

Comprehensive Study of the Evolution of Gas–Liquid Partitioning of Aroma Compounds during Wine Alcoholic Fermentation

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Calculating the gas–liquid partitioning of aromatic molecules during winemaking fermentation is essential to minimize the loss of aroma and to optimize the fermentation conditions. In this study, the effect of the main fermentation parameters on the partition coefficients (k_i) of higher alcohols (2-methylpropan-1-ol and 3-methyl butan-1-ol) and esters (ethyl acetate, 3-methyl-1-butyl acetate, and 2-ethyl hexanoate) was assessed. The values of k_i were first determined in synthetic media simulating must and wine. They varied considerably with both the hydrophobicity of the compound and the composition of the medium. Then, the effect of temperature on k_i was quantified. The absence of any effect of gas composition was also established by replacing air with CO₂. Finally, the impact of CO₂ stripping was assessed by running specific fermentations in which the rate of CO₂ production was kept constant by perfusion with assimilable nitrogen. These fermentations showed that in contrast to temperature and must composition, CO₂ stripping did not change the gas–liquid partitioning of higher alcohols and esters.

KEYWORDS: Aroma compounds; wine fermentation; volatility; PRV; partition coefficient; gas–liquid transfer

INTRODUCTION

The production of higher alcohols and esters during fermentation can have a major effect on wine quality. Higher alcohols are undesirable at high concentrations, although in smaller quantities they are thought to contribute positively to the overall quality of wine. Esters are the most abundant aromatic compounds produced by wine yeasts and are the main contributors to the bouquet of young wines. 3-Methyl-1-butyl acetate, hexyl acetate, and 2-ethyl hexanoate are considered to be the main components of a “fruity” flavor (1–3).

The volatile concentrations at the end of fermentation depend primarily on their synthesis by the yeasts but may also be significantly changed by losses in the exhaust CO₂. Estimating the transfer of aromatic compounds from the liquid to the gas phase is essential to calculate mass balances and to improve the management of fermentation, in particular through control of temperature.

The distribution of volatile compounds between the liquid and gas phases depends on the vapor–liquid equilibrium (VLE), which is defined by the gas–liquid partition coefficient, k_i . This coefficient is expressed as the ratio between the concentration of the compound in the gas phase [C_i^{gas} in (mol or g) m⁻³] and that in the liquid phase [C_i^{liq} in (mol or g) m⁻³] at equilibrium. The VLE may also be quantified by calculating the partition coefficient

expressed as (i) a molar fraction, also known as absolute volatility (K_i), or (ii) the activity coefficient (γ_i), representing the deviation from ideality. These two coefficients are related by the equation

$$K_i = \frac{y_i}{x_i} = \frac{\gamma_i P_{i(T)}^0}{P_T}$$

where x_i and y_i are molar fractions in the liquid and gas phases, respectively, $P_{i(T)}^0$ is the vapor pressure of pure component i at a given temperature T (Pa), and P_T is the total pressure (Pa) (4, 5).

Several authors have studied the vapor–liquid equilibrium in hydroalcoholic solutions (6–8) and in sugar solutions (9, 10), but their results cannot be extrapolated to winemaking fermentations. In these studies, the effects of sugar and ethanol on the gas–liquid repartition were studied independently, whereas during alcoholic fermentations, concentrations change simultaneously. In the must, the sugar concentration is close to 250 g L⁻¹. Then, it progressively decreases to 0. In parallel, ethanol concentration increases from 0 to 14%. Furthermore, during winemaking fermentations, CO₂ is produced and may have a stripping effect, in addition to effects on the VLE. Robinson et al. recently analyzed the composition of wines in detail, to improve our understanding of the perception of aroma by studying aroma compound partitioning in the headspace (11). They focused on potential interactions between aroma compounds and some wine components, such as ethanol and sugar (the content of which may differ between wines), but they did not calculate gas–liquid partition coefficients (k_i). Fermenting musts were also not considered.

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Table 1. Physical Properties of the Aroma Compounds Studied in This Work: Molecular Weight (MW), Boiling Point (T_b), Water Solubility at 25 °C (S), Log of the Octanol/Water Partition Coefficient at 25 °C (Log K_{ow}), and Vapor Pressure at 25 °C ($P_{i(T)}$)

family	CAS Registry No.	name	purity (%)	MW	T_b (°C) ^a	S (g L ⁻¹) ^b	log K_{ow} ^b	$P_{i(T)}$ (mmHg)
alcohols	78-83-1	2-methylpropan-1-ol	99.5	74.12	107.8	85	0.76	10.5
	123-51-3	3-methyl butan-1-ol	≥99	88.15	131.1	26.7	1.16	2.37
esters	141-78-6	ethyl acetate	99.8	88.11	77.1	80	0.73	93.2
	123-92-2	3-methyl-1-butyl acetate	≥99	130.19	142.5	2	20.25	5.6
	123-66-0	2-ethyl hexanoate	≥99	144.21	167	0.629	2.83	1.56

^aNIST web book (14) and SRC PhysProp database. ^b SRC PhysProp database (15).

