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1 **Modelling of the gas-liquid partitioning of aroma compounds during wine alcoholic**
2 **fermentation and prediction of aroma losses**

3

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15

16 **ABSTRACT**

17

18 A model was elaborated to quantify the gas-liquid partitioning of four of the most important
19 volatile compounds produced during winemaking fermentations, namely isobutanol, ethyl
20 acetate, isoamyl acetate and ethyl hexanoate. Analyses of constant rate fermentations
21 demonstrated that the partitioning was not influenced by the CO₂ production rate and was a
22 function of only the must composition and the temperature. The parameters of the model were
23 identified in fermentations run at different temperatures, including anisothermal conditions.
24 The prediction of the partition coefficient (k_i) by the model was very accurate for isobutanol,
25 isoamyl acetate and ethyl hexanoate. The technological potential of the model was confirmed
26 by using it to calculate the losses of volatiles in the gas phase during fermentation and
27 comparing them with experimental data. Up to 70% of the produced volatile compounds were
28 lost. The difference between observed losses and losses estimated from predicted k_i values
29 never exceeded 3%.

30

31 **Keywords:** gas-liquid transfer, online GC measurement, wine, aroma, dynamic modelling

32

33 **1. Introduction**

34

35 The synthesis of higher alcohols and esters during fermentation makes an important
36 contribution to wine quality and the control of the production of these volatile compounds is
37 one of the major ways to control the organoleptic characteristics of wine. The higher alcohols
38 are undesirable at high concentrations, but in smaller quantities they are thought to contribute
39 positively to overall wine quality. Esters have a significant effect on the fruity flavour in
40 wine. The esters making the largest olfactory impact are ethyl acetate, isoamyl acetate,
41 isobutyl acetate, ethyl hexanoate and 2- phenylethyl acetate [1]. Varietal aromas —volatile
42 compounds derived from non-volatile precursors in the grape which are released by the yeast
43 during fermentation, such as thiols— also play an essential role in wine aroma but they are
44 usually present at very low concentrations and are therefore more difficult to quantify.

45 The concentrations of volatiles at the end of fermentation depend primarily on their
46 synthesis by the yeasts but may also be significantly modified by losses into the exhausted
47 CO₂. Therefore, understanding and modelling the transfer of aroma compounds between the
48 gas and liquid phases would be extremely useful, and the calculation of balances
49 differentiating the microbiological process of production and the physicochemical process of
50 transfer into the exhausted CO₂ is central to this issue. The online monitoring of volatile
51 compounds in the tank headspace, as recently proposed by Mouret et al. [2], allows online
52 estimation of volatile concentrations in fermenting musts, provided that reliable models for
53 gas-liquid partitioning are available for all phases of the fermentation process. Finally, such
54 models could subsequently be coupled to predictive models of volatile compound synthesis,
55 based on knowledge of the biochemical pathways involved. Indeed, some authors have
56 already proposed such models in beer making conditions [3-5].

57 Several models have been developed to quantify the transfer of volatile molecules between
58 aqueous solutions and gas phases [6-8]. However, none of them is directly applicable to
59 winemaking conditions. Indeed, unlike the conditions considered in these established models,
60 the concentrations of volatile molecules, as well as the overall composition of the fermenting
61 must, are continuously changing during alcoholic fermentations. Another difference is the
62 production and release of CO₂, bubbles of which increase the transfer from the liquid to the
63 gas, by stripping. **Finally, only 3 molecules (isobutyl acetate, acetaldehyde and ethyl acetate)**
64 **out of 21 were of interest in wine fermentations.**

65 Several authors have studied flavour release in the context of oenology but mostly focused
66 on the partitioning properties of volatiles in final wines [9-11] and did not consider their
67 behaviour during fermentation. Recently, Morakul et al. [12] evaluated the effect of the
68 matrix changes (mainly corresponding to the consumption of sugar and the production of
69 ethanol) and of the temperature on gas-liquid partitioning in model conditions simulating
70 fermenting musts. Ferreira et al. [13] assessed volatile compound losses due to the CO₂
71 production and showed that up to 80% of some molecules could be blown off; however, the
72 experimental conditions **used in [13] were not completely representative of the fermentation**
73 **conditions, because changes in the matrix composition were not considered and the stripping**
74 **rate was much higher than usually observed in winemaking.**

75 In this paper, the objective was to **develop** a model of the evolution of the partition
76 coefficient between the gas and liquid phases of four major volatile molecules, (ethyl acetate,
77 isoamyl acetate, ethyl hexanoate and isobutanol) in winemaking fermentations. The partition
78 coefficient k_i is expressed as the ratio between the mass concentration of the compound in the
79 gas phase [C_i^{gas} in mg/L] and that in the liquid phase [C_i^{liq} in mg/L] at equilibrium.

80 The work was focused on these four molecules because they are representative of higher
81 alcohol and ester families. Isobutanol is one of the major fusel alcohols whose concentrations

82 in wines are several tens of mg/L. It is synthesized by the yeasts from amino acids, in
83 particular valine, and from keto-acids. As most higher alcohols are weakly volatile, its
84 partition coefficient (k_i), expressed as a mass concentration ratio, at 25°C in grape musts is
85 around 6.8×10^{-4} [12]. Despite its high concentration in the liquid phase, it is often below its
86 perception threshold in gas [1]. Ethyl acetate, isoamyl acetate, and ethyl hexanoate are well
87 known for their contribution to the fruity aroma of wines [14]. Their concentrations in wines
88 are usually low (20 - 60 mg/L for ethyl acetate and less than 10 mg/L for isoamyl acetate and
89 ethyl hexanoate) but nevertheless always above their perception thresholds [1]. The k_i values
90 at 25°C in grape musts are around 1.0×10^{-2} for ethyl acetate, 2.9×10^{-2} for isoamyl acetate and
91 4.5×10^{-2} for ethyl hexanoate [12].

