Influence of lactic acid bacteria strains on ester concentrations in red wines: specific impact on branched hydroxylated compounds

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Abstract

This research investigated the influence of lactic acid bacteria (LAB) strains on ester levels in Bordeaux red wines. These wines were made in five Bordeaux areas in two vintages, using three yeast strains. Malolactic fermentation (MLF) was carried out using industrial starters or indigenous strains, each in triplicate. Ester concentrations were determined by liquid-liquid-extraction- or HS-SPME-GC/MS at various stages in the winemaking process. The levels of most compounds were slightly impacted by LAB, depending on grape variety. Nevertheless, branched hydroxylated esters, such as ethyl 2-hydroxy-3methylbutanoate and ethyl 2-hydroxy-4-methylpentanoate were the only compounds to be strongly influenced by the bacteria strain, regardless of matrix composition or the yeasts used for alcoholic fermentation. Moreover, the effect observed after MLF persisted over time, for at least 12 months. These esters are apparently important markers of LAB esterase activity. To our knowledge, this was the first time they had been identified in this role.

Keywords

lactic acid bacteria; branched hydroxylated esters; red wine; fruity aroma

1. Introduction

Red wine is not only the result of the fermentation of sugars by yeasts, but is almost always followed by malolactic fermentation (MLF), conducted by lactic acid bacteria (LAB), which may occur spontaneously or be induced by inoculation with commercial starters (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006). Early works by Ribéreau-Gayon and Peynaud (1964) revealed the usefulness of this second fermentation, which usually ensures the stability of wines, as well as improving their aromas and flavors. The main result of MLF is to transform L-malic acid into L-lactic acid, accompanied by a release of carbon dioxide. Of all enological LAB species, *Oenococcus oeni* is preferred for MLF, as it is resistant to the harsh environmental conditions, decomposes the malic acid first, followed by the sugars, and forms little volatile acidity. This decarboxylation naturally reduces the total acidity and is accompanied by a slight increase in pH, which contributes to softening the flavor on the palate and enhancing its smoothness. MLF also promotes the microbial stability of wines by substrate depletion. These secondary bacterial metabolisms associated to bacterial development are responsible for chemical modifications affecting the olfactory and gustatory perception of wine (Bartowsky, Francis, Bellon, & Henschke, 2002; Henick-Kling, 1993; Matthews et al., 2004).

The most frequently-reported aromatic compound associated with MLF is diacetyl (butane-2,3-dione), mainly released by LAB (Bertrand, Zmirou-Bonnamour, & Lonvaud-Funel, 1984; de Revel, Martin, Pripis-Nicolau, Lonvaud-Funel, & Bertrand, 1999) and associated with an increase in buttery character (Bartowsky & Henschke, 2004). Ethyl lactate is another marker of bacterial activity (Boido et al., 1999), but its impact on fruity aroma is quite limited, contrary to other esters, which are considered some of the most important fruity compounds in wines (Ebeler, 2001; V. Ferreira, López, & Cacho, 2000).

From a qualitative point of view, all red wines contain the same set of ester compounds. However, their respective proportions vary considerably from one wine to another (Antalick, Perello, & de Revel, 2014). Generally, these molecules are present at concentrations well below their perception thresholds, so it would be logical to assume that they do not modulate wine aroma. Since 2009, new data has revealed that these compounds play a central role in the fruity expression of red wines, via synergistic phenomena (Lytra, Tempere, Le Floch, de Revel, & Barbe, 2013; Pineau, Barbe, Van Leeuwen, & Dubourdieu, 2009). Thus, small variations in the concentrations of one or more esters may have a significant effect on the perception of fruity aroma. In particular, previous research demonstrated the impact of ethyl esters, acetates, and branched ethyl esters on the fruity character of red wines (Falcao, Lytra, Darriet, & Barbe, 2012; Ferreira et al., 2016).

Since the late 1960's, studies have highlighted the capacity of LAB strains (*Lactobacillus*, *Pediococcus*, *Leuconostoc*) to increase concentrations of some esters in wine during MLF during MLF (Pilone, Kunkee, & Webb, 1966). Screening the enzyme activity of several wine LAB strains revealed that some of them were also able to hydrolyze esters (Davis, Wibowo, Fleet, & Lee, 1988). In that regard, several studies exploring the modulation of wine aromas revealed that ester concentration increased or decreased after MLF (Antalick, Perello, & de Revel, 2012; Delaquis et al., 2000; Zeeman, Snyman, & van Wyck, 1980). These results suggested that the esterase activity of wine LAB, like that found in the cheese industry, was capable of synthesizing and/or hydrolyzing these compounds. This hypothesis was recently validated by Sumby, Jiranek and Grbin (2013), highlighting the role of the synthesis and hydrolysis of two enzymes, EstA2 and EstB28, involved in the ester biosynthesis pathway in *O. oeni*. LAB ester metabolisms are apparently strongly influenced by several enological parameters. Maicas, Gil, Pardo, and Ferrer (1999) reported that the concentrations of some esters either increased or decreased during MLF, according to the type of bacterial strain used.

