Influence of different yeast/lactic acid bacteria combinations on the aromatic profile of red Bordeaux wine

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1   ABSTRACT

2

3   BACKGROUND: The typical fruity aroma of red Bordeaux wines depends on the grape variety

4   but also on microbiological processes, such as alcoholic and malolactic fermentations. These

5   transformations involve respectively the yeast *Saccharomyces cerevisiae* and the lactic acid

6   bacterium *Oenococcus oeni*. Both species play a central role in red winemaking but their

7   quantitative and qualitative contribution to the revelation of the organoleptic qualities of wine

8   has not yet been fully described. The aim of this study was to elucidate the influence of

9   sequential inoculation of different yeast and bacteria strains on the aromatic profile of red

10   Bordeaux wine.

11   RESULTS: All microorganisms completed fermentations and no significant difference was

12   observed between tanks regarding the main oenological parameters until 3 months' aging.

13   Regardless of the yeast strain, B28 bacteria required the shortest period to completely degrade

14   the malic acid, compared to the other strain. Quantification of 73 major components highlighted

15   a specific volatile profile corresponding to each microorganism combination. However, the

16   yeast strain appeared to have a predominant effect on aromatic compound levels, as well as on

17   fruity aroma perception.

18   CONCLUSION: Yeasts had a greater impact on wine quality and have more influence on the

19   aromatic style of red wine than bacteria.

20

21   KEYWORDS

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23   Red wine, yeast, lactic acid bacteria, aromatic compounds

INTRODUCTION

In the past, the aroma of red wines was characterized empirically by fruity notes and, more specifically to Bordeaux wines, descriptors referred to red and black berry fruit, such as raspberry, cherry, and blackcurrant. Recently, Pineau *et al.*(1) demonstrated the existence of a sensory space specific to Bordeaux red wines. These fruity notes are not detected in must, but are revealed during the various stages in winemaking and aging. Schematically, red winemaking includes three important steps: alcoholic fermentation (AF), maceration and malolactic fermentation (MLF). Fermentation processes play a central role in flavor development and microorganisms, which take part in the vinification, act more or less in-depth on the composition of wine and through their action are largely responsible for its taste and its aroma.(2)

During AF, yeasts such as *Saccharomyces cerevisiae* play a significant role in the formation and modulation of wine taste and aromas(3–5) by releasing varietal aromatic compounds from grape precursors,(6,7) as well as synthesizing *de novo* volatile compounds.(8,9) In contrast, the influence of MLF and lactic acid bacteria (LAB), such as *O. oeni*, on red wine fruity aroma is not as clear. MLF is often empirically associated with a decrease in the intensity of fruity notes. However, according to the literature, LAB enhance the fruity aroma of red wines in some cases, attenuate it in others, and sometimes have no influence on it at all.(10). These diverging results may be explained either by the use of different LAB strains in these studies or by a matrix effect involving the cultivar and the yeast strain used to carry out AF as well as the LAB. Indeed, it is well known that yeasts influence LAB growth during winemaking.(11,12) Therefore, it would not be surprising that they also influence LAB metabolism and thus the aromatic compounds in the wine. The few studies investigating these

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48 effects demonstrated significant differences in the aroma of Chardonnay(13) and Chancellor

49 wines(14) fermented with several yeast/LAB strain combinations, at different temperatures.

50   The lack of fundamental data on the aromatic markers responsible for the fruity aroma

51 of red wines is probably another reason for the lack of consensus. Recent studies suggested that

52 these fruity notes were due to perceptive interactions between different families of aromatic

53 compounds, rather than individual compounds.(15,16) Varietal compounds, such as C_{13}-

54 norisoprenoids,(17) lactones,(18) thiols,(19) sulfur-containing compounds such as dimethyl

55 sulfide,(20) and yeast- and LAB-derived compounds, including higher alcohols,(21) esters,(22)

56 volatile fatty acids,(23) and diacetyl(24) are examples of aromatic molecules that have a

57 negative or positive impact on red wine aroma.

58   This uncertainty surrounding the influence of fermentative microorganisms on wine

59 quality is problematic for winemakers. From a practical point of view, it would be useful to

60 know whether the influence of LAB strains on red wine quality is affected by some winemaking

61 variables, particularly the yeast strain used for AF. Thus the aim of this study was to analyze

62 the impact of different yeast/LAB combinations on the pool of aromatic markers potentially

63 responsible for the perception of fruity notes in red wines. Several combinations of yeast and

64 LAB were studied, using three commercial *Saccharomyces cerevisiae* strains and two

65 commercial *O. oeni* strains in sequential inoculation. Seventy-three compounds known to

66 contribute to the fruity notes of red wines were quantified using methods previously developed

67 in our laboratory.

68

69 **MATERIAL AND METHODS**

70

71 **Yeast and Bacteria Strains**

4

72 The three commercial *Saccharomyces cerevisiae* strains used in this work were Actiflore

73 cerevisiae® (522D), Zymaflore FX10® (Laffort, Floirac, France), and Excellence XR

74 (Lamothe-Abiet, Canéjan, France). Yeast implantation was verified by Polymerase Chain

75 Reaction (PCR) at the SARCO laboratory (Laffort, Floirac, France) (data not shown). Two

76 commercial *O. oeni* strains, Lactoenos 450 PreAc® and Lactoenos B28 PreAc® (Laffort,

77 Floirac, France), were used as MLF starters in this study. Bacteria implantation (data not

78 shown) was verified by the Microflora® laboratory (University of Bordeaux, France), using a

79 method developed by Claisse and Lonvaud-Funel.(25)

80

81 **Winemaking**

82 Cabernet Sauvignon grapes from the Bordeaux appellation in the 2011 vintage were manually

83 harvested, destemmed, crushed and homogenously distributed into nine 2 hL stainless-steel

84 tanks (150 kg grapes per tank). Grape must was treated by adding pectolytic enzyme (Lafase®

85 Fruit, 0.03 µg g^{-1}, Laffort, Floirac, France) and yeast assimilable nitrogen was corrected to

86 around 210 mg N L^{-1} by adding ammonium sulfate (Laffort, Floirac, France). Alcoholic

87 fermentation was conducted at 19-22 °C and initiated by inoculation with rehydrated dried

88 yeasts according to the manufacturer's recommendations. AF was performed in triplicate for

89 each yeast strain. Implantation in each tank was verified in the middle of AF (density close to

90 1.040). On completion of AF (<0.2 g L^{-1} glucose/fructose), each 2 hL tank was divided into two

91 30 L stainless steel barrels for MLF. Bacterial cells were rehydrated with bacterial nutrient

92 (Energizer®, Laffort, Floirac, France) according to the manufacturer's instructions and

93 inoculated into wines at the recommended rate. For the entire duration of MLF, the malic acid

94 concentration was measured once per week to monitor the bacterial metabolism. At the end of

95 MLF (<0.1 g L^{-1} malic acid), 50 g hL^{-1} SO$_2$ was added. Wines were drained into 20 L stainless

96 steel barrels for 3 months' aging. After 3 months, wine composition was analyzed (total and

97 volatile acidity, total and free SO_2 content, pH, alcohol content) (Table 1). Samples were

98 collected for volatile compound analysis in 0.75 L glass bottles and stored at 10 °C for 1 week.