92 After assessing the effects of the main factors involved, and in particular the impact of
93 stripping by CO₂, the model for the prediction of the partition coefficient (k_i) was developed
94 and then validated in different winemaking situations. The model was then used to estimate
95 the losses of volatile compounds in several winemaking situations.

96

97 **2. Material and methods**

98

99 *2.1. Fermentations*

100

101 *2.1.1. Yeast strains*

102 The *Saccharomyces cerevisiae* strains EC1118 and K1 were used. These commercial wine
103 yeasts are produced as active dry yeast by Lallemand SA. Each fermentation tank was
104 inoculated with 0.2 g/L of active dry yeast previously rehydrated for 30 minutes at 35 °C.

105

106 *2.1.2. Musts*

107 Various grape musts from the South of France were used. They were flash-pasteurised and
108 stored under sterile conditions. Their sugar concentrations were between 180-200 g/L and
109 their assimilable nitrogen concentrations were 40, 120, 140 and 240 mg/L.

110

111 *2.1.3. Tanks*

112 Fermentations were run at pilot scale in stainless steel tanks. The tanks contained 90 L of
113 must and the headspace represented 30% of the total volume.

114

115 *2.1.4. Control of fermentation*

116 The CO₂ released was automatically and continually measured with a gas mass flow meter
117 and the rate of CO₂ production (dCO₂/dt) was calculated with a high level of precision. The
118 fermentations were controlled in different ways:

119 (i) Constant rate fermentations (CRF): to control the stripping effect, constant rate
120 fermentations were run at 20°C. In these experiments, the rate of CO₂ production was kept
121 constant by a feedback control **system** involving the addition of ammoniacal nitrogen via a
122 peristaltic pump (Ismatec Reglo) [15].

123 (ii) Isothermal fermentations (IF): the temperature was maintained at a constant value (20
124 and 30°C), with a precision of 0.1°C.

125 (iii) Anisothermal fermentations (AF): the temperature was regulated according to the CO₂
126 production, which is proportional to the sugar degradation, with a slope of 0.2°C/(g/L) of
127 evolved CO₂. This evolution of temperature simulated anisothermal conditions observed in
128 industrial-size tanks when the temperature rises freely until the final setpoint is reached [16].

129 Two anisothermal fermentations were run between 15 and 30°C, thus covering the maximum
130 range of temperatures used in winemaking. Another fermentation was conducted between 20

131 and 30°C, simulating a common temperature profile for red winemaking. All parameters and
132 control conditions for the fermentation experiments in this study are summarized in **Table 1**.

133

134 *2.2 Analysis of volatile compounds*

135

136 *2.2.1. Online measurements in the gas*

137 The gas was pumped at a flow rate of 14 mL/min from the tank headspace through a
138 heated transfer line and concentrated in a cold trap (Tenax TM) for 6 min (desorption at
139 160°C for 1 min), and injected into a ZBWax (60 m × 0.32 mm × 0.5 µm, Phenomenex Inc.).
140 The injector was kept at 200°C. Helium was used as the carrier gas at a constant pressure of
141 120 kPa. The oven temperature program was 38°C for 3 min, 3°C/min up to 65°C, then
142 6°C/min to 160°C, held for 5 min, then 8°C/min up to 230°C and held for 5 min. A flame
143 ionisation detector (FID) was used at 260°C.

144 The on-line GC system was calibrated by using a Sonimix 6000C1 (LNI Schmidlin
145 SA). This equipment generates standard gases by dilution from standard gas bottles or
146 permeation tubes. Standard gas bottles (Air Product) containing 4004, 85.1 and 100
147 **mmol/Kmol** of ethyl acetate (CAS number 141-78-6), isoamyl acetate (CAS number 123-
148 92-2) and isobutanol (CAS number 78-83-1), respectively, were used. A permeation tube
149 with a permeation rate of 4831 ng/min at 45°C (LNI Schmidlin SA) was used to calibrate
150 ethyl hexanoate (CAS number 123-66-0) concentration. The permeation tube was placed
151 in an oven at 45°C, and diluted with air at 51 mL/min.

152

153 *2.2.2. Measurements in the liquid*

154 NaCl (1 g) was added to 3 **mL** of the fermentation sample in a 20 **mL** vial. To standardise
155 the equilibrium conditions between the liquid and the headspace, the ethanol concentration in

156 the vial was adjusted to 11% by adding 2 mL of a mixture of 12 g/L tartaric acid solution
157 diluted either in water or a ethanol/ water mix (30% v/v). Fifty μ L of 4-Methylpentan-2-ol at
158 a concentration 3 g/L was added to the vial as an internal standard. The sample vial was
159 heated and agitated for 5 min at 50°C in a headspace autosampler HT200 equipped with a
160 gastight syringe, preheated to 60°C. One mL of headspace gas was analysed by using a
161 HP6890 GC coupled with a FID detector. The injector temperature was 240°C. The GC oven
162 was equipped with a BP20 column (30 m \times 0.53 mm \times 1.0 μ m, SGE). H₂ was used as the
163 carrier gas at a constant flow rate of 4.8 mL/min. The oven temperature programme was 40°C
164 for 3 min, 3°C/min to 80°C, 15°C/min to 160°C held for 1 min, then 30°C/min to 220°C and
165 then held at 220°C for 2 min. The detector was set at 250°C. Peak areas were acquired with
166 Agilent Chemstation software.