In this study, the effects of the main fermentation parameters on the partition coefficients (k_i) of several higher alcohols (2-methylpropan-1-ol and 3-methylbutan-1-ol) and esters (ethyl acetate, 3-methyl-1-butyl acetate, and 2-ethyl hexanoate) were assessed. The values of k_i were first determined in synthetic media that simulated must and wine. The effect of temperature on k_i was then quantified, and the hypothetical effect of the gas composition was determined by replacing air with CO₂ in the gas phase. Finally, the effect of CO₂ stripping was studied. The stripping effect is complex because together with other factors, the rate of CO₂ production usually varies throughout the fermentation process. It increases during the growth phase and then steadily decreases during the stationary phase, mostly due to the inhibition of yeasts by ethanol (12). Using a previously described method (13), we overcame the problems associated with this complexity and isolated the effects of stripping by keeping the rate of CO₂ production constant through a controlled perfusion of ammoniacal nitrogen.

MATERIALS AND METHODS

Volatile Compounds. Ethyl acetate, 3-methyl-1-butyl acetate, 2-methylpropan-1-ol, and 3-methyl butan-1-ol were purchased from Merck (Darmstadt, Germany); 2-ethyl hexanoate was purchased from Aldrich (Steinheim, Germany). The main physicochemical properties of these products are summarized in **Table 1**. Solutions of aroma compounds were prepared from concentrated stock solutions in absolute ethanol (BDH, France) of 20000 ppm (v/v) for esters and 100000 ppm (v/v) for higher alcohols. These stock solutions were stored at -80 °C and used to prepare model solutions and to enrich the natural must (if necessary, for the phase ratio variation (PRV) method), to concentrations of 20 ppm (v/v) for esters and 100 ppm (v/v) for higher alcohols.

Media. *Model Solutions Simulating Must and Wine.* For the model solution simulating the must, the volatile compounds listed above and 220 g L⁻¹ glucose were added to a buffer solution containing 6 g L⁻¹ citric acid and 6 g L⁻¹ malic acid adjusted to pH 3.3 with sodium hydroxide. For the model solution simulating the wine, the buffer solution was supplemented with 13% (v/v) ethanol and the volatile compounds, without the addition of glucose.

Model Solutions Simulating Musts at Different Stages of Fermentation. Changes in k_i during fermentation were compared with those observed for the natural must described below, by supplementing the buffer solution containing volatile compounds described above with (1) 180 g L⁻¹ glucose, (2) 144 g L⁻¹ glucose and 2.2% (v/v) ethanol, (3) 107.5 g L⁻¹ glucose and 4.4% (v/v) ethanol, (4) 72.5 g L⁻¹ glucose and 6.5% (v/v) ethanol, or (5) 10.8% (v/v) ethanol. The resulting media correspond to 0, 20, 40, 60, and 100% progression of the fermentation.

Natural Must. A grape must from southern France was used. It was flash pasteurized and stored under sterile conditions (16). The sugar content was 180 g L⁻¹, assimilable nitrogen concentration, 40 mg L⁻¹, and pH, 3.52.

The samples from the fermenting were centrifuged at 8000g for 10 min before storage at cold temperature (-18 °C) and analysis.

Determinations of Gas-Liquid Partition Coefficients (Static Conditions). The gas-liquid partition coefficients of volatile compounds in model solutions and natural fermenting musts were determined by the PRV method, as previously described by Athes et al. (5).

Sample Preparation. Aliquots of 50 μL–2 mL of solution were introduced into four different headspace vials (22 mL, Chromacol, France), which were closed with Teflon/silicone septa in metallic caps, to give volume ratios (β) from 9.8 to 437; the vials were then equilibrated at the temperatures studied (15, 20, 25, and 30 °C) for at least 1 h (the time required to reach equilibrium in static conditions). The gas in the headspaces was then analyzed by gas chromatography.

Gas Chromatography. After equilibration, 1 mL of headspace gas was automatically withdrawn using a gastight syringe, preheated to a temperature of 15 °C above the studied temperature, in an automatic headspace sampler CombiPal (Shimadzu AOC-5000) and analyzed on a Hewlett-Packard gas chromatograph coupled to a HP 5973 mass spectrometer. The injection was direct, and the injector temperature was settled at 245 °C and injection rate at 100 μL s⁻¹. Only one headspace injection was made per vial, and three vials were analyzed for each solution. An Optima-Wax capillary column (30 m × 0.25 mm × 0.25 μm) from Macherey-Nagel was used with a carrier gas (helium) flow rate of 1 mL min⁻¹. The oven temperature program was set from 40 to 80 °C at 4 °C/min and from 80 to 200 °C at 8 °C/min. Peak areas were acquired with Hewlett-Packard Chemstation software.