Delaquis et al. (2000) reported that the aromatic composition of wines was influenced by both yeast and LAB strains, as well as winemaking conditions. Finally, Knoll et al. (2011) demonstrated the influence of ethanol and pH on MLF and ester profiles.

One of the difficulties in finding a consensus is that the previous work on this topic focused mainly on a few cases of bacterial strains or wines, whereas many enological parameters may affect the influence of LAB strains on the ester composition of red wines. Thus, it was essential to conduct a comprehensive study. To investigate the influence of LAB strains on ester levels, MLF was triggered using two different commercial *O. oeni* starters and compared with spontaneous MLF. To elucidate the influence of the yeast strain on LAB metabolism, alcoholic fermentation (AF) was triggered by inoculation with three different commercial *Saccharomyces cerevisiae* starters. To evaluate the impact of the matrix on ester metabolism by LAB, experiments were conducted during two vintages, using two cultivars, Merlot and Cabernet Sauvignon. Finally, to confirm the influence of LAB strains on some ester levels, particularly in micro-vinification, some of the wines tested were made on an industrial scale.

2. Material and methods

2.1. Winemaking.

Two different experimentations were conducted in the Bordeaux region during the 2011 and 2012 vintages. Microvinifications were carried out with Cabernet Sauvignon grapes (named WEC 2011 and WEC 2012). Vinifications in four wineries were conducted with Cabernet Sauvignon or Merlot grapes at industrial scale (MRGX 2011, MDC 2011, PCLN 2012, and STEM 2012) (Table 1). In all six experiments, AF was initiated by inoculation with rehydrated dried yeast, according to the manufacturer's recommendations (*S. cerevisiae* yeasts

strains: Actiflore cerevisiae, 522D; Zymaflore FX10, Biolaffort, Floirac, France; and Excellence XR, Lamothe-Abiet, Canéjan, France). AF was performed in 2 h L stainless steel tanks in triplicate under micro-vinification conditions. In wineries, AF was completed in stainless steel tanks in bigger volume (Table 1). Implantation in each tank under all experimental conditions was checked at the middle of AF (density close to 1.040). Yeast starter culture implantation was monitored by PCR at SARCO laboratory (Biolaffort, Floirac, France) (data not shown). It confirms that, for each wine, AF was carried out by the yeast strain implanted. MLF was triggered using starters (*O. oeni* bacterial strains: Lactoenos 450 PreAc and Lactoenos B28 PreAc, Biolaffort, Floirac, France) or indigenous strains (spontaneous flora), in triplicate for all experimental conditions (Table 1) at the end of AF. In wines inoculated with bacteria, starters were rehydrated with bacterial nutrient (Energizer®, Biolaffort, Floirac, France), according to the manufacturer's instructions, and added to wines at the recommended dose. Malic acid concentrations were measured once a week throughout MLF under the various conditions, to monitor the bacterial metabolism. Implantation control of commercial bacterial starter cultures (data not shown) was performed by the Microflora® laboratory (ISVV, Bordeaux University, France), based on a method developed by Claisse & Lonvaud-Funel (2012). This analysis also confirmed that the indigenous strains (IND1 and IND2) responsible for MLF in wineries, MRGX 2011 and MDC 2011, were different from each other and from the commercial strains used in this study (data not shown). At the end of MLF (<0.1 g/L malic acid), 5 g/h L SO2 were added. Wines made under winery and micro-vinification conditions were sampled for oenological and volatile compound analyses at the end of AF (<0.2 g/L glucose/fructose) and after completion of MLF (malic acid ≤0.1 g/L). Samples were collected for volatile compound analysis in 0.75 L glass bottles, stored at 10 °C for 1 week, decanted, and frozen at −18 °C prior to analysis. The remaining wine was stored in a 30 L stainless-steel barrel for aging. SO_2 content was measured and adjusted if necessary. Samples

were collected for chemical analyses after 3, 6, and 12 months' aging under the same conditions as those applied after AF and MLF.

2.2. Standard Chemical Analysis.

The standard chemical parameters of wine (total acidity, sugar, malic acid, yeast assimilable nitrogen, SO_2, pH, and alcohol) were analyzed by SARCO laboratory (Biolaffort, Floirac, France), using the official methods or those recommended by the International Organization of Viticulture and Wine (OIV).