167

168 2.3. Determinations of gas-liquid partition coefficients (k_i)

169

170 The gas-liquid partition coefficients (k_i) during fermentation were followed by dividing the
171 volatile concentrations in the tank headspace by the concentrations in the liquid at various
172 times. Several k_i were also determined in samples taken at different stages of fermentation by
173 using the Phase Ratio Variation (PRV) method in static conditions as previously described
174 [12, 17].

175

176 2.4. Modelling

177

178 The equations of the mathematical model (listed in the section Results) were implemented
179 in a program written under Matlab 7 (The Matworks Inc., Natick, MA). The parameters were
180 identified by nonlinear regression under Matlab, using the Statistic Toolbox.

181

182 **3. Results and discussion**

183

184 *3.1. Model development*

185

186 *3.1.1. Effects of CO₂ stripping and of must composition*

187 Morakul et al. [12] used synthetic solutions with increasing ethanol concentrations and
188 decreasing sugar concentrations to simulate the evolution of the composition of must during
189 fermentation. They showed that the gas-liquid partition coefficients (k_i) of higher alcohols and
190 esters continuously decreased as the composition of a model wine fermentation medium
191 changed because the sugar induces ‘salting out’ of volatile compounds (at the beginning of a
192 fermentation) whereas the ethanol increases their solubility, and thereby decreases their
193 volatility. The authors also observed a decrease of the relative gas-liquid ratio of these
194 molecules during fermentation. However, the ratios were expressed in arbitrary units and
195 could not be directly compared to the values of k_i obtained in synthetic solutions, without any
196 release of CO₂.

197 To complete this previous preliminary study, the first aim of the present work was to
198 clarify the effect of stripping on gas-liquid partitioning of aroma **compounds**. The stripping
199 effect is complex because, in usual fermentations, both the rate of CO₂ production and other
200 factors vary throughout the fermentation process. The problems associated with this
201 complexity were overcome by controlling the rate of CO₂ release. The effect of stripping was
202 indeed isolated by running defined fermentations, in which the rate of CO₂ production was
203 kept constant by perfusion of ammoniacal nitrogen controlled. **By modifying the amount of**
204 **assimilable nitrogen initially present in the must i.e. 40 mg/L or 120 mg/L (no addition or**
205 **addition of 80 mg/L of ammoniacal nitrogen), it was possible to set up two fermentations**

206 (CRF-0.3 and CRF-0.6) in which the rates of CO₂ production were kept constant at 0.3 g /L.h
207 and 0.6 g /L.h respectively. The rate of CO₂ production was regulated between 10 and 85% of
208 the fermentation progress. Fig. 1 compares the evolution of the CO₂ production rate (i) in
209 these two fermentations and in (ii) an isothermal fermentation at 20°C, without any control of
210 the CO₂ production rate (IF-20-B).

211 Changes in gas-liquid concentration ratios of ethyl acetate, isoamyl acetate, ethyl
212 hexanoate and isobutanol were compared in these three different fermentation conditions.
213 They were also compared to the values of k_i calculated by the static headspace PRV method
214 in samples taken during fermentation. In these samples, k_i was measured at equilibrium, in the
215 absence of CO₂ release. **Fig. 2** shows the results obtained for isobutanol and isoamyl acetate,
216 the following observations being also valid for the other volatile molecules (data not shown).
217 A very remarkable result was that (i) almost identical decreases in k_i with increasing ethanol
218 **concentration** were observed whatever the CO₂ production rate and (ii) values of the partition
219 coefficients were close to those obtained at equilibrium without any CO₂ release. It can
220 therefore be concluded that stripping did not significantly change the gas-liquid partitioning
221 of aroma compounds during fermentation and that the two phases always remained at
222 equilibrium throughout the process in spite of the CO₂ flux.

223 Therefore, at constant temperature, the values of k_i **which** reflect changes in the gas-liquid
224 partitioning of aroma compounds in fermenting musts only result from changes in the
225 composition of the liquid phase, that is the decreasing sugar concentration and increasing
226 ethanol concentration.

227 Consequently, at constant temperature, the evolution of k_i can be written as follows:

$$228 \quad k_i = A \times E + B \quad \text{(Equation 1)}$$

229 where **A and B are constants depending on the considered compound i**, and E is the ethanol
230 concentration (g/L) in the liquid phase, which is proportional to the sugar consumption and

231 CO₂ production. E is therefore representative of the whole matrix effect corresponding to the
232 modification of the ethanol and sugar concentrations.

233

234 3.1.2 Effect of temperature

235 Gas-liquid partitioning not only depends on the composition of the liquid phase; it is also
236 strongly affected by the temperature. For a constant medium composition, the Clausius-
237 Clapeyron law is usually applied to the changes in partition coefficient (k_i) with temperature
238 [19]:

$$239 \quad -\frac{d(\ln k_i)}{d(1/T)} = \frac{\Delta H_{vap}}{R} \quad \text{or} \quad \ln k_i = C - \frac{\Delta H_{vap}}{R \times T} \quad (\text{Equation 2})$$

240 Where ΔH_{vap} is the phase change enthalpy of the volatile compound expressed in J/mol, T
241 is the absolute temperature (K), R is the perfect gas constant (8.413 J/mol.K) and C is a
242 constant.