Calculation of Partition Coefficients by the PRV Method. Partition coefficients were calculated from mass balance, as described by Etre et al. (17)

$$\frac{1}{A} = \frac{1}{f_i C_i^{\text{liq}}} \times \frac{1}{k_i} + \frac{1}{f_i C_i^{\text{liq}}} \times \beta \quad (1)$$

where A is the peak area obtained at equilibrium, f_i is a proportional factor, and β is the volume ratio, V_g/V_l , where V_g is the headspace volume and V_l is the liquid volume of the sample. Using the linear equation $1/A = \alpha\beta + b$, where

$$a = \frac{1}{f_i C_i^{\text{liq}}}$$

and

$$b = \frac{1}{f_i \times C_i^{\text{liq}}} \times \frac{1}{k_i}$$

and a/b is the gas-liquid partition coefficient expressed as the concentration ratio (k_i):

$$k_i = \frac{C_i^{\text{gas}}}{C_i^{\text{liq}}}$$

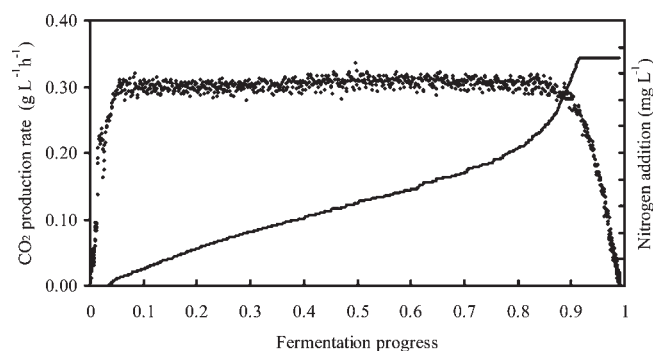
Effect of Carbon Dioxide. The effect of replacing air with carbon dioxide was investigated, using the model solution containing 220 g L⁻¹ glucose. CO₂ was bubbled through the solution at a rate of 100 mL min⁻¹ before adding the volatile compounds and adjusting the volume to reach the concentration indicated above. The model solution was dispensed into headspace vials that had previously been saturated with CO₂, and the partition coefficient was determined by the PRV method, as described above.

Changes in Gas-Liquid Concentration Ratio during Fermentation (Dynamic Conditions). *Fermentation Conditions and Control.* Yeast fermentations were carried out in stainless steel tanks, using the natural must. The tanks contained 90 L of must, and the headspace represented 30% of the total volume. The temperature was kept constant at 20 °C. The CO₂ released was automatically and continually measured

Table 2. Gas–Liquid Partition Coefficients ($\times 10^3$; Concentration Ratio) of Volatile Compounds in Acidic Aqueous Solution at pH 3.3 and 25 °C for Our Study and in Water for Literature and Estimated Data

compound	experimental values		estimated values ^a	
	this study	literature	bond contribution ^{b,c}	group contribution ^b
esters				
ethyl acetate	6.26 ± 0.18	5.49 ^d 7.86 ^e 7.09 ± 0.59 ^f	9.52	6.46
3-methyl-1-butyl acetate	21.8 ± 1.06	24 ^b	22.3	21.9
2-ethyl hexanoate	34.2 ± 4.03	29.2 ^e 43.0 ± 2.8 ^f 34.0 ± 2.4 ^g	29.6	25.7
alcohols				
2-methylpropan-1-ol	0.50 ± 0.01	0.40 ^h	0.41	0.48
3-methylbutan-1-ol	0.56 ± 0.03	0.58 ⁱ	0.54	0.68

^a Estimated partition coefficient values calculated from a bond contribution^b or a group contribution^c method (EPIWEB 4.0 software, developed by U.S. EPA Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC)). ^b Hine and Mookerjee (25). ^c Meylan and Howard (26). ^d Butler and Ramchandani (20). ^e Aprea, Biasioli, Märk, and Gasperi (19). ^f Landy, Druaux, and Voilley (23). ^g Landy, Courthaudon, Dubois, and Voilley (22). ^h Snider and Dawson (24). ⁱ Butler, Ramchandani, and Thompson (21).

**Figure 1.** Constant rate fermentation at 0.3 g L⁻¹ h⁻¹: evolution of the CO₂ production rate (◆) and added nitrogen (—).