2.3. Volatile Compound Analyses.

2.3.1. Chemicals.

Compounds used as internal standards, including octan-3-ol (99%), were obtained from Sigma-Aldrich (Steinheim, Germany); deuterated compounds, including ethyl butyrate-4,4,4-d_3 (>99%), ethyl hexanoate-d_{11} (>98%), ethyl octanoate-d_{15} (>98%), and ethyl *trans*-cinnamate-d_5 (phenyl-d_5) (>99%), were obtained from Cluzeau (Sainte-Foy-la-Grande, France). Dichloromethane (>99%) and sodium chloride (norma pure) were from VWR Chemicals (Fontenay-sous-Bois, France), anhydrous sodium sulfate (99%) was supplied by Scharlau Chemie (Sentmenat, Spain), and ethanol (≥99.9%) was obtained from Merck (Damstadt, Germany). R-ethyl 2-hydroxy-3-methylbutanoate (>98%), S-ethyl 2-hydroxy-3methylbutanoate (>98%), R-ethyl 2-hydroxy-4-methylpentanoate (>98.7%), and S-ethyl 2-hydroxy-4-methylpentanoate (>98.7%) were synthesized by Hangzhou Imaginechem Co., (Hangzhou, China).

140 *2.3.2. Ester quantification by HS-SPME-GC/MS.*

141 The method developed and validated by Antalick, Perello & de Revel (2010) was used
142 to quantify thirty-two esters: six ethyl fatty acid esters, seven higher alcohol acetates, four
143 branched acid ethyl esters, four methyl esters, three isoamyl esters, three ethyl esters with odd
144 numbers of carbon atoms, two ethyl cinnamates, and some other minor esters. A mixture of
145 ethyl butyrate-4,4,4-d_3, ethyl hexanoate-d_{11}, ethyl octanoate-d_{15}, and ethyl *trans*-cinnamate-d_5
146 (phenyl-d_5) at about 200 mg/L in ethanol was used as internal standard. In accordance with this
147 method, 20 µL internal-standard solution was added to 25 mL wine. An aliquot of 10 mL of the
148 wine mixture was put into a 20 mL standard headspace vial with 3.5 g sodium chloride. Samples
149 were extracted by HS-SPME and analyzed by GC/MS. The fiber used was 100 mm
150 polydimethylsiloxane (PDMS-100) (Supelco, Bellefonte, PA, USA), conditioned before use as
151 recommended by the manufacturer. Quantification was performed with calibration curves built
152 using red wines.

153

154 *2.3.3. Branched hydroxylated ester quantification by liquid-liquid extraction and GC/MS*
155 *analysis.*

156 The method developed and validated by Lytra, Tempere, de Revel & Barbe (2012) was
157 used to quantify two branched hydroxylated esters: ethyl 2-hydroxy-3methylbutanoate
158 (E2H3MB) and ethyl 2-hydroxy-4-methylpentanoate or ethyl leucate (E2H4MP). According to
159 this method, 100 mL wine were spiked with 20 µL internal standard solution (octan-3-ol at 1.04
160 g/L in ethanol). The mixture was extracted once with 8 mL and twice with 4 mL
161 dichloromethane. The organic phases were blended, dried over sodium sulfate, and
162 concentrated under nitrogen flow (100 mL/min) to obtain 250 µL wine extract
163 Total ester content was quantified using an Agilent 7890A gas chromatograph coupled to a
164 mass spectrometer (MSD 5975C, Agilent Technologies Inc., Santa Clara, CA). A 1 µL sample

of organic extract was injected in splitless mode (injector temperature, 250 °C; splitless time, 0.75 min) on a BP21 capillary column (50 m × 0.32 mm, 0.25 µm film thickness, SGE, Courtaboeuf, France). The oven was programmed at 40 °C for the first minute, heated to 220 °C at 3 °C/min, and then held at that temperature for 20 min. The mass spectrometer was operated in electron impact mode at 70 eV with selected-ion-monitoring (SIM), using 3 characteristic ions for E2H3MB: *m/z* 73 as quantifier and *m/z* 55 and 76 as qualifiers, as well as 3 characteristic ions for E2H4MP: *m/z* 69 as quantifier and *m/z* 87 and 104 as qualifiers. Quantifications were performed with calibration curves built using red wines.

Enantiomers of both esters were assayed using an Agilent 6890N gas chromatograph coupled to a mass spectrometer (MSD 5973i, Agilent Technologies Inc., Santa Clara, CA). A 1 µL sample of organic extract was injected in split mode (injector temperature, 200 °C; split flow, 15 mL/min) on a Chiraldex Gamma-TA column (50 m × 0.25 mm, 0.12 µm film thickness, Astec, Whippany, NJ). The oven was programmed at 40 °C for the first minute, heated to 100 °C at 1 °C/min, and then at 3 °C/min to a final isotherm at 180 °C, which was maintained for 5 min. The mass spectrometer was operated in electron impact mode at 70 eV with SIM mode, selecting the same ions as previously described. After enantiomeric synthesis by an external collaborator, the R- and S-forms and a mixture of both (50:50) were injected separately to identify its LRI, and the peaks of the reference products were compared with those naturally present in wines.