243

244 Nevertheless, Morakul et al. [12] showed that, in synthetic media, the value of the
245 parameter ΔH_{vap} is not constant. For example, the ΔH_{vap} of isobutanol was 71.4 kJ/mol in a
246 synthetic medium simulating a grape juice and 37.8 kJ/mol in a synthetic medium simulating
247 a wine. As a consequence, the effect of the temperature on the gas-liquid partitioning not only
248 depends on the temperature but is also a function of the composition of the liquid phase. So,
249 the classical Clausius-Clapeyron **expression was** modified to introduce the dependence of the
250 values C and ΔH_{vap} on the medium composition:

$$251 \quad \ln k_i = D1 + D2 \times E - \frac{D3 + D4 \times E}{R \times T} \quad (\text{Equation 3})$$

252 Where T is the absolute temperature and D1, D2, D3 and D4 are constants. To give a
253 clearer physical meaning to the parameters of the model, we modified the previous equation
254 by including a reference temperature (T_{ref}), so the model expression became:

$$\ln k_i = F1 + F2 \times E - \frac{F3 + F4 \times E}{R} \left(\frac{1000}{T} - \frac{1000}{T_{ref}} \right) \quad (\text{Equation 4})$$

Where T is the **current absolute** temperature, T_{ref} corresponds to the absolute reference temperature, i.e 293 K (20°C) in this study and F1, F2, F3 and F4 are constants. F1 is the logarithm of the partition coefficient ($\ln k_i$) at the reference temperature in the initial must (E = 0). F2 represents the sensitivity of the partition coefficient to medium composition at the reference temperature. F3 corresponds to the value of ΔH_{vap} in the initial must (E = 0), ΔH_{vap} giving the sensitivity of k_i to changes in temperature. F4 represents the sensitivity of ΔH_{vap} to changes in medium composition, described here as the ethanol concentration. The arbitrary factor 1000 was introduced for numerical convenience, to have numeric parameter values (F1-F4) of order of one. This generally favours reliable identification with nonlinear regression software. This factor can be of course absorbed into the values of F3 and F4.

The mathematical expression detailed in Equation 4 was then used in the subsequent steps to model the evolution of the k_i values for ethyl acetate, isoamyl acetate, ethyl hexanoate and isobutanol throughout the wine fermentation as a function both of the ethanol production and of the temperature.

270

271 *3.2 Model identification*

272

Model parameters in Equation 4 were determined **simultaneously** by nonlinear regression based on the values of the k_i (in concentration ratio) obtained from three experiments (i) isothermal fermentation at 20°C (IF-20-A) (ii) isothermal fermentation at 30°C (IF-30) and (iii) anisothermal fermentations between 15 and 30°C (**AF-15-30**). All k_i measurements (**41 values, including 14 from IF-20-A, 11 from IF-30 and 16 from AF-15-30**) were used to determine the parameters F1-F4 together with their standard **errors (Table 2)**.

279 The estimated values of F1 were consistent with the values of $\ln k_i$ measured by the PRV
280 method [12] in static conditions in the must at the beginning of fermentation (-5.11, -3.94, -
281 3.68 and -7.72 for ethyl acetate, isoamyl acetate, ethyl hexanoate and isobutanol,
282 respectively). Among the volatile compounds studied, ethyl hexanoate had the highest $\ln k_i$
283 consistent with the higher volatility of this compound whereas isobutanol, which had a lower
284 value of $\ln k_i$, is always less volatile than esters.

285 F2 values were negative, indicating that k_i decreased as the ethanol concentration
286 increased. The most negative value indicates the greatest sensitivity of k_i to the changes in
287 liquid composition. Ethyl hexanoate was the molecule most affected by the liquid
288 composition ($F2 = -1.39 \times 10^{-2}$) and ethyl acetate and isobutanol were less sensitive with F2
289 values of -2.90×10^{-3} and -4.10×10^{-3} , respectively. This sensitivity is seemingly related to the
290 hydrophobicity of the molecule. Indeed, the hydrophobicity constant values ($\text{Log}K_{ow}$ at
291 25°C), i.e. 0.76, 0.73, 2.25 and 2.83, for isobutanol, ethyl acetate, isoamyl acetate and ethyl
292 hexanoate, respectively (SRC Interactive PhysProp database, Syracuse), are in the same order
293 as F2.

294 The values of F3 representing the sensitivity of k_i to the temperature, were compared to
295 previously reported ΔH_{vap} values [12]. The ΔH_{vap} was 39, 39.4, 67.5 and 71.4 kJ/mol for ethyl
296 acetate, isoamyl acetate, ethyl hexanoate and isobutanol, respectively. Although the values
297 are in the same order of magnitude as our F3 values, there are differences of about 20% for
298 isoamyl acetate, ethyl hexanoate and isobutanol. These differences between F3 and ΔH_{vap}
299 may be a consequence of the differences in the matrix used, as the F3 values were identified
300 using the natural must whereas ΔH_{vap} were calculated using a synthetic medium which
301 contained only sugar and weak acids to simulate the must at the start of the fermentation. The
302 difference between F3 and ΔH_{vap} for ethyl acetate is higher than 40%, and this might be due
303 to an atypical behaviour of this compound. Indeed, temperature had little influence on the

304 value of k_i of ethyl acetate and therefore, it is difficult to determine precisely the values of F3
305 and F4.