99 SO_2 content was measured and adjusted, if necessary. Wines were then decanted and frozen at

100 -18 °C until analysis.

101

102 **Standard Chemical Analyses**

103 The standard chemical parameters of the wines (total acidity, sugar, malic acid, yeast

104 assimilable nitrogen, SO_2 content, pH, and alcohol) were analyzed by SARCO laboratory

105 (Laffort, Floirac, France), which has been accredited by COFRAC since 1995 (NF EN ISO

106 17025, accreditation N°1-0588). Analyses were carried out using the official methods or those

107 recommended by the International Organization of Viticulture and Wine (OIV)(26).

108

109 **Volatile Compound Analyses**

110 Each wine sample was analyzed simultaneously after defrosting, which did not affect the aroma

111 compound concentrations in the racked wine. Eighty molecules were analyzed, using eight

112 different methods developed and validated in the laboratory.

113

114 **Chemicals**

115 Commercial compounds were used as internal standards: butan-1,4-diol was obtained from

116 Merck (Damstadt, Germany); 4-methylpentan-2-ol (99%), octan-3-ol (99%), thiophene

117 (>99%), hexan-2,3-dione (97%), and ethyl-2-hydroxyisobutyrate (98%) were supplied by

118 Sigma-Aldrich (Steinheim, Germany), as well as 1,2-diaminobenzene (98%), used for

119 derivatization. Methanol (>99.9%), dichloromethane (>99%), phosphoric acid (85%), sodium

120 hydroxide (98%), sulfuric acid (98%) and sodium chloride (norma pure) were purchased from

121 VWR Chemicals (Fontenay-sous-Bois, France). Diethyl ether (>99%) and isohexane (>99%)

122 were obtained from Carbo Erba Reactif-SDS (Val de Reuil, France) and ethanol (≥99.9%) from

123 Merck (Damstadt, Germany). Anhydrous sodium sulfate (99%) was supplied by Scharlau

124 Chemie (Sentmenat, Spain).

125

126 **Higher alcohols and ethyl acetate (direct injection and GC/FID analysis)**

127 Propan-1-ol, 2-methylpropanol, 2-methylbutan-1-ol, 3-methylbutan-1-ol and ethyl acetate were

128 quantified using a modified version of the official OIV method (OIV-MA-AS315-02A).

129 According to this method, 5 mL wine was spiked with 50 µL internal standard solution (4-

130 methylpentan-2-ol at 14.062 g L^{-1} in 50% hydroalcoholic solution). The vials were filled with

131 this solution for direct injection into an HP 5890 gas chromatograph coupled to a flame

132 ionization detector. The column was a CP-WAX 57 CB (50 m x 0.25 mm x 0.2 µm, Varian).

133 Quantification was performed using a calibration curve obtained from 12% hydroalcoholic

134 solution.

135

136 **Acetoin and butanediols (direct injection and GC/FID analysis)**

137 The method developed by de Revel *et al.*(27) was used to quantify acetoin, D-butan-2,3-diol

138 and meso-butan-2,3-diol. As specified in this method, 1 mL wine was spiked with 50 µL

139 internal standard solution (butan-1,4-diol at 1 g L^{-1} in 40% hydroalcoholic solution) and diluted

140 with 2 mL methanol. The vials were filled with this solution for direct injection into an Agilent

141 6890N gas chromatograph coupled to a flame ionizsation detector. The column was an FFAP

142 type (BP21, 50 m x 0.25 mm x 0.2 µm, SGE). Quantification was performed using a calibration

143 curve obtained from 12% hydroalcoholic solution.

144

145 **Volatile fatty acids (liquid-liquid extraction and GC/FID analysis)**

146 Butyric, hexanoic, octanoic, decanoic and dodecanoic acids were quantified using the method

147 developed by Bertrand.(28) In accordance with this method, 50 mL wine was spiked with 200

148 µL internal standard solution (octan-3-ol at 400 mg L^{-1} in 40% hydroalcoholic solution) and 0.3

149 mL phosphoric acid (diluted 1/3). Samples were successively extracted with 4 mL, 2 mL and 2

150 mL of a diethyl ether-isohexane mix (1:1, v/v). The organic phases were collected, dried with

151 anhydrous sodium sulfate and injected into an HP5890 gas chromatograph coupled to a flame

152 ionization detector. The column was an FFAP type (BP 21, 50 m x 0.25 mm x 0.2 µm, SGE).

153 Quantification was performed with calibration curves obtained from red wines.

154

155 **Volatile sulfur compounds (headspace-gas chromatography-flame photometric detection**

156 **(HS-GC/FPD analysis))**

157 Dimethyl sulfide (DMS) and hydrogen sulfide (H$_2$S) were quantified using the method

158 developed and validated by Anocibar-Beloqui *et al.*.(20) According to this method, 100 mL

159 wine was spiked with 10 µL internal standard solution (thiophene at 300 mg L^{-1} in ethanol) in

160 a 125 mL headspace vial. After 24 h at 22 °C, 1 mL of the gas phase was taken from the

161 headspace and injected into an HP5890 gas chromatograph coupled to a flame photometric

162 detector. The column was an HP5 (30 m x 0.25 mm x 0.25 µm, Agilent). Quantification was

163 performed using a calibration curve obtained from 12% hydroalcoholic solution.

164

165 **Diacetyl (liquid-liquid extraction after derivatization and gas chromatography-mass**

166 **spectrometry (GC-MS) analysis)**

167 The method developed by de Revel *et al.*(27) was used for direct quantification of diacetyl,

168 glyoxal, methylglyoxal and pentan-2,3-dione. In accordance with this method, 50 mL wine was

169 spiked with 100 µL internal standard solution (hexan-2,3-dione at 3.80 g L^{-1} in 50%

170 hydroalcoholic solution). Then, 5 mL 1,2-diaminobenzene was added and pH was adjusted to

171 8 with NaOH (10 mol L^{-1}). After a 3 h derivatization reaction at 60 °C, the pH of the mixture

172 was adjusted to 2 with sulfuric acid (2 mol L^{-1}) and it was extracted twice with 5 mL

173 dichloromethane. The organic phases were collected, dried with anhydrous sodium sulfate and

174 injected into an Agilent 6890N gas chromatograph coupled to a mass spectrometer (Agilent

175 5973). GC-MS analysis conditions were as previously described.[27] Quantification was

176 performed with a calibration curve obtained from 12% hydroalcoholic solution.