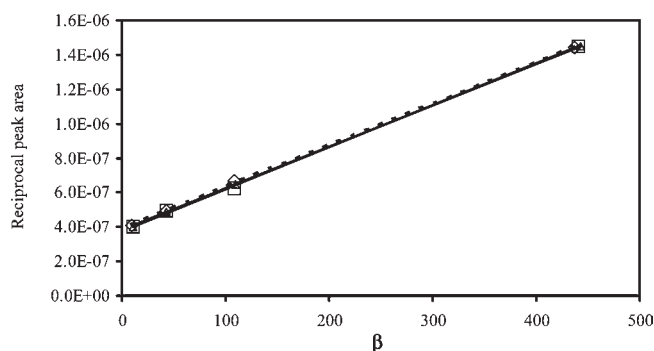
with a gas mass flow meter (16). The many acquisitions and the precision of the flow meter made it possible to calculate the rate of CO₂ production ($d\text{CO}_2 dt^{-1}$) with a high level of precision.

To control the stripping effect, constant rate fermentations were run. In these experiments, the rate of CO₂ production was kept constant by a feedback control mechanism involving the addition of ammoniacal nitrogen via a peristaltic pump (Ismatec Reglo) (13). By modifying the amount of assimilable nitrogen (no addition or addition of 80 mg/L of ammoniacal nitrogen) initially present in the must, that is, 40 mg/L, we set up two fermentations in which the rates of CO₂ production were kept constant at, respectively, 0.3 g L⁻¹ h⁻¹ (Figure 1) and 0.6 g L⁻¹ h⁻¹ (data not shown). The rates of CO₂ production were regulated between 10 and 85% progression of the fermentation reaction.

Gas Chromatography. The composition of the gas phase was analyzed with the online device described by Mouret et al. (18). The gas was pumped at a flow rate of 14 mL min⁻¹ from the tank headspace. Carbon volatile compounds were concentrated in a cold trap (Tenax) for 6 min (desorption at 160 °C for 1 min), injected into a column ZBWax (60 m × 0.32 mm × 0.5 μm) from Phenomenex Inc. at a pressure of 120 kPa and with a temperature profile of 38 °C for 3 min, increasing by 3 °C min⁻¹ to 65 °C, and then by 6 °C min⁻¹ to 160 °C, with the temperature kept constant at 160 °C for 5 min and then increasing by 8 °C min⁻¹ to 230 °C, at which it was maintained for 5 min. Flame ionization detection (FID) was used.

The concentrations of the volatile compounds in the liquid phase were determined by the headspace method, using a GC with a temperature profile and a column similar to those used for online measurements. For standardization of the equilibrium conditions between the liquid and the headspace, the ethanol concentration in the sample was fixed at 11%.

Statistical Analysis. R version 2.8.1 (The R Foundation for Statistical Computing) was used for statistical analysis. A one-way analysis of variance was carried out. The Wilcoxon test was used to compare medians, to determine the differences between the gas–liquid partition coefficients of volatile compounds, to evaluate the effect of CO₂ in static conditions, in

**Figure 2.** Reciprocal of peak area versus volume ratio (β) for ethyl acetate in acidic (pH 3.3) aqueous solution at 25 °C: (□, △, ◇) triplicates.

particular, to compare the k_i measurements for model solutions and natural musts, and to assess the extent to which the model media were representative.

RESULTS AND DISCUSSION

Study of Gas–Liquid Partition Coefficients in Model Solutions.

Determination of Gas–Liquid Partition Coefficients. A representative plot of the reciprocal peak area of ethyl acetate versus volume is presented in Figure 2. The k_i value was obtained by using the PRV method (eq 1) and averaging the results of different series.

Partition coefficients and standard deviations are reported in Table 2. The results are consistent with published results (Table 2) (19–24) and estimates from the chemical structure of the compounds, based on bond contribution or group contribution methods (25, 26). It is notable that the presence of malic acid and citric acid and adjustment of the pH had no significant effect on the partitioning properties of the compounds, as shown by comparisons with published data obtained with pure water at 25 °C. Indeed, the volatile compounds used in this study were unlikely to be affected by the pH of the solution because they are not in a dissociated form, unlike volatile carboxylic acids. Small differences in reported values are nonetheless observed (Table 2), but may be attributed to differences in the measurement techniques used (5).

Within each chemical family, the gas–liquid partition coefficient increased with molecular weight. This finding is consistent with those of previous studies (23, 27–30). Indeed, within a group of chemicals, the molecules become more hydrophobic (the log K_{ow} increases) as molecular weight increases; this results in a

Table 3. Comparison of k_i Values in Model Solutions and Natural Musts at Different Fermentation Stages Using the Wilcoxon Range Sum Test^a

fermentation progress (%)	p values				
	ethyl acetate	3-methyl-1-butyl acetate	2-ethyl hexanoate	2-methylpropan-1-ol	3-methylbutan-1-ol
0	0.10	0.70	0.70	0.70	0.40
20	0.40	1.00	0.10	0.20	0.10
40	0.10	1.00	0.70	0.70	0.40
60	0.10	0.70	0.40	0.40	0.10
100	0.86	1.00	0.11	0.10	0.10

^a Differences are significant if the p value is <0.05.