306 A sensitivity analysis of the model was conducted to assess the effect and the relative
307 importance of the model parameters. Average conditions ($T=25^\circ\text{C}$ and ethanol concentration
308 $E=45\text{g/L}$) were selected and each parameter (F1-F4) was arbitrarily increased by 30%. As
309 expected, parameter F1 (directly related to the partition value) had the higher sensitivity,
310 comprised between 64% for ethyl hexanoate and 92% for isobutanol. Its knowledge is thus
311 the most important for accurately predicting k_i . The second most important parameter
312 (between 10 and 16% sensitivity) was F3, confirming the usually reported fact that
313 temperature has a significant effect on volatility. The effect of the medium composition
314 expressed via F2 was similar (between 4% for ethyl acetate and 17% for ethyl hexanoate).
315 Finally, parameter F4 was found to have some effect only for ethyl acetate (4%), and less than
316 1% for the other compounds studied; this is consistent with the model identification results
317 indicating that a significantly different from zero value of F4 could only be determined for
318 ethyl acetate.

319

320 *3.3. Model validation*

321

322 After parameter identification, the variation of k_i as a function of ethanol concentration and
323 temperature, according to equation 4, was plotted. **Fig. 3** shows the plot for fermentations
324 used for parameter identification and **Fig. 4** that for independent fermentations: (i) isothermal
325 fermentation at 20°C (IF-20-B) and (ii) anisothermal fermentation between 20 and 30°C (AF-
326 20-30).

327 The mean relative error between model prediction values and the measured values was
328 calculated as follows:

$$\varepsilon = \frac{1}{n} \sum \frac{|k_{ij}^{measured} - k_{ij}^{predicted}|}{k_{ij}^{measured}} \times 100\% \quad (\text{Equation 5})$$

Where n is the number of k_i measurements used for model validation. **Table 3** indicates that (i) the average differences between the experimental and the calculated values were less than 10% for isoamyl acetate, ethyl hexanoate and isobutanol and (ii) the precision of the k_i estimations was comparable for data from experiments not used for parameter identification. These results demonstrate the value of the model to predict k_i with a good accuracy for these 3 compounds. The prediction was much less satisfactory for ethyl acetate with differences up to 33%, due to an atypical behaviour of this compound.

One of the main reasons why predicting k_i is valuable is that it allows calculation of the concentrations of volatiles in the gas phase from measurements in the liquid, and the reverse. It is therefore possible to calculate the global production by adding the volatile concentration in the liquid to the amount lost in the gas phase (Equation 6):

$$Losses = \frac{\int_0^{t_{end}} C^{gas}(t) \times Q(t) \times dt}{C^{liq}(t_{end}) + \int_0^{t_{end}} C^{gas}(t) \times Q(t) \times dt} \times 100\% \quad (\text{Equation 6})$$

Where t is the current time (h), t_{end} is the final time (h), $C^{gas}(t)$ is the concentration of volatile compound in the gas phase at time t expressed in mg/L of CO₂, $Q(t)$ is the CO₂ specific flow rate at time t expressed in (L of CO₂/L of must)/h and C^{liq} is the total concentration of the volatile compound in the must at the end of the fermentation (mg/L of must).

The relative amount of volatiles lost, i.e the ratio of losses to total production, is of particular technological interest. **Table 4** compares measured (using concentrations in the gas) and predicted (using k_i values and concentrations in the liquid) loss values. The predicted losses were very close to the values measured, illustrating the accuracy of the model. The

351 amounts of lost volatile in the gas phase varied with the volatility of the compounds: it was
352 negligible in the case of isobutanol but was 70% for ethyl hexanoate at 30°C.

353

354 **4. Conclusion**

355

356 The gas-liquid partitioning of the main aroma compounds produced during winemaking
357 fermentations, namely isobutanol, isoamyl acetate, ethyl hexanoate and to a lesser extent ethyl
358 acetate, was accurately predicted by the model. The model, based on the effects of changes to
359 the matrix and temperature during fermentation, allowed estimation of the partition
360 coefficient (k_i) with less than 10% error, except for ethyl acetate. The benefits of predicting k_i
361 include allowing the calculation of the total production of the volatile compounds from a
362 single measure (concentration in the gas or in the liquid phase). This is particularly
363 advantageous in the case of on-line monitoring of the main aroma compounds in the gas, as
364 described by Mouret et al. [2]. The ability to calculate the total production and to differentiate
365 between the amounts remaining in the liquid and those lost in the CO₂ are major issues for
366 improving our understanding of yeast metabolism and optimising fermentation control. From
367 a microbiological point of view, the total amount produced needs to be considered whereas,
368 from a technological point of view, the concentration remaining in the wine is more
369 important. For some molecules, such as isobutanol, the losses in the gas are negligible but for
370 more volatile compounds, in particular esters, such losses can represent a very significant
371 proportion of the total production. **Minimising these losses, by optimizing the fermentation
372 control, particularly the temperature profile, is a significant challenge. The objective is to find
373 the best compromise between fermentation kinetics and aroma production. The development
374 of metabolic models predicting the synthesis of aroma compounds [20], in combination with**

375 the model of gas-liquid partitioning and with a kinetic model [21] represents a complex but
376 very promising prospect.

377

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379

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382

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434 assimilable nitrogen and temperature on fermentation kinetics in enological conditions.
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- 436

437 **Figure captions**

438

439 **Fig 1-1.** Evolution of the CO₂ production rate as a function of ethanol concentration, for a
440 standard fermentation IF-20-B (...), and for constant rate fermentations at CO₂ production
441 rates of 0.3 g/L.h CRF-0.3 (+) and 0.6 g/L.h CRF-0.6 (— —) and assimilable nitrogen
442 concentration added to control the CO₂ production rate (—). Initial assimilable nitrogen
443 concentrations in the musts: 240, 40 and 120 mg/L. Temperature: 20°C.