177

178 **C$_{13}$-Norisoprenoids and lactones (stir bar sorptive extraction GC/MS)**

179 This method, developed and validated by Antalick *et al.*,[29] was used to quantify four C$_{13}$-

180 norisoprenoids (*β*-damascenone, *β*-damascone, *β*-ionone, and *α*-ionone) and six lactones (*γ*-

181 octalactone, *γ*-nonalactone, *γ*-decalactone, *γ*-undecalactone, *γ*-dodecalactone, and *δ*-

182 decalactone). According to the method, 25 mL wine was spiked with 25 μL internal standard

183 solution (ethyl-*d5* cinnamate at 1.74 g L^{-1} in ethanol) and a 20 mL sample was introduced into

184 a 25 mL vial. A 20 mm × 1 mm (length × film thickness) PDMS stir bar (Twister®, 126 μL

185 coating) (Gerstel, Müllheim an der Ruhr, Germany) was dropped into the vial, which was

186 capped with a PTFE-faced rubber stopper. The closed vial was stirred at 900 rpm for 1 h at

187 room temperature. At the end of the extraction time, the Twister® was removed from the vial,

188 washed quickly with Milli-Q water, and dried with lint-free tissue. Each Twister® was then

189 transferred into a glass tube for thermal desorption (Gerstel) and GC-MS analysis, under the

190 conditions described previously.[29] Quantification was performed using calibration curves

191 obtained from red wines. Ethyl-*d5* cinnamate was synthesized using the method described by

192 Antalick *et al.*[30]

193

194 **Apolar esters (HS-SPME-GC-MS)**

195 The method developed and validated by Antalick *et al.*(30) was used to quantify 32 esters: six

196 ethyl fatty acid esters, seven higher alcohol acetates, four ethyl branched acid esters, four

197 methyl esters, three isoamyl esters, three ethyl esters with odd numbers of carbon atoms, two

198 ethyl cinnamates and some other minor esters. A mixture of ethyl-d_5 butyrate, ethyl-d_5

199 hexanoate, ethyl-d_5 octanoate and ethyl-d_5 cinnamate at about 200 mg L^{-1} in ethanol was used

200 as internal standard. Deuterated esters were synthesized as described by Antalick *et al.*(30) In

201 accordance with this method, 20 µL internal standard solution was added to 25 mL wine. An

202 aliquot of 10 mL was introduced into a 20 mL standard headspace vial containing 3.5 g sodium

203 chloride. The samples were extracted by HS-SPME and analyzed by GC-MS. The fiber used

204 was 100 µm polydimethylsiloxane (PDMS-100) (Supelco, Bellefonte, PA, USA), conditioned

205 before use as recommended by the manufacturer. Quantification was performed with calibration

206 curves obtained from red wines.

207

208 **Additional volatile compounds (liquid-liquid extraction and GC/MS analysis)**

209 The method developed and validated by Antalick *et al.*(29) was used to quantify seven polar

210 esters (ethyl lactate, ethyl leucate, ethyl succinates and hydroxylated ethyl esters), three

211 branched acids (isobutyric acid, isovaleric acid and 2-methylbutyric acid), frambinone and

212 linalol. According to this method, 50 mL wine was spiked with 10 µL internal standard solution

213 (ethyl-2-hydroxyisobutyrate at 0.96 g L^{-1} in ethanol). The mixture was successively extracted

214 with 4 mL, 2 mL, and 2 mL dichloromethane. The organic phases were combined, dried with

215 anhydrous sodium sulfate, and then analyzed by GC-MS, under the conditions described

216 elsewhere.(29) Quantification was performed with calibration curves obtained with red wines.

217

218 **Statistical Analyses**

219   Volatile compound concentrations and oenological parameters (milligrams or micrograms per

220   liter) were expressed as mean value ± standard deviation. The effects of yeast/LAB

221   combinations were tested using one-way and two-way analysis of variance. Principal

222   component analysis (PCA) was also carried out on the concentrations quantified for certain

223   compounds. Statistical analyses were performed using XL-STAT (Addinsoft, Paris, France),

224   whereas graphical representations of PCA were obtained using R v2.15.0 (R Development Core

225   Team 2009, Vienna, Austria; R Foundation for Statistical Computing).

226

227   **RESULTS AND DISCUSSION**

228

229   **Fermentation conditions and chemical composition of wines**

230   Six combinations of yeast/LAB starter cultures (three yeasts, two bacteria) were tested in

231   Cabernet Sauvignon wines made under micro-vinification conditions (2 hL). The whole

232   winemaking process, including AF and MLF, highlighting the kinetic performance of the

233   microorganisms, is presented in Fig. 1. Since no significant difference was observed between

234   the triplicate experiments, one representative fermentation curve is presented for each modality.

235   As shown in Fig. 1a, all AF followed the same pattern and were completed in 7 days

236   (170 h). Total reducing sugar in the must was around 218 g L^{-1} and no differences were found

237   between wines after AF (<1 g L^{-1}). There was a negligible difference in the ethanol

238   concentrations of the wines, with an average of 13.2% (v/v). The pH value of the must was

239   3.48, which had increased slightly after AF (around 3.51). No significant differences in total or

240   volatile acidity were found between wines. Finally, concentrations of L-malic acid in musts

241   fermented with the 522D and XR yeast strains decreased during AF (0.29 g L^{-1} and 0.18 g L^{-1}

242   respectively). This suggested that these two strains had the ability to metabolize malic acid in

243   the presence of glucose or other assimilable carbon sources.(31)

244   After alcoholic fermentation was completed, LAB were inoculated. As shown in Figure

245 1b, MLF was completed in every case, irrespective of the bacteria strain used. However, the

246 degradation kinetics of L-malic acid during the course of MLF varied depending on the LAB

247 strain. All *O. oeni* 450 samples completed MLF in 26 days (L-malic acid < 0.1 g L^{-1}),

248 irrespective of the yeast strain. In contrast, all B28 samples required much longer to complete

249 MLF: 31 days for XR/B28 and 522D/B28 and 33 days for FX10/B28. It is important to note

250 that dissimilarities in the kinetics of these two bacteria strains were not due to a difference in

251 the L-malic acid degradation rate. The latency phase of B28 strain was longer than that of the

252 450 (5 days), suggesting a differential adaptation to growth in wine.(32)

253   After 3 months' aging, differences between most of the oenological parameters of the

254 various modalities were negligible. Only volatile acidity, expressed in grams of acetic acid per

255 liter, was significantly affected by the LAB cultures. The largest increase was measured in

256 522D/B28 (0.23 g L^{-1}), XR/B28 (0.29 g L^{-1}), and FX10/B28 (0.30 g L^{-1}) samples, with

257 statistically significant differences depending on the LAB strain used. The influence of bacterial

258 strains on volatile acidity has already been reported.(33,34) Acetic acid is produced from citric

259 acid by some genera of LAB,(10) and the statistically significant differences in acetic acid

260 content observed may be due to degradation of larger quantities of citric acid by *O. oeni* B28.

261

262 Influence of yeast/LAB combination on wine aromatic compounds

263 Seventy-three major volatile compounds were quantified, including eight acids, six alcohols,

264 six aldehydes and ketones, six lactones, four C_{13}-norisoprenoids, two sulfur-containing

265 compounds, one terpene, and 40 esters, using analytical methods that previously developed and

266 validated in our laboratory. Concentrations measured in the different modalities are presented

267 in Tables 2 and 3. First, a one-way analysis of variance (ANOVA) was used to study the

268 yeast/LAB combination parameter. Results revealed a significant effect of the microorganism

269 combination on the concentrations of 51 volatile compounds, mainly alcohols, acids and esters.

270 Varietal compounds and α-dicarbonyl compounds were less affected. Concentrations of

271 aldehydes (glyoxal, methylglyoxal) and volatile sulfur compounds (DMS, H_2S) did not vary

272 according to the microorganism combination.

273 Larger quantities of higher alcohols are present in alcoholic beverages than any other

274 group of aroma compounds. Their concentrations were significantly affected by the

275 yeast/bacteria combination used in winemaking. The total amount of higher alcohols was

276 strongly associated with the concentration of 3-methylbutan-1-ol, which constituted over 60%

277 of the total alcohol for each modality. However, this was the only higher alcohol not affected

278 by the yeast/LAB combination, while concentrations of other alcohols, such as propan-1-ol, 2-

279 methypropan-1-ol and 2-methylbutan-1-ol differed significantly differences in their according

280 to the yeast/LAB combination (0.1%, 5% and 0.1%, respectively).