Table 4. Gas–Liquid Partition Coefficient ($\times 10^3$; Concentration Ratio) Measured in Model Solution with either Glucose or Ethanol: Effect of Temperature

compound	model solution	15 °C	20 °C	25 °C	30 °C
esters					
ethyl acetate	glucose 22%, ethanol 0%	5.66 ± 0.20	8.30 ± 0.58	10.13 ± 0.90	12.98 ± 0.90
	glucose 0%, ethanol 13%	3.85 ± 0.21	4.20 ± 0.55	5.79 ± 0.48	7.06 ± 0.89
3-methyl-1-butyl acetate	glucose 22%, ethanol 0%	18.44 ± 0.49	25.81 ± 2.31	29.11 ± 5.22	44.62 ± 7.91
	glucose 0%, ethanol 13%	8.88 ± 1.17	11.35 ± 1.02	13.17 ± 1.28	18.62 ± 4.25
2-ethyl hexanoate	glucose 22%, ethanol 0%	12.54 ± 1.85	43.92 ± 6.86	45.25 ± 13.4	85.79 ± 27.44
	glucose 0%, ethanol 13%	9.71 ± 1.26	14.14 ± 0.70	16.80 ± 2.05	26.18 ± 9.99
alcohols					
2-methylpropan-1-ol	glucose 22%, ethanol 0%	0.22 ± 0.03	0.45 ± 0.03	0.68 ± 0.11	0.99 ± 0.08
	glucose 0%, ethanol 13%	0.21 ± 0.02	0.19 ± 0.02	0.33 ± 0.06	0.43 ± 0.10
3-methylbutan-1-ol	glucose 22%, ethanol 0%	0.26 ± 0.05	0.60 ± 0.08	0.71 ± 0.11	1.17 ± 0.12
	glucose 0%, ethanol 13%	0.30 ± 0.05	0.33 ± 0.05	0.28 ± 0.06	0.38 ± 0.13

weakening of the interaction between the volatile molecule and water, leading to an increase in volatility from aqueous solution for the most hydrophobic molecules.

Difference in volatility may also be accounted for by differences in the vapor pressures of the pure compounds as illustrated by the comparison of ethyl acetate and 2-methylpropan-1-ol, which have very similar hydrophobicities.

Comparison of Partition Coefficients Measured in Natural Musts and Model Solutions. The representativeness of the gas–liquid partition coefficients measured in model solutions was assessed by preparing different solutions in which sugar and ethanol concentrations were changed simultaneously to mimic different stages of fermentation of the natural must used. Using the Wilcoxon range sum test to compare the k_i values, p values of >0.05 were obtained for all of the aroma compounds studied, indicating an absence of significant difference between the k_i values in model solutions and those in natural musts (Table 3). Thus, the model solution used in this experiment provided a satisfactory simulation of natural musts, at least for musts used in white winemaking, which contain only small amounts of polyphenolic compounds.

Effect of Glucose and Ethanol on Gas–Liquid Partition Coefficients. The effect of glucose and ethanol on the partition coefficients of the volatile compounds was measured in model solutions simulating the fermenting must at the beginning (22% (w/v) glucose and no ethanol) and end (no glucose and 13% (v/v) ethanol) of fermentation.

The partition coefficients of aroma compounds in both model solutions were different from those in aqueous solution (Table 4). The effects of the composition of the solution on the partitioning properties of volatile compounds were compared by calculating the release ratio, expressed as

$$\% k_{i\text{m/aq}} = \frac{k_i^{\text{m}} - k_i^{\text{aq}}}{k_i^{\text{aq}}} \times 100 \quad (2)$$

where k_i^{m} is the partition coefficient of the molecule in the model solution and k_i^{aq} is the partition coefficient of the molecule in aqueous solution. A positive value indicates that the solute

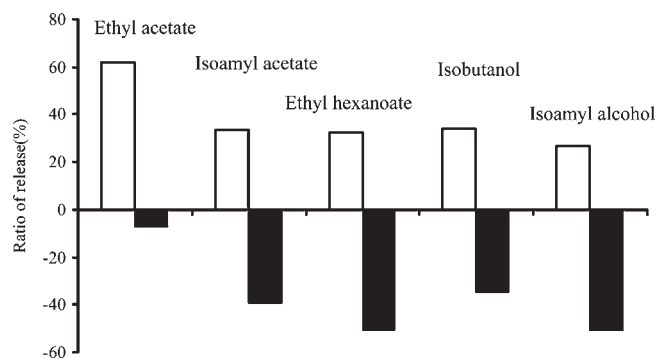


Figure 3. Ratio of release ($\% k_{i\text{m/aq}} = (k_i^{\text{m}} - k_i^{\text{aq}})/k_i^{\text{aq}} \times 100$, where k_i^{m} is the partition coefficient of the molecule in model solution and k_i^{aq} is the partition coefficient of the molecule in aqueous solution) of aroma compounds in two model solutions with 22% (w/v) glucose, 0% (v/v) ethanol (open bars), and 0% (w/v) glucose, 13% (v/v) ethanol (solid bars).

enhances release, whereas a negative value is associated with retention.