2.4. Statistical analyses

Volatile compound concentrations (micrograms per liter) were expressed as mean ± standard deviation. For each experiment, a first one-way ANOVA was performed between esters levels quantified before and after MLF to study esters levels variation. A second one-way

ANOVA was carried out to study the influence of LAB strain on ester levels. ANOVA were followed by a Tukey *post hoc* test to identify differences between groups, using a 95% confidence interval. by Statistical analyses were performed using XLSTAT 2015.1.03.15659 (Addinsoft, Paris, France).

3. Results and discussion

A total of thirty-four esters were quantified before and after MLF in wines made under six different experimental conditions, using three yeasts and two commercial LAB strains. First, a two-way ANOVA was used to detect significant differences due to the yeast and LAB strains present during both fermentations for each experimentation. No yeast × LAB interaction effect was detected in any of the assays, indicating that the yeast strain responsible for AF did not influence the bacterial ester metabolism (Gammacurta, Marchand, Albertin, Moine, & de Revel, 2014). Therefore, for greater clarity, the results presented in this article will focus on the different bacteria strains used but only one yeast strain (522D). The results obtained with XR and FX10 yeasts are included in the supplementary data (Tables S2 and S3). As an overview of the results, mean variations (percentage) in post-MLF ester levels in wines made under winery conditions (PCLN and STEM), using yeast strain 522D are presented in Figure 1. Table 2 lists ester levels (in µg/L) in wines made under all experimental conditions. A one-way ANOVA followed by a Tukey test was applied to each assay to detect changes in wine composition before and after MFL (Table 3). Data were also processed using one-way ANOVA to highlight the effect of the LAB strain on ester concentrations (Table 4). Results revealed three principal groups: the first and the second group with a decrease or an increase general trend respectively, and the third group where ester levels increased regarding to LAB strain.

215 *3.1. Decrease in ester levels after malolactic fermentation.*

216

217 Two groups of esters were distinguished in terms of their contribution to red wine fruity
218 aroma: one consisted of major esters, such as ethyl fatty acid esters and higher alcohol acetates
219 and the other contained minor esters, including ethyl esters with an odd number of carbon
220 atoms, methyl esters, isoamyl esters, and cinnamates (Guillaume Antalick et al., 2014).

221 A significant decrease in the concentrations of all esters in the "major esters" group was
222 observed in all experiments (Table 2 and Table 3) irrespective of the vintage and the cultivar
223 studied. Ethyl fatty acid esters (4–12 carbons) were significantly affected, with a decrease in
224 the range of 2–15% (C4), 15–29% (C6), 2–59% (C8), 13–75% (C10), and 30–78% (C12)
225 (Figure 1). These compounds are generally considered to make a key contribution to the flavor
226 of red wines (Ebeler, 2001; Ferreira et al., 2000). As for higher alcohol acetates, concentrations
227 of isoamyl acetate, the most powerful odorant in this group, characterized by banana notes,
228 decreased following MLF, which is consistent with previous findings (Malherbe, Tredoux,
229 Nieuwoudt, & Toit, 2011). The decrease was proportionately greater for long-chain acetates, as
230 observed for ethyl esters. A similar decrease in ethyl fatty acid ester and acetate levels was
231 observed in wines fermented with FX10 and XR yeasts, irrespective of the vintage or cultivar
232 considered (Supplementary data).

233 The results were less clear for minor compounds. In particular, variations in ethyl propanoate
234 level were highly-dependent on the experimental conditions, with post-MLF concentrations
235 increasing in WEC-11, WEC-12, and STEM-12 and decreasing in MRGX-11 and MDC-11.
236 Finally, no changes were observed in ethyl propanoate levels in PCLN-12 wines after MLF.
237 Concentrations of other ethyl esters with odd numbers of carbon atoms (5–9 carbons) and
238 methyl esters (4–10 carbons) remained stable in WEC-11 and LP-11 wines, whereas post-MLF
239 concentrations decreased in the other wines.