281 Eleven varietal compounds known to contribute to the fruity aroma of red wines,

282 including C_{13}-norisoprenoids, lactones and terpene, were quantified. For C_{13}-norisoprenoids,

283 differences between the six combinations were low or non-existent and only α-ionone presented

284 small, but significant, variations (1%). Lactones were mainly represented by γ-octalactone, with

285 significant variations in concentration (5%) according to the yeast/LAB combination. The

286 concentrations of other lactones were not significantly affected by the microorganism

287 combinations.

288 Eight volatile acids known to contribute to the balance of fruity aroma were assayed.

289 Concentrations of branched acids (isobutyric, isovaleric and 2-methylbutyric acids) were

290 significantly modulated by the yeast/LAB combination (0.1%). Similarly, levels of linear acids

291 (butyric, hexanoic, decanoic and dodecanoic) all varied depending on the microorganism

292 combinations (0.1%, except for decanoic acid, significant at 5%).

293   Finally, esters are considered one of the most important families of aromatic compounds

294   for modulating red wine fruity aromas. Among the 40 esters quantified, only seven were not

295   affected by the yeast/LAB combination. Concentrations of over half of the compounds (33

296   esters) differed significantly according to the microorganisms used.

297

Predominant impact of yeast on concentrations of aromatic compounds

299   Principal component analysis (PCA) was used to refine these observations. Among 73

300   molecules quantified, 22 did not exhibit any significant combination effect and were not

301   included in the PCA. Using 51 analytical variables (volatile data) and 18 objects (3 yeasts x 2

302   bacteria in triplicate), PCA explained over 65% of the total variance on the first two axes (Fig.

303   2). Triplicates of each modality were all represented close to each other, indicating good

304   reproducibility of the experiment. According to this PCA, the yeast strain alone had a greater

305   impact on volatile compound levels than the yeast/LAB combination. Indeed, triplicate samples

306   fermented with FX10/450 and FX10/B28 were separated from the other wines along axis 1.

307   Samples inoculated with XR/450 and XR/B28 combinations were at the bottom of the two-

308   dimensional plot, whereas the 522D/B28 and 522D/450 samples were higher on axis 2. Ethyl

309   lactate and diacetyl were the only compounds strongly represented on axis 3 (10.37% of total

310   variance; data not shown), which separated the wines according to the LAB strain, as expected.

311   In contrast, no yeast/LAB combination effect was revealed.

312   These observations were confirmed with a two-way ANOVA (yeast/bacteria/yeast x

313   bacteria interaction) (Table 4). Among the fifty-one compounds previously highlighted, only

314   eight were actually affected by the yeast/LAB interaction, while a yeast strain effect was

315   observed for 48 of these aromatic compounds.

316   The concentration of higher alcohols was only modulated by the yeast strain. Wines

317   fermented by the 522D strain contained significantly more 2-methylpropan-1-ol, propan-1-ol,

318   and 2-methylbutan-1-ol than FX10 or XR wines (1%, 0.1% and 0.1%, respectively).

319   Yeast strains also influenced the C_{13}-norisoprenoid and lactone concentrations, but their

320   impact was not as clear (Table 4). Indeed, only small variations were measured in lactone

321   concentrations. Among these compounds, γ-octalactone was the most representative, with

322   levels ranging from 15.86 µg L^{-1} (XR/B28 wine) to 22.50 µg L^{-1} (522D/B28 wine), but is

323   unlikely to have had any aromatic impact in view of its perception threshold (35 µg L^{-1}).[35]

324   Little information is available concerning lactone formation pathways in wine, but they are

325   assumed to be mainly synthesized from hydroxylated fatty acids or esters *via* an enzymatic or

326   chemical pathway.[18,36] The results of this study were consistent with previous observations

327   that yeasts were capable of enzymatic esterification but not, apparently, LAB[37]. However,

328   lactones are mainly synthesized during wine aging[38] and some differences in concentrations

329   may occur depending on the LAB strain used during MLF. Indeed, some studies have indicated

330   the possibility of a late synthesis of these compounds, related to bacterial β-glycosidase and

331   oxidase activities.[36,39] Among the C_{13}-norisoprenoids, only α-ionone presented small

332   variations in concentration with different yeast or LAB strains, as well as yeast/LAB

333   interactions (from 0.11 µg L^{-1} for FX10/450 to 0.22 µg L^{-1} for XR/B28). Although levels found

334   in this study were below the perception threshold (2.6 µg L^{-1}),[40] which is highly dependent

335   on the matrix, some studies have highlighted the potential implication of these compounds in

336   modulating fruity aroma *via* perceptive interactions.[17] These results are in accordance with

337   numerous data presented in the literature, demonstrating the ability of both yeast and LAB to

338   hydrolyze glycosidic precursors of C_{13}-norisoprenoids.[36,41]

339   Two groups were identified among the 40 esters quantified in this study. Major esters,

340   including ethyl acetate, ethyl lactate, and monoethyl succinate, were present at higher

341 concentrations (mg L^{-1}) compared to other esters, which are nevertheless considered "odorant

342 esters", due to their lower perception threshold in wine.

343 In the major ester group, diethyl succinate and ethyl acetate concentrations were slightly

344 impacted by yeast strains. However, in view of its perception threshold (154 mg L^{-1})[40] and

345 the variations measured in this study (<20 mg L^{-1}), ethyl acetate probably did not affect wine

346 aroma. Ethyl lactate levels varied significantly among the different modalities, reaching higher

347 concentrations in wines inoculated with LAB strain B28 (0.1%), confirming the literature

348 reporting the capacity of LAB to synthesize this compound during MLF.[42,43]

349 Concentrations of other esters, known as "odorant esters", were also mainly influenced by the

350 yeast strain. Three groups may be identified in terms of their contribution to fruity aroma. Fatty

351 acid ethyl esters were the least influenced by the yeast/LAB combination. Ethyl butyrate,

352 decanoate and dodecanoate, as well as their corresponding acids, were mainly synthesized by

353 FX10 and 522D yeasts. Higher concentrations of most acetates were found in wines fermented

354 with 522D and FX10 (except hexyl acetate). Higher concentrations of branched esters, such as

355 ethyl 2-methylbutyrate and ethyl isovalerate, were found in wines fermented with 522D or XR

356 (significant at 0.1%). Similarly, significantly higher levels of the corresponding acids, such as

357 isobutyric, isovaleric and 2-methylbutyric acids, were also found in these last two wines (also

358 at 0.1%). The concentrations of the other esters (esters with an odd number of carbon atoms,

359 methyl esters, isoamyl esters, cinnamates and minor esters) were also affected by the different

360 combinations used, particularly the yeast strain, as reported in previous studies.[9,44] Although

361 the variations measured for these esters were below the perception threshold, some studies have

362 demonstrated that they may still be perceived by a trained panelist.[15,45]

363 While the majority of these compounds were synthesized by yeast during AF, the esterase

364 activity of wine LAB has also been reported.[46] Besides diacetyl, known to be synthesized

365 during MFL by LAB,[24] these results suggest that microorganisms may be capable of