The presence of glucose in the model solution raised the partition coefficient in all of the volatile compounds studied. In our conditions, simulating a grape must, the relative increase of k_i ranged between 26.6% for 3-methylbutan-1-ol and 61.8% for ethyl acetate (Figure 3). Thus, glucose decreases the solubility of volatile compounds in water (9, 31) and increases the partition coefficients of volatile compounds as observed with sucrose (32, 33). Lubbers and Guichard (32) determined the partition coefficients of 2-methylpropan-1-ol in a syrup consisting of a mixture of 54% w/w glucose and 21% w/w sucrose. This gave a partition coefficient that was 4.5 times higher than that in water.

The release of aroma compounds from glucose solution observed in this study resulted from a salting-out effect of glucose, which forms hydrogen bonds with water molecules, thereby decreasing the activity of the water, lowering the free water content and decreasing the solubility of aroma compounds (34).

Unlike glucose, the presence of ethanol in the solution tended to favor retention. The ratio of aroma compound release from

matrix/aqueous solutions of volatile compounds was negative (Figure 3). The presence of 13% ethanol (i.e., the average concentration in wines) decreased k_i values by up to 50% for 2-ethyl hexanoate and 3-methylbutan-1-ol. As previously described (6, 7, 35–38), ethanol increases the solubility of volatile compounds in the matrix, thereby decreasing their headspace concentration. Aznar et al. (35) also established a relationship between headspace volatile compound concentration and hydrophobicity ($\log K_{ow}$), by describing the decrease in headspace concentration with increases in the ethanol concentration of the solution from 4 to 42% (v/v). A correlation between the decrease in headspace volatile compound concentration and $\log K_{ow}$ values was observed for $\log P$ values below 3, as with the molecules we studied. Our results confirmed that, for the same chemical family, the k_i decrease was related to the hydrophobicity of the molecules as illustrated by the comparison between ethyl acetate (no effect of ethanol at the 95% confidence level) and 2-ethyl hexanoate.

On the other hand, ethanol had very different retention effects on 2-methylpropan-1-ol and ethyl acetate, despite the similarity of their $\log K_{ow}$ values, both around 0.7. This confirms that, in aqueous solution, the availability of OH groups on a volatile compound affects the release of that compound through hydrogen bonding with ethanol.

Effect of Temperature on Gas–Liquid Partition Coefficients. The effect of temperature on partition coefficients in the range of enological interest (i.e., between 15 and 30 °C) was studied in both model solutions containing either glucose or ethanol (Table 4). The Clausius–Clapeyron law was applied to partition coefficient (K_i) change with temperature (39)

$$-\frac{d \ln K_i}{d(1/T)} = \frac{\Delta H_{vap}}{R} \quad (3)$$

where ΔH_{vap} is the enthalpy of vaporization expressed in J mol^{-1} , R is the ideal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$), T is the temperature in Kelvin, and K_i is the partition coefficient expressed as y_i/x_i . Values of $\ln K_i$ were plotted against the inverse of temperature (Figure 4; Van't Hoff representation). Relationships were linear (8, 30, 39–41), except for 3-methylbutan-1-ol in the model solution containing 13% ethanol, for which the r^2 was below 0.5 due to the slight variation of K_i as a function of ethanol content and temperature. Enthalpies of vaporization were calculated from these plots (Table 5). These values reflect the minimum energy required for a switch from the liquid to the vapor phase. The enthalpy of vaporization was between 30.1 for acetate esters and 71.4 kJ mol^{-1} for alcohols. The values obtained for ethyl acetate, 3-methyl-1-butyl acetate, 2-ethyl hexanoate, and 3-methylbutan-1-ol were consistent with published data (Table 5). The values obtained in water were intermediate between those measured in the presence of glucose and those measured in the presence of ethanol.

The enthalpies of vaporization of all of the volatile compounds in the model solution containing 22% (w/v) glucose were higher than in the presence of ethanol or in water. Presumably, the

physicochemical interactions between aroma compounds and glucose were stronger.