240 Earlier studies revealed that MLF resulted in significant increases in the concentrations
241 of individual esters potentially involved in modulating red wine fruity aroma, such as ethyl
242 esters and acetates (Delaquis et al., 2000; Maicas et al., 1999). In contrast with these
243 observations, Davis et al. (1988) indicated that enological LAB had esterase activities likely to
244 degrade esters during MLF. Consistent with this study, several authors reported a decrease in
245 ester concentrations following inoculation with *O. oeni* (Gámbaro et al., 2001) or spontaneous
246 MLF (Du Plessis, Steger, Du Toit, & Lambrechts, 2002). Moreover, Knoll et al. (2011) recently
247 highlighted the important role of pH during MLF, reporting an increase in ethyl ester and acetate
248 level in wines at pH 3.2 following this bacterial fermentation, but a decrease in wines with
249 higher pH values. In agreement with these results, our data indicated that MLF may result in a
250 significant decrease in concentrations of these compounds in wines at pH 3.5–3.8, following
251 either inoculated or spontaneous MLF (Supporting Information, Figure S1). Finally, no LAB
252 effect was detected (Table 4), irrespective of the vineyard or the vintage considered, indicating
253 that the ester metabolisms of the two LAB tested in this study were not influenced by the matrix.
254
255 *3.2. Influence of LAB strain on branched ester levels.*
256
257 In contrast with linear esters, concentrations of the four branched esters quantified in
258 this study increased after MLF under all experimental conditions. Concentrations of ethyl 2-
259 methylpropanoate, ethyl 2-methylbutanoate, and ethyl 3-methylbutanoate increased to varying
260 extents after MLF, according to a matrix effect (Table 2 and Table 3). Indeed, a 10–200%
261 increase was observed for ethyl 2-methylpropanoate, 12–190% for ethyl 2-methylbutanoate,
262 and 10–150% for ethyl 3-methylbutanoate. Ethyl phenylacetate content varied less after MLF,
263 remaining relatively stable in WEC-11, MRGX-11, and MDC-11, but increasing by 12–50% in
264 WEC-12, PCLN-12, and STEM-12 (Table 3). Quantification of these compounds during wine

aging revealed that concentrations continued to increase over time (Figure 2), in a range of 20–40%, depending on the esters and matrix considered. Moreover, no significant difference was correlated with the LAB strain, irrespective of the experimental conditions or the yeast strain used (Supplementary data). These esters, derived from the catabolism of amino acids, are mainly synthesized during wine aging, by esterification with ethanol and the corresponding branched acid (Díaz-Maroto, Schneider, & Baumes, 2005). Antalick et al. (2012) recently demonstrated that LAB synthesized branched ethyl esters during MLF. Quantification of these compounds before and after MLF, as well as during wine aging confirmed these results. However, in this study, the LAB strain used to conduct MLF was not found to be an important factor for the synthesis of these aromatic molecules (Table 4).

Two other branched ethyl esters with a hydroxyl group, ethyl 2-hydroxy-3-methylbutanoate (E2H3MB) and ethyl 2-hydroxy-4-methylpentanoate (E2H4MP), were also quantified. Concentrations increased after MLF in all wines, under all experimental conditions (Table 3), as described for branched esters. Variations observed were influenced by the matrix (Table 1), as well as the LAB strain used for MLF (Table 4). Indeed, E2H3MB concentrations were multiplied by 200–1000% in Lactoenos B28 wines, whereas those in wines fermented with Lactoenos 450 LAB increased by 60–150%, depending on the matrix considered. E2H3MB concentrations in spontaneous MLF wines also increased by 100 and 160% in MDC-11 and MRGX-11 wines, respectively. Similar observations were made concerning E2H4MP, with concentrations increasing by 100–550% in Lactoenos B28 wines, 50–100% in Lactoenos 450 wines, and 70–100% in spontaneous MLF wines. Samples fermented with FX10 or XR yeasts also developed higher concentrations of these two compounds when they were inoculated with Lactoenos B28 LAB than Lactoenos 450 LAB (Supplementary data). Quantification of these two aromatic compounds during wine aging revealed that concentrations increased over time (Figure 2). These results agreed with those of previous studies (Bordiga, Piana, Coïsson,

290 Travaglia, & Arlorio, 2014; Lytra et al., 2012). However, E2H3MB and E2H4MP clearly have
291 one stereogenic center in position 2, indicating the potential existence of two enantiomers.
292 Chromatograms analysis revealed that only the R forms of E2H3MB (Figure 3) and E2H4MP
293 (data not shown) were found in all wines in this study after MLF. Lytra et al. (2012) previously
294 demonstrated that young red wines contained only the R form of E2H4MP, whereas aged wines
295 presented both enantiomeric forms in varying ratios, according to age. However, to our
296 knowledge, this was the first time that the influence of the LAB strain on concentrations of
297 these two aromatic compounds had been clearly demonstrated. In a previous publication, Lloret
298 et al. (2002) reported that larger amounts of the (S)-enantiomer of ethyl lactate were produced
299 by *O. oeni*. In the case of these branched hydroxylated ethyl esters, the (R)-enantiomeric
300 pathway of LAB was apparently preferred. Campo, Cacho, & Ferreira (2006) revealed the
301 presence of these two compounds in wine and hypothesized that they contributed significantly
302 to some of its specific fruity notes. Falcão et al. (2012) then assessed the organoleptic impact
303 of E2H4MP, suggesting that this compound contributed to fresh blackberry aromas. However,
304 the results of sensory analyses of these wines, presented in a previous article, demonstrated that
305 the yeast strain had a significant impact on fruity aroma modulation, whereas no LAB strain
306 impact was observed (Gammacurta et al., 2014). Considering the significant difference in
307 E2H3MB and E2H4MP concentrations observed between wines fermented with Lactoenos B28
308 and 450 LAB strains, and the absence of sensory variations, these two compounds apparently
309 have little direct impact on overall red wine flavor.
310
311 **4. Conclusion**
312
313 These findings indicate that MLF has a significant influence on the ester composition
314 of red wines from different Bordeaux vineyards, made with different cultivars – Cabernet