366   modulating the concentrations of esters and their corresponding acids. The carbon chain length

367   seemed to be an important parameter in the synthesis of these compounds by LAB. Indeed, the

368   longer the carbon chain, the more the esters and acids were affected by the LAB strain (Table

369   4). Hexanoic, octanoic, decanoic and dodecanoic acids were all found in significantly higher

370   concentrations in wines inoculated with LAB strain 450 (1%). This was also true of the

371   corresponding esters, ethyl decanoate and ethyl dodecanoate (significant at 0.1%). These results

372   contradicted some data in the literature. Matthews *et al.*(47) reported that the hydrolytic activity

373   of esterases in different species or genera (*O. oeni, Lactobacillus, Pediococcus*) had greater

374   specificity for substrates with short carbon chains (C2, C4). In particular, the esterase activity

375   of *O. oeni* was reported to be greater for substrates in C4. In contrast, other recent studies

376   reported the ability of LAB to synthesize ethyl esters and acetates with long carbon chains (C8,

377   C10, C12).(29) In all cases, these long carbon chain esters play a minor role in red wine fruity

378   aroma. It is therefore unlikely that the small variations in concentration observed between

379   samples inoculated with different LAB strains would be perceived by a tasting panel.

380   This study examined the influence of *S. cerevisiae* and *O. oeni* strains on the production

381   of Bordeaux red wines using six different yeast/LAB combinations. Results obtained for

382   standard chemical parameters revealed that the level of volatile acidity varied significantly

383   according to the LAB strain. For aromatic compounds, each microorganism combination

384   resulted in a specific volatile profile. However, the yeast strain was apparently the predominant

385   component in the yeast/LAB combination in modulating aromatic compound levels. In

386   particular, the 522D and FX10 strains exhibited a similar capacity to produce esters, acids and

387   higher alcohols. These results showed that yeasts had a more significant effect on wine quality

388   and are thus likely to have a greater impact on wine style than the LAB used. A previous study

389   had already demonstrated the predominant impact of yeast strain rather than yeast/LAB

390   combination on cherry wines.(48)

Sensory analyses were performed on these six wines and presented in a previous study,[49] using a Napping® test. According to Napping® results obtained with wines at two different aging steps (3 and 12 months), the differences observed between modalities seemed to be correlated with the yeast strain use for AF. Most descriptors used to discriminate wines referred to fruity notes. In both cases, the trained panel composed of 20 judges perceived FX10 and XR wines as being fruitier than 522D wines. To confirm these preliminary results, a ranking test and a comparison profile were performed with wines from the 2012 vintage fermented with the same yeast/LAB combinations. In this study,[49] the yeast strain appeared to be a dominant factor involved in the modulation of fruity notes in Bordeaux red wines. Wines inoculated with FX10 were perceived as fruitier, regardless of the vintage or grape cultivar, after 3 and 12 months of aging.

If we consider the volatile composition of these wines, samples fermented with the yeast FX10 had higher values for the attributes referring to ''fruity'', due to their large quantities of fruity ethyl esters. Surprisingly, 522D wines, described as fruitless, also contained important levels of these aromatic compounds, as well as high amounts of higher alcohols. These compounds, recognized by their strong, pungent smell, influence the taste and character of wine depending on their concentration: below 300 mg L^{-1}, they contribute to the desirable complexity of wine but at concentrations exceeding 400 mg L^{-1}, they are regarded as a negative influence on wine quality.[21] The high alcohol levels found in this study, particularly in 522D/B28 and 522D/450 samples (577 mg L^{-1} and 570 mg L^{-1}, respectively), may have had a negative effect on fruity aroma perception in these wines.

While these experiments offer new insights into the organoleptic effect of fermentations, the chemistry underlying the sensory interactions is highly complex. Further investigations are necessary to elucidate the influence of yeast- and LAB-derivative compounds on fruity aroma. Moreover, in light of recent articles dealing with the interactions between volatile and non-

416   volatile compounds,(50) the impact of both microorganisms on the non-volatile matrix should

417   also be investigated as a potential modulating factor of wine aroma.

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TABLES

Table 1. Mean Concentration with Standard Deviation of Oenological Parameters of Wines after 3 months' aging.

	XR/B28[*]	522D/B28	FX10/B28	XR/450	522D/450	FX10/450
Fermentation duration (days)	47	47	47	40	42	35
Alcoholic degree (% v/v)	13.2 ± 0.2	13.2 ± 0.3	13.2 ± 0.2	13.2 ± 0.1	13.2 ± 0.3	13.1 ± 0.2
pH	3.68 ± 0.03	3.67 ± 0.01	3.60 ± 0.02	3.64 ± 0.03	3.64 ± 0.01	3.59 ± 0.02
Total acidity (g L^{-1} H$_2$SO$_4$)	3.4 ± 0.04 cd	3.43 ± 0.08 cd	3.56 ± 0.03 b	3.5 ± 0.1 c	3.54 ± 0.04 bc	3.67 ± 0.02 a
Volatile acidity (g L^{-1} acetic acid)	0.29 ± 0.02 a	0.23 ± 0.03 ab	0.30 ± 0.02 a	0.13 ± 0.02 c	0.09 ± 0.01 d	0.16 ± 0.01 c
Total sulfur dioxide (mg L^{-1})	41 ± 2	43 ± 6	42 ± 4	33 ± 9	39 ± 13	29 ± 1
Free sulfur dioxide (mg L^{-1})	30 ± 4	28 ± 2	30 ± 2	20 ± 5	25 ± 7	21 ± 1

[*] Values with different superscript roman letter (a-d) in the same row are significantly different according to the Tuckey *post hoc* test (P < 0.05).

Table 2. Mean concentrations with standard deviation (mg L^{-1}, n=3) of fermentation-derived compounds in wines made by different yeast/LAB combinations.

