic volatile compound tested with Log P = 4.13 and was the most highly bound. This result suggested that the bonds between aroma compounds and yeast walls were of a hydrophobic nature.

Analysis of Fould Springer yeast walls gave lipid concentration at 18 mg/100 mg of dry weight (Table 2), a higher value than that found in the literature (1 to 10% of dry weight) (1,6). The presence of a high concentration of lipids in the industrial yeast walls resulted from the fabrication process. The yeast walls were obtained after autolysis of whole cells. The plasma membrane was destroyed and lipids were able to adsorb onto the yeast wall surface. Some authors showed the great capacity of lipids and proteins to bind volatile compounds and their ability to form hydrogen bonds and hydrophobic interactions (5,15). The importance of lip-

ids on the level of interactions between walls and aroma compounds was, therefore, investigated.

Effect of lipids. Yeast walls free of lipids were studied by the equilibrium dialysis method with ß-ionone and ethyl hexanoate (Fig. 2). It was found that the lipid-free yeast walls bound volatile compounds less. The level of binding decreased by 28% for ß-ionone and 18% for ethyl hexanoate. In a water-oil system, the partition of volatile compounds between the aqueous phase and the lipid phase depends on their solubility in the lipids. Lebert (11) showed that the volatility of *n*-alcohols and *n*-alcanes in a homologous series decreases with an increase of the length of the carbon chain in a water-oil system. This highest decrease for ß-ionone can be attributed to its greater solubility in lipids. However, lipid-free yeast walls always bound volatile compounds, 22% for ethyl hexanoate and 50% for ß-ionone. Binding capacity of yeast walls was not only due to lipid matter. Other components of the wall such as proteins could create bonds with volatile substances. This hypothesis should be confirmed. These results were in accordance with the studies of Simpson and Miller (20). They observed high losses of ethyl esters of C 6-12 acids during the production of Barossa Valley Riesling wine. These compounds are lipophilic substances and were readily adsorbed onto the surface of yeast matter. Greater quantities of these aroma compounds were held by the yeast walls at lower temperature because of their greater degree of adsorption (20).

Conclusions

This theoretical study using a model wine showed that the effect of yeast walls on the volatility of aroma depends on the physico-chemical nature of volatile substances. Yeast walls led to a decrease of volatility of all the aroma compounds. However, the level of binding increased with the hydrophobic nature of the aroma. The capacity of yeast walls to bind was partly explained by the lipid matter. The insolubility of walls in model wine and in water was probably the major factor in these interactions. The binding of volatile compounds by adsorption onto the surface of walls needs to be confirmed. The data obtained in model wine permit us to suggest that great quantities of aroma compounds, especially the most hydrophobic, could be lost if musts are processed with large amounts of yeast walls. The quantity of yeast walls required for treatments is, therefore, important. Studies should be developed with experiments on must and wine to investigate the effect of different amounts of yeast walls on the whole bouquet of the wine.

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