444 **Fig 2.** Changes in gas-liquid ratio (k_i) as a function of ethanol concentration for isoamyl
445 acetate (A) and isobutanol (B); standard fermentation IF-20-A (×), constant rate fermentation
446 at 0.3 g/L.h CRF-0.3 (□) and constant rate fermentation at 0.6 g/L.h CRF-0.6 (Δ).
447 Comparison with k_i measured in static conditions by the PRV method (◇). Temperature:
448 20°C.

449 **Fig 3.** Comparison of predicted and measured k_i for isoamyl acetate (B) and isobutanol (C) in
450 fermentations run at different fermentation temperatures (A) for model identification. (B) and
451 (C) show predicted (...) and measured (□) k_i during an isothermal fermentation at 20°C (IF-
452 20-A); predicted (—), measured (●) k_i during an anisothermal fermentation run between 15-
453 30°C (AF-15-30); predicted (—), measured (×) k_i during an isothermal fermentation at
454 30°C (IF-30).

455 **Fig 4.** Comparison of predicted and measured values for k_i for isoamyl acetate (B) and
456 isobutanol (C) in fermentations run at different fermentation temperatures (A) for model
457 validation. (B) and (C) show predicted (...) and measured (□) k_i during an isothermal
458 fermentation at 20°C (IF-20-B); predicted (—), measured (●) k_i during an anisothermal
459 fermentation run between 20-30°C (AF-20-30). The (A) graph shows temperature profiles for
460 the two fermentation runs IF-20-B and AF-20-30.

1 **Table 1.** Experimental conditions of the fermentation trials, used for model identification and
 2 for model validation.

Fermentations	Initial assimilable nitrogen (mg/L)	Regulated temperature (°C)	Identification / validation
Constant rate fermentations (CRF) ^a			
1) CRF-0.3	40	20	
2) CRF-0.6	120	20	
Isothermal fermentations (IF) ^b			
3) IF-20-A	140	20	Model identification
4) IF-20-B	240	20	Model validation
5) IF-30	240	30	Model identification
Anisothermal fermentations (AF) ^c			
6) AF-15-30	140	15 to 30	Model identification
7) AF-20-30	140	20 to 30	Model validation

3 ^aThe rate of CO₂ production was kept constant at 0.3 and 0.6 g/L.h by addition of ammoniacal
 4 nitrogen

5 ^bThe temperature during fermentation was regulated at the indicated constant values

6 ^cThe fermentation temperature was increased by 0.2°C per g/L of CO₂ produced

- 1 **Table 2.** Numerical values for the model parameters identified from Equation 4 and given
 2 with their standard error.

Parameter	Ethyl acetate	Isoamyl acetate	Ethyl hexanoate	Isobutanol
F1 (-)	-6.11 ± 0.07	-3.98 ± 0.03	-3.09 ± 0.31	-8.45 ± 0.03
F2 (g/L)	$-2.9 \times 10^{-3} \pm 1.2 \times 10^{-3}$	$-9.6 \times 10^{-3} \pm 0.5 \times 10^{-3}$	$-1.39 \times 10^{-2} \pm 0.06 \times 10^{-2}$	$-4.1 \times 10^{-3} \pm 0.5 \times 10^{-3}$
F3 (kJ/mol ^l)	71 ± 9	49 ± 4	55 ± 4	53 ± 4
F4 (kJ mol/g.L)	$-4.4 \times 10^{-1} \pm 1.5 \times 10^{-1}$	$-1.7 \times 10^{-3} \pm 59 \times 10^{-3}*$	$8.6 \times 10^{-2} \pm 6.1 \times 10^{-2}*$	$6.4 \times 10^{-3} \pm 60 \times 10^{-3}*$

- 3 * A standard error leading to the value zero being included in the 95% confidence interval
 4 means that the parameter is not significantly different from zero.

5

- 1 **Table 3. Mean relative errors (%)** between predicted and measured k_i calculated according to
 2 equation 5, with n: number of k_i measurements per fermentation.

Fermentations	Ethylacetate		Isoamylacetate		Ethyl hexanoate		Isobutanol	
	%	n	%	n	%	n	%	n
Fermentations used for model identification								
Anisothermal 15-30°C	12.8	16	4.13	16	4.1	16	3.28	16
Isothermal at 20°C	13.5	14	5.15	14	6.5	14	6.23	14
Isothermal at 30°C	14.5	11	3.47	10	7.0	12	4.63	12
Mean	13.5		4.25		5.7		4.65	
Independent fermentations used for model validation only								
Anisothermal 20-30°C	17.2	11	9.07	11	6.6	11	7.94	11
Isothermal at 20°C	32.8	10	5.77	10	4.2	10	4.92	10
Mean	24.7		7.42		5.4		6.50	

3

- 1 **Table 4. Volatile** compound losses (%). Comparison of predicted* (pred.) and measured**
 2 (meas.) values for losses, in %.

Experiments	Ethyl acetate		Isoamyl acetate		Ethyl hexanoate		Isobutanol	
	pred.	meas.	pred.	meas.	pred.	meas.	pred.	meas.
Anisothermal 15-30°C	7.48	6.87	33.3	33.7	54.2	54.3	0.66	0.65
Isothermal at 20°C	5.86	6.02	25.2	25.6	44.6	44.4	0.55	0.54
Isothermal at 30°C	13.6	13.0	44.1	42.2	70.9	71.0	1.33	1.33
Anisothermal 20-30°C	10.5	12.5	45.0	46.7	66.3	64.7	0.93	1.01
Isothermal at 20°C	5.59	8.73	27.1	26.2	46.2	45.3	0.63	0.63

- 3 *Predicted losses were calculated from k_i values and concentrations of the volatiles in the
 4 liquid.
 5 **Measured losses were calculated from concentrations of the volatiles in the gas.