Compounds	XR/B28	XR/450	522D/B28	552D/450	FX10/B28	FX10/450	One-way ANOVA
Alcohols							
propan-1-ol	35 ± 5	37 ± 3	55 ± 5	55 ± 5	38 ± 2	40 ± 2	***
2-methylpropan-1-ol	54 ± 2	56 ± 2	61 ± 3	62 ± 3	57 ± 1	58.4 ± 0.4	*
2-methylbutan-1-ol	88 ± 5	90 ± 4	115 ± 4	114 ± 8	79 ± 1	80 ± 3	***
3-methylbutan-1-ol	319 ± 17	330 ± 10	346 ± 13	341 ± 21	326 ± 8	334 ± 7	NS
Sum higher alcohols	496	514	577	570	499	513	
butan-2,3-diol (D)	127 ± 31	99 ± 16	140 ± 17	98 ± 16	189 ± 66	116 ± 9	*
butan-2,3-diol (M)	49 ± 17	56 ± 9	54 ± 8	52 ± 11	66 ± 21	52 ± 4	NS
Aldehydes & ketones							
glyoxal	0.14 ± 0.03	0.1 ± 0.01	0.16 ± 0.05	0.19 ± 0.05	0.15 ± 0.02	0.13 ± 0.03	NS
methylglyoxal	0.4 ± 0.06	0.42 ± 0.07	0.46 ± 0.06	0.45 ± 0.06	0.55 ± 0.08	0.43 ± 0.03	NS
acetoin	19 ± 4	24 ± 3	24 ± 5	19 ± 2	30 ± 10	21 ± 2	NS
diacetyl	11 ± 1	7.4 ± 0.6	10 ± 1	7 ± 2	10 ± 1	5.6 ± 0.5	***
pentan-2,3-dione	1.5 ± 0.1	1.68 ± 0.09	1.47 ± 0.01	1.9 ± 0.2	1.1 ± 0.2	1.4 ± 0.2	***
frambinone (µg/L)	15 ± 5	14 ± 3	14 ± 3	13 ± 3	12 ± 2	11.1 ± 0.7	NS
Sulfur-containing compounds							
hydrogen sulfide	0.8 ± 0.1	0.7 ± 0.2	0.9 ± 0.3	1.0 ± 0.4	1.2 ± 0.6	1.2 ± 0.2	NS
dimethyl sulfide	3.7 ± 0.2	3.7 ± 0.2	4.0 ± 0.5	3.6 ± 0.7	4.1 ± 0.3	4.0 ± 0.4	NS
Acids							
butyric acid	7.5 ± 1.0	5.5 ± 0.5	6.7 ± 0.6	4.6 ± 0.4	5.2 ± 0.3	3.7 ± 0.2	***
isobutyric acid	1.22 ± 0.09	1.2 ± 0.1	1.22 ± 0.03	1.22 ± 0.03	1.01 ± 0.01	1.00 ± 0.02	***
isovaleric acid	1.11 ± 0.07	1.1 ± 0.07	1.11 ± 0.04	1.12 ± 0.01	0.76 ± 0.01	0.78 ± 0.02	***

2-methylbutyric acid	0.82 ± 0.04	0.8 ± 0.05	0.94 ± 0.06	0.93 ± 0.03	0.51 ± 0.02	0.51 ± 0.01	***
hexanoic acid	7.8 ± 0.4	8.0 ± 0.4	8.7 ± 0.4	9.4 ± 0.3	8.4 ± 0.5	9.2 ± 0.5	***
octanoic acid	2.6 ± 0.1	3.0 ± 0.3	2.9 ± 0.1	3.4 ± 0.2	3.3 ± 0.2	3.62 ± 0.05	***
decanoic acid	0.7 ± 0.04	0.76 ± 0.06	0.75 ± 0.05	0.88 ± 0.09	0.72 ± 0.04	0.82 ± 0.07	*
dodecanoic acid (µg/L)	9 ± 3	6 ± 1	6.7 ± 0.8	11 ± 1	6.8 ± 0.7	12.1 ± 0.8	***
C₁₃-norisoprenoids, lactones, & terpene							
β-damascone (µg/L)	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.04	0.02 ± 0.01	NS
β-damascenone (µg/L)	6.6 ± 0.2	6.0 ± 0.4	5.5 ± 0.6	6.3 ± 0.9	6.1 ± 0.5	6.5 ± 0.5	NS
α-ionone (µg/L)	0.22 ± 0.02	0.12 ± 0.02	0.15 ± 0.02	0.12 ± 0.03	0.14 ± 0.04	0.11 ± 0.02	**
β-ionone (µg/L)	0.09 ± 0.02	0.09 ± 0.01	0.08 ± 0.01	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.01	NS
γ-octalactone (µg/L)	16 ± 3	17 ± 3	23 ± 2	21 ± 2	19 ± 3	18 ± 2	*
γ-nonalactone (µg/L)	7.7 ± 0.4	7.1 ± 0.5	8 ± 1	8 ± 1	8 ± 2	7.1 ± 0.5	NS
γ-decalactone (µg/L)	0.91 ± 0.01	0.8 ± 0.2	0.63 ± 0.08	0.62 ± 0.06	0.8 ± 0.3	0.7 ± 0.01	NS
δ-decalactone (µg/L)	1.06 ± 0.04	1.3 ± 0.3	1.2 ± 0.1	1.3 ± 0.1	1.9 ± 0.5	1.57 ± 0.09	*
γ-undecalactone (µg/L)	0.07 ± 0.01	0.09 ± 0.02	0.08 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	NS
γ-dodecalactone (µg/L)	0.05 ± 0.01	0.05 ± 0.02	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	NS
linalol (µg/L)	12 ± 2	9.7 ± 0.7	13 ± 2	12 ± 1	8 ± 2	8.0 ± 0.4	**

Significant effect: NS, Not Significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 3. Mean concentrations with standard deviation ($\mu g\ L^{-1}$, n=3) of ester compounds in wines made with different yeast/LAB combinations.

Compounds	XR/B28	XR/450	522D/B28	552D/450	FX10/B28	FX10/450	One-way ANOVA
Major polar esters							
ethyl lactate (mg/L)	56 ± 5	31 ± 1	55 ± 2	31 ± 2	55 ± 1	38 ± 4	***
monoethyl succinate (mg/L)	22 ± 1	20 ± 2	22 ± 3	20 ± 1	21 ± 2	20 ± 1	NS
diethyl succinate	683 ± 29	586 ± 38	793 ± 105	697 ± 115	621 ± 81	588 ± 50	*
Polar esters							
ethyl leucate	70 ± 5	58.2 ± 0.3	94 ± 6	96 ± 13	85 ± 13	80 ± 16	**
ethyl 3-hydroxybutyrate	333 ± 10	323 ± 12	384 ± 29	387 ± 18	454 ± 26	454 ± 21	***
ethyl 2-hydroxyhexanoate	0.9 ± 0.3	1.6 ± 0.6	1.27 ± 0.04	1.2 ± 0.2	1.2 ± 0.2	1.0 ± 0.1	NS
ethyl 6-hydroxyhexanoate	3.12 ± 0.06	3.4 ± 0.8	3.1 ± 0.7	4 ± 1	3.9 ± 0.4	3.9 ± 0.7	NS
Ethyl fatty acid esters							
ethyl butyrate	185 ± 17	179 ± 8	218 ± 22	218 ± 28	198 ± 9	194 ± 17	*
ethyl hexanoate	286 ± 11	294 ± 5	319 ± 13	320 ± 23	313 ± 18	324 ± 29	NS
ethyl octanoate	289 ± 20	282 ± 25	307 ± 27	302 ± 34	330 ± 21	334 ± 15	NS
ethyl decanoate	71 ± 6	94 ± 3	91 ± 10	103 ± 8	87 ± 6	115 ± 7	***
ethyl dodecanoate	4.8 ± 0.4	6.8 ± 0.4	7.4 ± 0.6	9.9 ± 0.9	8 ± 1	13 ± 2	***
Ethyl branched acid esters							
ethyl isobutyrate	62 ± 6	58 ± 6	63.5 ± 0.9	49.0 ± 15.0	61 ± 4	59 ± 6	NS
ethyl 2-methylbutyrate	11.7 ± 0.7	12 ± 1	13.7 ± 0.3	13.8 ± 0.8	7.4 ± 0.2	7.1 ± 0.3	***
ethyl isovalerate	18.4 ± 0.4	17 ± 1	18 ± 1	19 ± 1	11.6 ± 0.4	11.7 ± 0.6	***
ethyl phenylacetate	3.22 ± 0.02	3.2 ± 0.4	5.0 ± 0.6	5.5 ± 0.5	3.0 ± 0.3	3.1 ± 0.1µ	***
Acetate of higher alcohols							
ethyl acetate (mg/L)	90 ± 2	78 ± 3	72 ± 1	71 ± 4	88 ± 4	80 ± 3	***
propyl acetate	17.1 ± 0.5	15.5 ± 0.5	23 ± 2	21 ± 1	21 ± 2	19.3 ± 0.3	***