Effect of Carbon Dioxide on Gas–Liquid Partition Coefficients. To mimic the headspace composition during fermentation, the headspaces above model solutions containing 22% (w/v) glucose were saturated with carbon dioxide, and the partition coefficient of each volatile compound was measured at 30 °C. The Wilcoxon test was used to compare the results with control

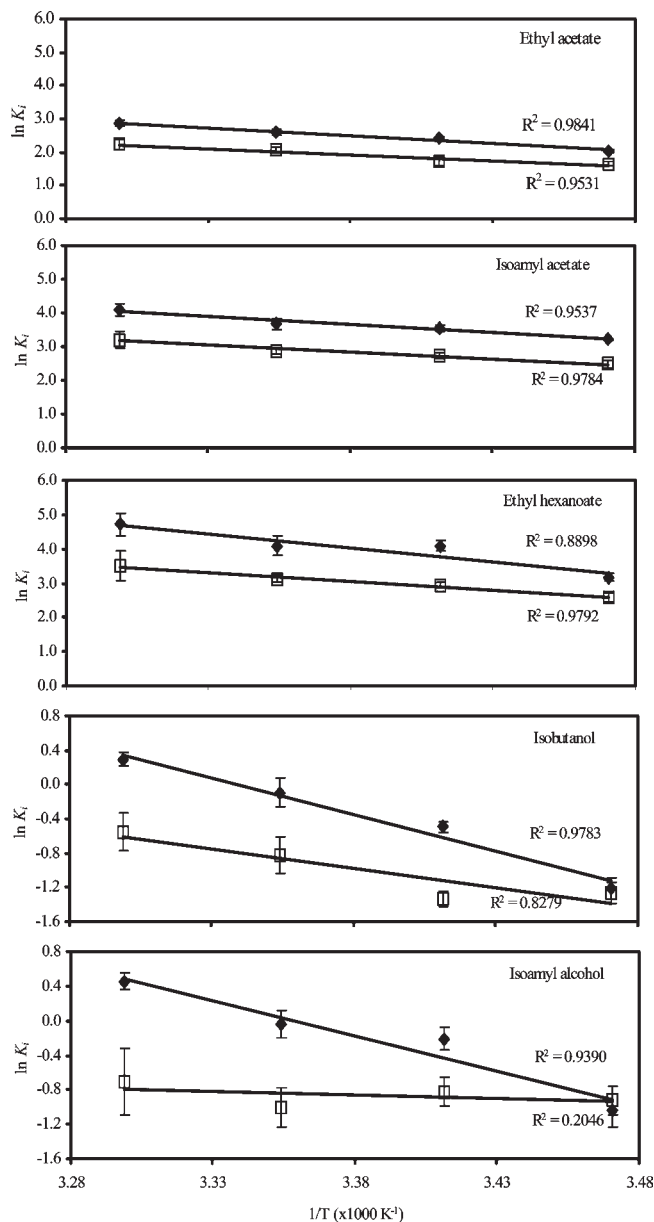


Figure 4. \ln of the partition coefficient (K_i ; expressed as y/x_i) as a function of $1000/T$ (K^{-1}), 22% (w/v) glucose, 0% (v/v) ethanol (\diamond), and 0% (w/v) glucose, 13% (v/v) ethanol (\square).

Table 5. Enthalpy of Vaporization of the Five Aroma Compounds in Model Solutions

ΔH_{vap} (kJ mol^{-1})	ethyl acetate	3-methyl-1-butyl acetate	2-ethyl hexanoate	2-methylpropan-1-ol	3-methylbutan-1-ol
glucose 22% (w/v)	39.0	39.4	67.5	71.4	67.6
ethanol 0% (v/v)					
glucose 0% (w/v)	30.1	33.4	42.6	37.8	nd ^a
ethanol 13% (v/v), water	35.7 ^b	35.7 ^c	45.5	50.8 ^b	
		46.3 ^b	51.7 ^b		

^a Not determined ($r^2 < 0.5$). ^b Plyasunov, Plyasunova, and Shock (42). ^c Meynier et al. (39).