Sauvignon or Merlot – in two vintages, and fermented with three different yeast strains. The concentrations of the key esters known to play a major role in wine aroma, such as acetates and ethyl esters, decreased after MLF, whereas levels of branched esters increased, irrespective of the LAB strain considered. However, the matrix was apparently an important factor in variations in ester concentrations. Conversely, LAB strains had a strong influence on concentrations of branched hydroxylated esters. These results also revealed that commercial and indigenous LAB only synthesized the R forms of E2H3MB and E2H4MP. This effect was observed in samples vinified under experimental conditions and confirmed in wines made in wineries. Further experiments are required to elucidate the mechanisms involved in the biosynthesis of these aromatic compounds by *O. oeni*, as well as the impact of different bacterial starters, to confirm their interest as aromatic markers of MLF. Sensory investigations are also required to fully elucidate their impact on red wine fruity aroma, in order to establish a correlation between their synthesis by *O. oeni* and the flavor modifications associated with MLF.

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Tables

Table 1. Experimental design carried out for this study according to alcoholic and malolactic fermentations parameters.

		Alcoholic fermentation		Malolactic fermentation	
Wine	Cultivar	Stainless steel tank (replicate × hL)	Yeast strain	Stainless steel tank (replicate x L)	LAB strain
WEC 2011	CS	3 × 2.5	522D, XR, FX10	3 × 30	B28, 450
MRGX 2011	CS	1 × 120	522D, FX10	3 × 30	IND1
WEC 2012	CS	3 × 2.5	522D, XR, FX10	3 × 30	B28, 450
PCLN 2012	CS	1 × 65	522D, FX10	3 × 30	B28, 450
MDC 2011	Merlot	1 × 120	522D, XR	3 × 30	B28, 450, IND2
STEM 2012	Merlot	1 × 190	522D, XR	3 × 30	B28, 450

IND, indigenous bacterial strain; CS, Cabernet Sauvignon.

Table 2. Concentration of ester (µg/L) after AF (522D) and mean values in the different wines studied after MLF.