isobutyl acetate	41 ± 1	37 ± 2	53 ± 4	51 ± 5	50 ± 2	51 ± 1	***
butyl acetate	0.8 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	1.2 ± 0.2	1.26 ± 0.07	0.8 ± 0.2	**
isoamyl acetate	1105 ± 61	1064 ± 37	1484 ± 181	1339 ± 196	1367 ± 143	1317 ± 73	**
hexyl acetate	6 ± 1	9 ± 2	7 ± 1	9 ± 2	8.1 ± 0.9	8.7 ± 0.3	NS
octyl acetate	0.08 ± 0.01	0.12 ± 0.02	0.1 ± 0.01	0.15 ± 0.04	0.15 ± 0.03	0.18 ± 0.01	***
2-phenylethyl acetate	87 ± 8	89 ± 3	144 ± 24	145 ± 17	117 ± 10	120 ± 9	***

Methyl esters

methyl butyrate	0.86 ± 0.05	0.78 ± 0.02	1.22 ± 0.01	1.09 ± 0.08	1.0 ± 0.1	0.84 ± 0.04	***
methyl hexanoate	1.9 ± 0.1	1.71 ± 0.06	2.2 ± 0.2	2.16 ± 0.09	1.9 ± 0.2	2.0 ± 0.1	**
methyl octanoate	1.26 ± 0.02	1.34 ± 0.05	1.4 ± 0.1	1.5 ± 0.1	1.44 ± 0.09	1.5 ± 0.1	**
methyl decanoate	0.33 ± 0.01	0.42 ± 0.03	0.42 ± 0.03	0.48 ± 0.03	0.4 ± 0.01	0.48 ± 0.01	***

Ethyl esters with odd number of carbon atoms

ethyl propanoate	306 ± 12	292 ± 8	425 ± 25	384 ± 58	281 ± 22	258 ± 16	***
ethyl valerate	0.67 ± 0.03	0.54 ± 0.04	1.0 ± 0.1	0.87 ± 0.03	0.84 ± 0.06	0.9 ± 0.2	***
ethyl heptanoate	0.9 ± 0.1	0.9 ± 0.1	0.92 ± 0.06	0.95 ± 0.06	0.64 ± 0.01	0.66 ± 0.03	***
ethyl nonanoate	0.61 ± 0.01	0.69 ± 0.03	0.89 ± 0.03	0.9 ± 0.04	0.89 ± 0.08	1.07 ± 0.09	***

Isoamyl esters

isoamyl butyrate	0.66 ± 0.06	0.67 ± 0.05	0.75 ± 0.02	0.8 ± 0.1	0.71 ± 0.05	0.57 ± 0.03	***
isoamyl hexanoate	1.9 ± 0.1	1.88 ± 0.06	2.0 ± 0.1	1.9 ± 0.2	2.1 ± 0.1	2.18 ± 0.07	*
isoamyl octanoate	2.8 ± 0.3	2.9 ± 0.2	3.1 ± 0.3	3.2 ± 0.2	3.4 ± 0.1	3.48 ± 0.07	**

Cinnamates

ethyl cinnamate	2.55 ± 0.02	2.57 ± 0.05	2.4 ± 0.1	2.4 ± 0.2	2.56 ± 0.07	2.71 ± 0.06	**
ethyl dihydrocinnamate	1.7 ± 0.1	1.69 ± 0.05	1.5 ± 0.1	1.52 ± 0.06	1.51 ± 0.07	1.57 ± 0.05	**

Minor esters

ethyl hexenoate	1.8 ± 0.2	1.6 ± 0.1	1.41 ± 0.07	1.51 ± 0.09	1.3 ± 0.1	1.68 ± 0.09	***
isobutyl hexanoate	0.16 ± 0.00	0.17 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.2 ± 0.01	0.21 ± 0.01	***

| methyl *trans*-geranate | 0.15 ± 0.02 | 0.21 ± 0.01 | 0.19 ± 0.02 | 0.22 ± 0.00 | 0.24 ± 0.01 | 0.27 ± 0.02 | *** |

Significant effect: NS, Not Significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 4. Results of the two-way ANOVA (yeast/LAB/yeast x LAB interaction).

Compounds	PCA abbreviation	two-way ANOVA		
		yeast	bacteria	yeast x bacteria
Alcohols				
propan-1-ol	C3OH	***	NS	NS
2-methylpropan-1-ol	2mC3OH	**	NS	NS
2-methylbutan-1-ol	2mC4OH	***	NS	NS
butan-2,3-diol (D)	C4-2,3OH	NS	**	NS
Aldehydes & ketones				
diacetyl	diacetyl	NS	***	NS
pentan-2,3-dione	pentan-2,3-dione	***	***	NS
Acids				
butyric acid	C4	***	***	NS
isobutyric acid	iC4	***	NS	NS
isovaleric acid	iC5	***	NS	NS
2-methylbutyric acid	2mC4	***	NS	NS
hexanoic acid	C6	***	**	NS
octanoic acid	C8	***	***	NS
decanoic acid	C10	*	**	NS
dodecanoic acid	C12	*	**	***
C₁₃-norisoprenoids, lactones, & terpene				
α-ionone	α-i	*	***	*
γ-octalactone	γ-oct	**	NS	NS
δ-decalactone	δ-dec	**	NS	NS
linalol	linalol	**	NS	NS
Major polar esters				
ethyl lactate	C2lac	NS	***	NS
diethyl succinate	DES	*	NS	NS
Polar esters				
ethyl leucate	C2leu	**	NS	NS
ethyl 3-hydroxybutyrate	C2 3OHC2	***	NS	NS
Ethyl fatty acid esters				
ethyl butyrate	C2C4	**	NS	NS
ethyl decanoate	C2C10	**	***	NS
ethyl dodecanoate	C2C12	***	***	*

Table 4. Continued

Compounds	PCA abbreviation	two-way ANOVA		
		yeast	bacteria	yeast x bacteria
Ethyl branched acid esters				
ethyl 2-methylbutyrate	C2 2mC4	***	NS	NS
ethyl isovalerate	C2iC5	***	NS	NS
ethyl phenylacetate	C2PhC2	***	NS	NS
Acetate of higher alcohols				
ethyl acetate	C2C2	***	***	**
propyl acetate	C3C2	***	**	NS
isobutyl acetate	iC4C2	***	NS	NS
butyl acetate	C4C2	*	NS	**
isoamyl acetate	iC5C2	**	NS	NS
octyl acetate	C8C2	***	**	NS
2-phenylethyl acetate	2-PhC2C2	***	NS	NS
Methyl esters				
methyl butyrate	C1C4	***	**	NS
methyl hexanoate	C1C6	***	NS	NS
methyl octanoate	C1C8	**	*	NS
methyl decanoate	C1C10	***	***	NS
Ethyl esters with odd number of carbon atoms				
ethyl propanoate	C2C3	***	*	NS
ethyl valerate	C2C5	***	NS	NS
ethyl heptanoate	C2C7	***	NS	NS
ethyl nonanoate	C2C9	***	**	*
Isoamyl esters				
isoamyl butyrate	iC5C4	***	NS	**
isoamyl hexanoate	iC5C6	**	NS	NS
isoamyl octanoate	iC5C8	**	NS	NS
Cinnamates				
ethyl cinnamate	C2cin	**	NS	NS
ethyl dihydrocinnamate	C2dhicin	***	NS	NS
Minor esters				
ethyl hexenoate	C2hex	**	*	**
isobutyl hexanoate	iC4C6	***	NS	NS
methyl *trans*-geranate	C1ger	***	***	NS