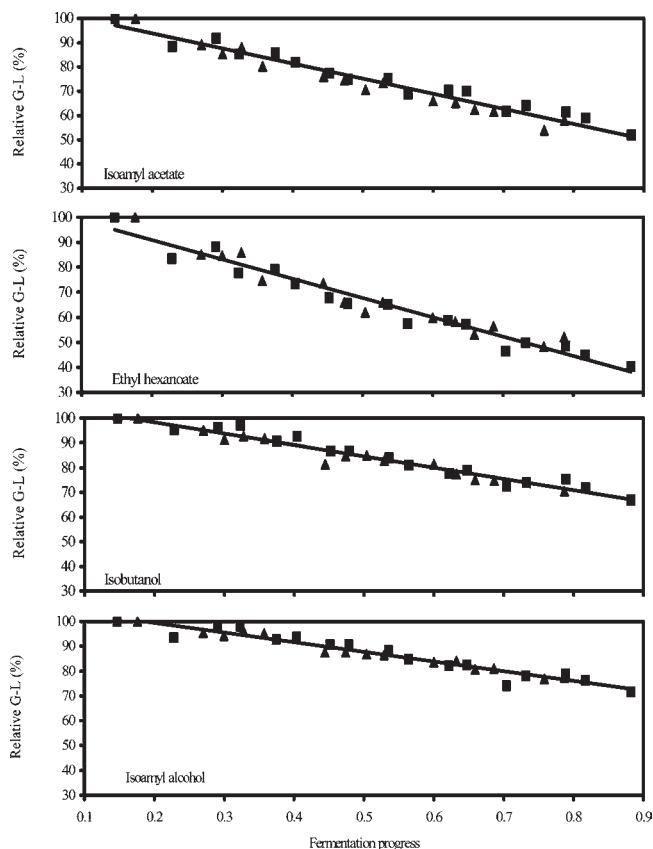


Figure 5. Evolution of the gas–liquid ratio during fermentations at constant CO_2 production rate $0.3 \text{ g L}^{-1} \text{ h}^{-1}$ (■) and $0.6 \text{ g L}^{-1} \text{ h}^{-1}$ (▲). At each stage of fermentation the gas–liquid ratio is normalized by the ratio at 20% of fermentation progress (considered as the 100% reference).

values. The p values obtained were >0.05 (ethyl acetate, 0.31; 3-methyl-1-butyl acetate, 0.48; 2-ethyl hexanoate, 0.13; 3-methylbutan-1-ol, 0.24). Thus, there was no significant difference between the mean partition coefficients with and without CO_2 saturation, at a confidence level of 95%, for the volatile compounds studied.

Changes in the Gas–Liquid Ratio of Aroma Compound Concentration during Alcoholic Fermentation (Dynamic Conditions). After determining the effects of medium composition and temperature in model conditions, the impact of CO_2 stripping was assessed in real fermentations to avoid possible artifacts due to the use of spargers (size of the bubbles). Another interest was the possibility to run fermentations (i) at a constant rate by perfusing ammoniacal nitrogen and (ii) with different rates by changing the initial amounts of assimilable nitrogen, to study the effect of stripping independently.

Changes in the relative gas–liquid ratio of aromatic compound concentrations during the regulated-rate phase were plotted versus the fermentation progress (Figure 5). This relative ratio remained almost constant for ethyl acetate and decreased linearly, by 28% for 3-methylbutan-1-ol, by 35% for 2-methylpropan-1-ol, by 50% for 3-methyl-1-butyl acetate, and by 65% for 2-ethyl hexanoate. A very remarkable result is that identical decreases were observed for CO_2 production rates of 0.3 and $0.6 \text{ g L}^{-1} \text{ h}^{-1}$ for all of the compounds studied. It can therefore be deduced that stripping did not change the gas–liquid partitioning of aroma compounds during fermentation and that the two phases remain in equilibrium throughout the process. The stripping of aromas from the fermenting must depends on both vapor–liquid equilibrium and mass transfer rates. Our results show that mass

transfer rates were very fast; hence, the stripping rate of aromas from the fermenting must depended only on the VLE. This result is remarkable and could be hardly anticipated. Indeed, with respect to other studies (43, 44), we expected that esters and higher alcohols would be “blown off” by CO_2 . However, Ferreira et al. (43) and Aguera et al. (44) used higher CO_2 flow rates, beyond the natural rates of production by yeasts, which certainly explained the observed differences with our work.

It was also observed that the decrease in this ratio during fermentation was (i) larger for esters than for the less volatile higher alcohols and (ii) for all the compounds considered, consistent with the decrease in partition coefficient (k_i) observed in static conditions. Therefore, at constant temperature, changes in the gas–liquid partitioning of aroma compounds in fermenting musts result mostly from changes in the composition of the liquid phase (increase of ethanol concentration, decrease of sugar concentration).

The fact that the CO_2 production does not affect gas–liquid aroma partitioning requires confirmation, in different winemaking situations, with higher CO_2 production rates, but it is nonetheless of prime importance, particularly for developing models that describe the relationship between the liquid and gas phase concentrations of volatile compounds. Once measurements in the gas phase have been calibrated and such models have been developed, online measurement devices should make it possible to precisely describe changes in the concentrations of the main esters and higher alcohols in both the gas and liquid phases.

An online monitoring device for higher alcohols and esters in exhaust CO_2 has already been described by Mouret et al. (17), and its use for improving the understanding of yeast metabolism has already been demonstrated. Coupling such a device with online monitoring of the CO_2 production rate (reviewed in ref 45) will allow the dynamics of loss and production of aroma compounds to be estimated during fermentation. From a microbiological point of view, this will provide a useful means of calculating metabolic fluxes on the basis of the total production of volatile molecules (i.e., the concentration remaining in the fermenting must plus the losses in the exhaust CO_2). This technique will also be useful to help design innovative strategies to optimize aroma production and loss through controlling temperature and nutrient supplementation.

ACKNOWLEDGMENT

We thank Magaly Angenieux, Evelyne Aquera, Pamela Nicolle, Marc Perez, Peggy Rigou, and Isabelle Sanchez for their help and technical assistance.

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