Fig 1

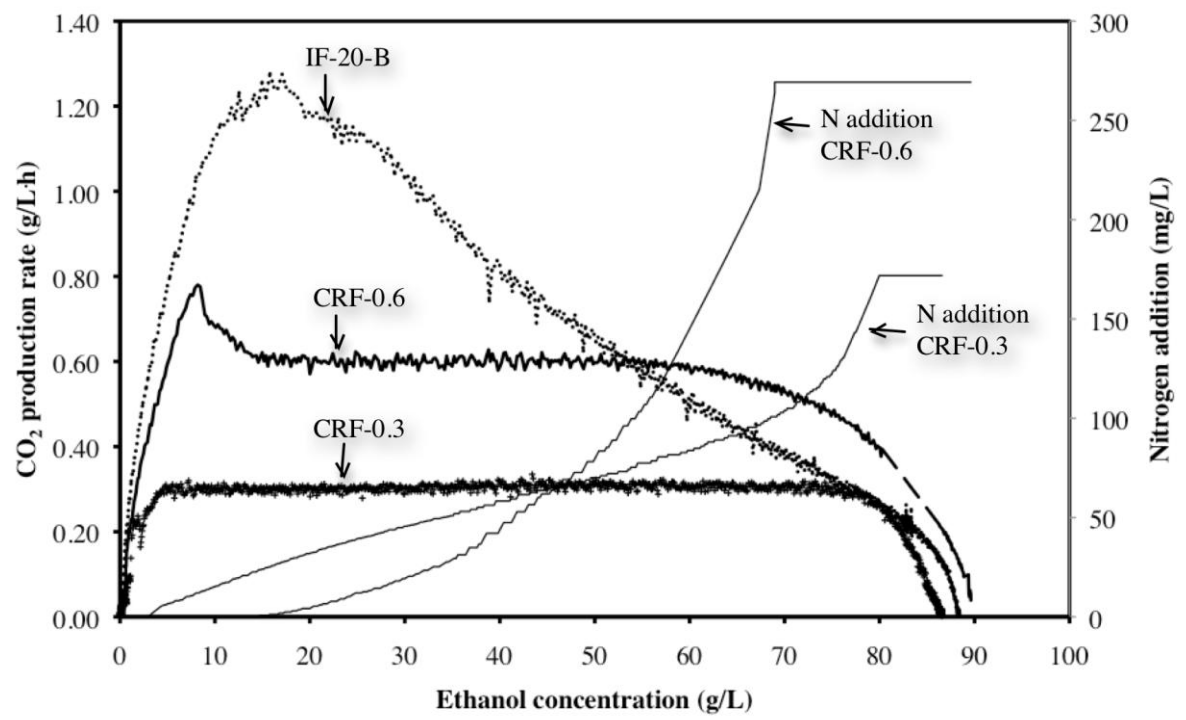


Fig 2.