	Cabernet Sauvignon											Merlot						
	WEC 2011			MRGX 2011		PCLN 2012			WEC 12			MDC 2011				STEM 2012		
	522D	522D/B28	522D/450	522D	522D/IND1	522D	522D/B28	522D/450	522D	522D/B28	522D/450	522D	522D/B28	522D/450	522D/IND2	522D	522D/B28	522D/450
ethyl fatty acid esters																		
C2C4	192.8	218.1	208.1	133.0	120.5	155.4	150.2	151.7	209.8	203.3	205.2	187.2	157.3	168.3	176.6	179.2	171.5	176.9
C2C6	406.4	310.4	310.7	205.2	174.2	304.0	237.6	238.4	746.5	540.4	540.4	228.8	178.0	184.1	193.5	438.7	310.4	322.3
C2C8	371.1	332.8	355.6	235.7	181.6	308.4	153.5	154.1	2089.0	1914.5	1872.4	307.0	231.3	239.4	263.1	764.7	322.9	315.6
C2C10	186.1	159.2	160.4	106.9	63.9	185.3	53.4	46.5	1063.6	1218.6	1136.9	169.3	97.0	101.9	170.4	412.9	113.0	114.8
C2C12	17.9	11.1	11.2	10.6	5.4	9.8	2.3	2.4	94.2	124.6	105.6	30.6	8.9	10.0	20.9	39.7	9.0	8.9
higher alcohols acetates																		
C3C2	25.2	23.8	20.1	6.9	5.7	16.0	15.4	15.7	35.5	31.5	32.0	7.7	6.2	6.7	6.4	11.0	10.7	10.6
iC4C2	64.9	55.7	50.7	28.4	22.8	47.5	42.2	42.9	190.0	159.8	161.8	23.1	18.8	20.2	19.7	40.9	38.1	38.6
C4C2	1.8	1.6	1.5	1.6	1.1	1.3	1.0	1.1	3.6	2.6	3.8	1.0	0.9	1.0	0.9	1.1	1.0	0.9
iC5C2	1868.7	1521.2	1330.2	795.9	678.4	1234.7	999.3	973.8	7072.9	5193.6	5356.0	305.9	272.3	281.0	271.4	822.4	662.5	721.6
C6C2	10.0	9.1	10.3	2.8	2.3	7.9	5.1	5.0	267.1	134.9	142.8	0.4	0.3	0.3	0.3	2.5	1.6	1.7
C8C2	0.4	0.2	0.2	0.1	0.1	0.1	0.0	0.0	3.1	0.6	0.6	NQ	NQ	NQ	NQ	0.1	0.0	0.0
2-PhC2C2	186.6	131.6	151.4	57.2	43.9	88.1	70.5	66.7	1483.5	1132.1	1119.5	18.5	17.4	17.8	15.5	48.4	39.1	39.3
ethyl esters with odd number of carbon																		
C2C3	425.0	444.5	437.9	136.2	119.6	187.3	197.6	197.8	47.0	63.0	64.0	166.2	134.2	145.0	147.2	142.3	145.4	149.1
C2C5	1.0	1.0	1.0	0.1	0.1	0.7	0.7	0.6	0.6	0.8	0.8	0.4	0.2	0.4	0.5	1.1	0.9	1.0
C2C7	1.2	1.3	1.3	1.5	1.1	0.6	0.4	0.4	0.9	0.6	0.6	0.4	0.5	0.5	0.6	0.8	0.4	0.4
C2C9	1.3	2.7	2.1	1.7	1.0	1.0	0.3	0.3	1.5	1.4	1.4	1.5	1.1	1.2	1.3	4.4	0.8	0.8
methyl esters																		
C1C4	1.1	1.2	1.1	3.2	0.4	0.8	0.9	0.7	0.0	0.0	0.2	1.1	0.9	0.9	1.0	1.4	1.3	1.3
C1C6	2.5	2.0	1.9	0.7	0.6	1.1	0.9	0.9	0.7	0.5	0.5	1.0	0.7	0.7	0.8	2.1	1.5	1.6

C1C8	1.9	2.0	2.0	0.8	0.6	0.9	0.5	0.5	1.5	1.2	1.3	1.1	1.0	1.0	1.1	3.4	1.6	1.6
C1C10	0.9	0.9	1.0	0.3	0.2	0.5	0.1	0.1	0.9	0.9	0.9	0.6	0.3	0.4	0.6	1.8	0.4	0.4
isoamyl esters																		
iC5C4	0.8	1.0	1.0	0.5	0.4	0.5	0.3	0.4	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.6	0.4	0.5
iC5C6	3.1	2.7	2.3	1.5	1.0	1.8	0.7	0.8	4.4	3.6	3.5	1.2	1.3	1.4	1.5	3.7	1.6	1.6
iC5C8	7.9	8.0	7.2	3.1	2.2	3.0	1.5	1.3	10.3	12.1	11.6	3.0	3.1	3.6	3.6	9.0	4.3	4.4
minor esters																		
C2hex	1.9	1.9	1.7	2.1	2.1	2.3	2.2	2.1	3.0	3.4	3.3	1.0	0.7	0.7	0.8	2.4	2.0	2.0
iC4C6	0.3	0.2	0.3	0.1	0.1	0.1	0.1	0.1	0.3	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.1
C1ger	0.4	0.3	0.4	0.6	0.5	0.2	0.1	0.1	3.4	5.6	5.5	2.0	0.6	0.6	1.2	0.9	0.3	0.3
C2dhcinn	2.2	1.5	1.6	0.4	0.3	0.8	0.6	0.6	0.9	0.9	0.9	0.3	0.3	0.3	0.3	0.7	0.6	0.6
C2cin	2.4	2.2	2.4	1.0	0.8	0.9	0.6	0.6	0.1	0.2	0.2	0.4	0.8	0.4	0.6	0.6	0.5	0.6
ethyl branched acid esters																		
C2 2-mC3	32.3	45.4	45.6	40.3	41.1	43.7	67.7	67.7	19.2	57.8	56.2	42.6	43.2	47.3	51.4	34.8	50.7	51.4
C2 2-mC4	6.8	10.6	10.9	13.5	13.5	11.4	15.9	16.1	1.9	5.5	5.4	10.2	11.4	12.2	13.4	6.1	8.3	8.5
C2 3-mC4	8.4	13.3	11.8	17.0	15.0	15.2	20.6	20.7	3.5	8.9	8.8	12.8	14.2	15.7	15.9	8.5	12.0	12.0
C2PhC2	3.6	3.8	3.7	3.7	3.2	2.8	3.4	3.3	1.5	2.3	2.1	2.6	2.7	2.8	2.9	1.7	1.9	1.9
ethyl branched hydroxylated esters																		
E2H3MB	NQ	NQ	NQ	105.2	177.2	69.5	169.7	117.0	13.4	83.8	26.6	61.2	90.4	66.2	71.5	40.7	91.9	52.9
E2H4MP	NQ	NQ	NQ	4.0	10.8	3.0	8.8	5.1	0.6	6.6	1.5	2.1	5.6	3.0	3.8	1.8	6.6	3.0