Significant effect: NS, Not Significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$
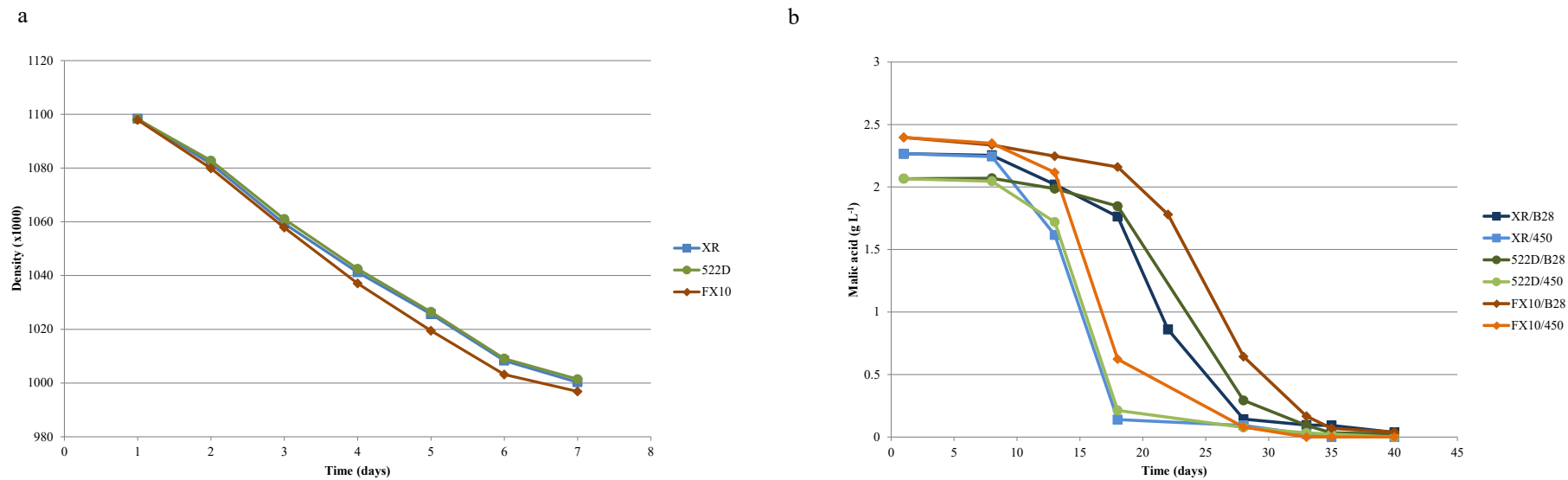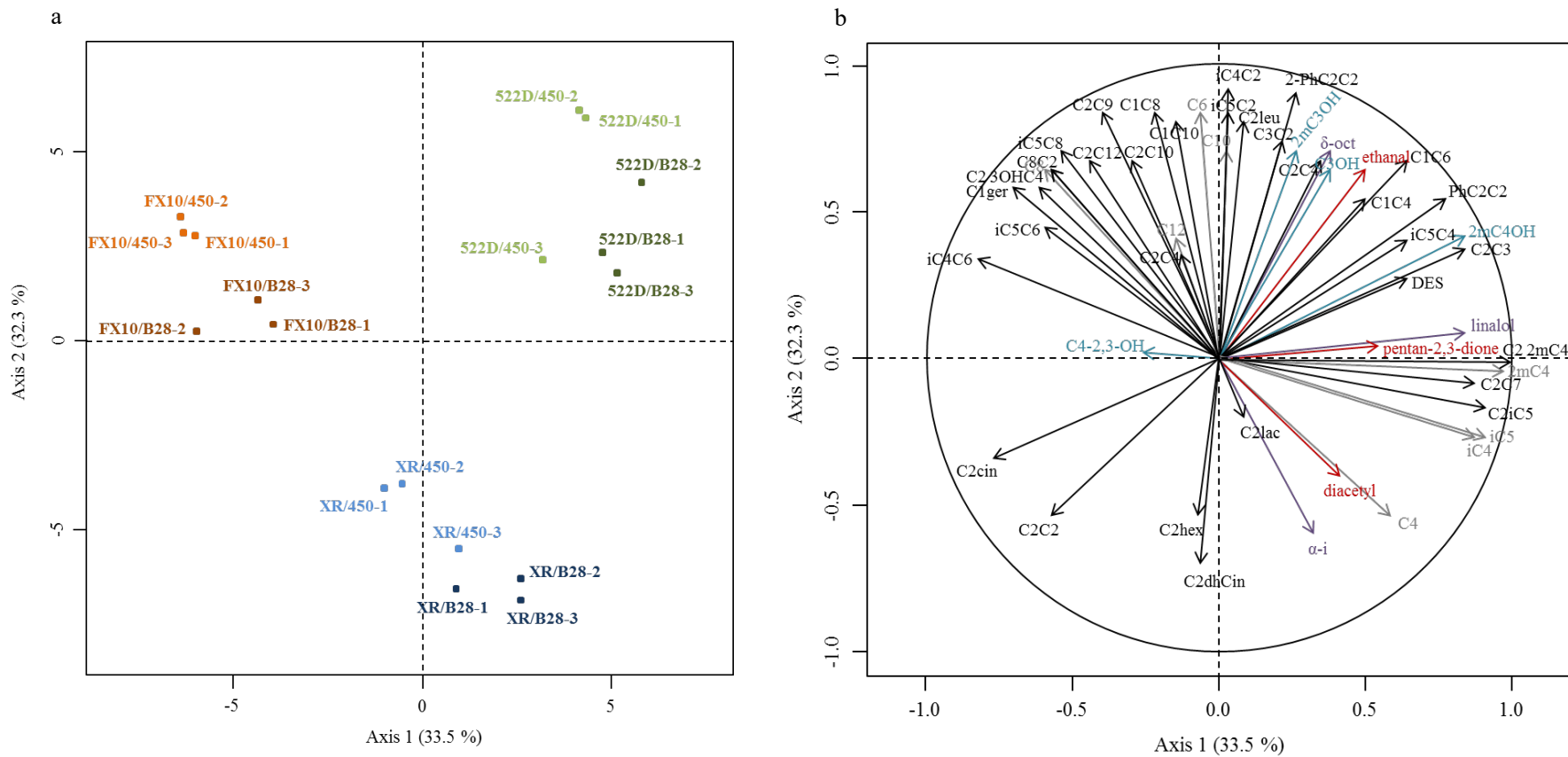
FIGURE GRAPHICS

a

b

Figure 1. Kinetics of alcoholic (a) and malolactic (b) fermentations in wines fermented with different yeast/LAB combinations.

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Figure 2. Principal component analysis represented as a scatter point plot (a) and 51 parameters (b) on axes 1 x 2. Abbreviations for the various parameters are presented in Table 4.