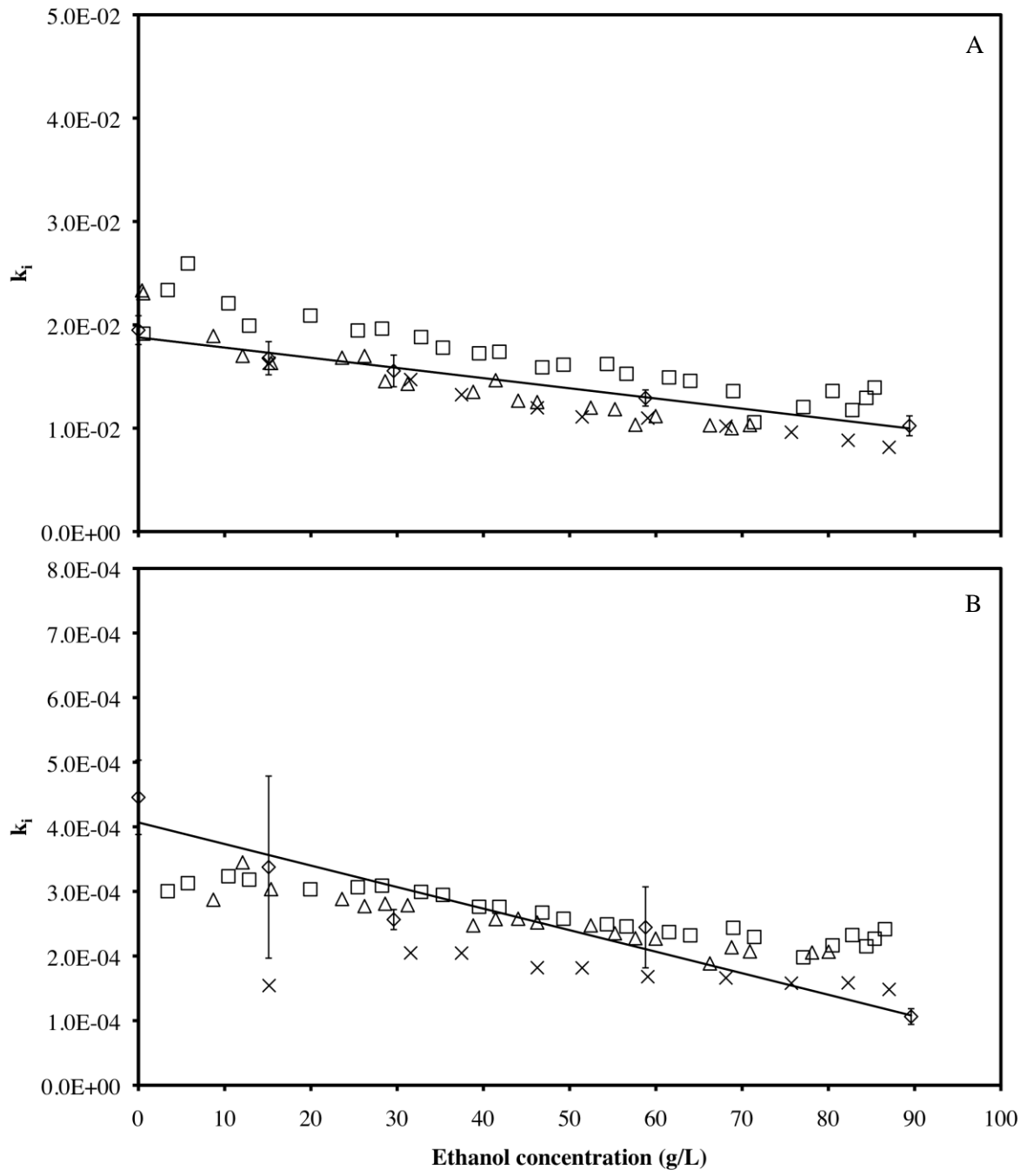


Fig 3

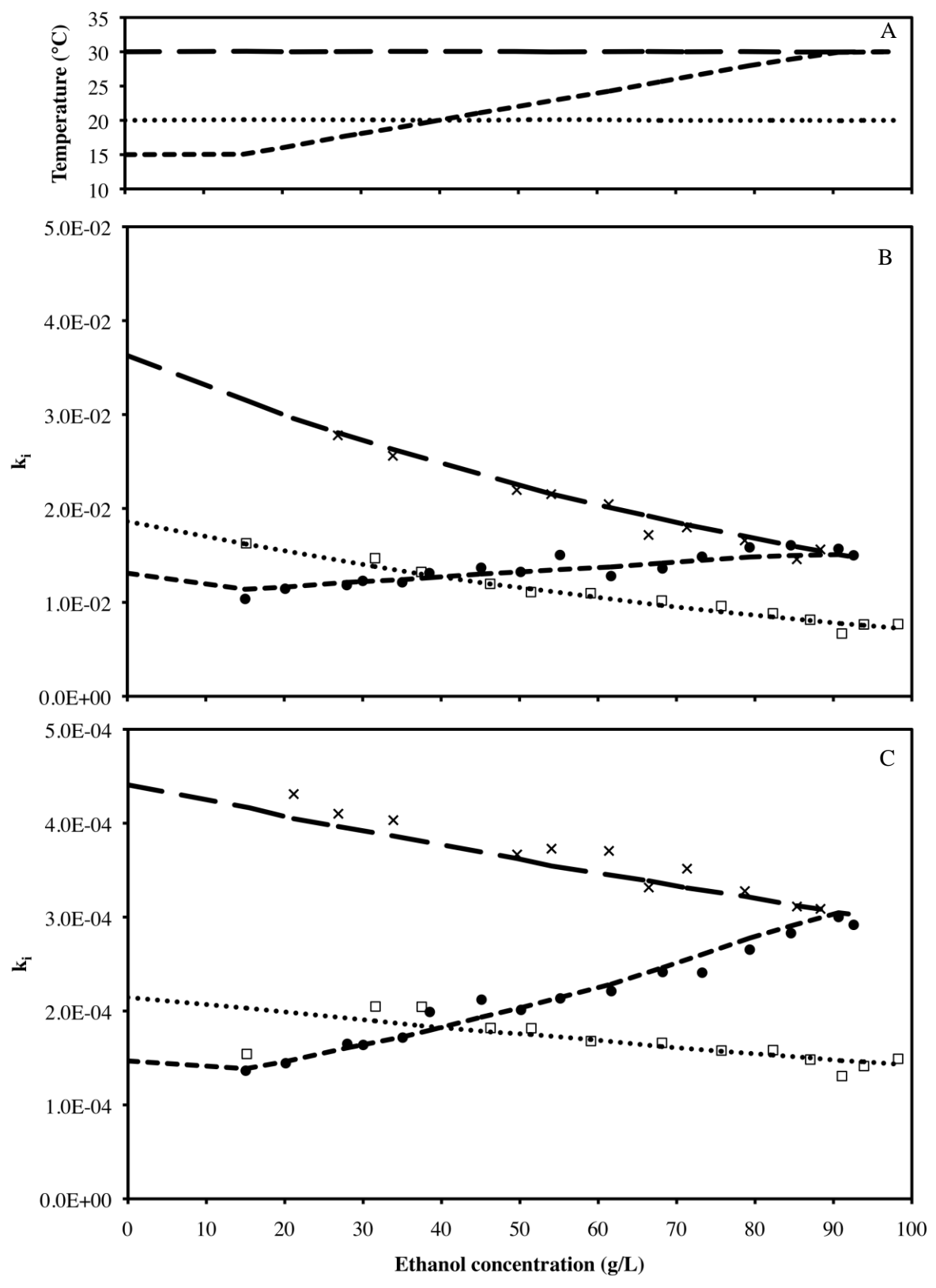


Fig 4.