NQ: not quantified; ND: not detected

449

Table 3. General trend of esters level variation observed after a one-way ANOVA ($p < 0.05$): ↑ ester level significantly increased, ↓ ester concentration significantly decreased, ↔ ester concentration remained unchanged.

		Cabernet Sauvignon				Merlot	
Compounds	Abbreviations	WEC 2011	MRGX 2011	PCLN 2012	WEC 2012	MDC 2011	STEM 2012
ethyl fatty acid esters							
ethyl butanoate	C2C4	↓	↓	↓	↓	↓	↓
ethyl hexanoate	C2C6	↓	↓	↓	↓	↓	↓
ethyl octanoate	C2C8	↓	↓	↓	↓	↓	↓
ethyl decanoate	C2C10	↓	↓	↓	↓	↓	↓
ethyl dodecanoate	C2C12	↓	↓	↓	↓	↓	↓
higher alcohols acetates							
propyl acetate	C3C2	↓	↓	↓	↓	↓	↓
isobutyl acetate	iC4C2	↓	↓	↓	↓	↓	↓
butyl acetate	C4C2	↓	↓	↓	↓	↓	↓
isoamyl acetate	iC5C2	↓	↓	↓	↓	↓	↓
hexyl acetate	C6C2	↓	↓	↓	↓	↓	↓
octyl acetate	C8C2	↓	↓	↓	↓	NQ	↓
2-phenylethyl acetate	2-PhC2C2	↓	↓	↓	↓	↓	↓
ethyl esters with odd number of carbon							
ethyl propanoate	C2C3	↑	↓	↔	↑	↓	↑
ethyl valerate	C2C5	↔	↓	↓	↓	↔	↓
ethyl heptanoate	C2C7	↔	↓	↓	↓	↔	↓
ethyl nonanoate	C2C9	↔	↔	↓	↓	↓	↓
methyl esters							
methyl butyrate	C1C4	↔	↓	↔	↔	↓	↓

methyl hexanoate	C1C6	↓	↔	↓	↓	↓	↓
methyl octanoate	C1C8	↔	↓	↓	↓	↔	↓
methyl decanoate	C1C10	↔	↓	↓	↔	↓	↓
isoamyl esters							
isoamyl butanoate	iC5C4	↔	↓	↓	↔	↔	↓
isoamyl hexanoate	iC5C6	↓	↓	↓	↓	↔	↓
isoamyl octanoate	iC5C8	↔	↓	↓	↔	↔	↓
minor esters							
ethyl *trans* 2-hexenoate	C2hex	↔	↔	↓	↔	↓	↓
isobutyl hexanoate	iC4C6	↔	↔	↓	↓	↔	↓
methyl *trans*-geranate	C1ger	↔	↓	↓	↔	↓	↓
ethyl dihydrocinnamate	C2dhcinn	↓	↓	↓	↔	↔	↓
ethyl cinnamate	C2cin	↔	↓	↓	↔	↔	↓
ethyl branched acid esters							
ethyl 2-methylpropanoate	C2iC4	↑	↑	↑	↑	↑	↑
ethyl 2-methylbutanoate	C2 2-mC4	↑	↑	↑	↑	↑	↑
ethyl 3-methylbutanoate	C2iC5	↑	↑	↑	↑	↑	↑
ethyl phenylacetate	C2PhC2	↔	↔	↑	↑	↔	↑
ethyl branched hydroxylated esters							
ethyl 2-hydroxy-3-methylbutanoate	2OH3C1C4C2	↑	↑	↑	↑	↑	↑
ethyl 2-hydroxy-4-methylpentanoate	2OH4C1C5C2	↑	↑	↑	↑	↑	↑

NQ: not quantified

Table 4. Influence of LAB strain on esters levels after MLF determined with a one-way ANOVA.

	Cabernet Sauvignon			Medoc	
Compounds	WEC 2011	PLCN 2012	WEC 2012	MDC 2011	STEM 2012
ethyl fatty acid esters					
C2C4	NS	NS	NS	NS	NS
C2C6	NS	NS	NS	NS	*
C2C8	NS	NS	NS	NS	NS
C2C10	NS	NS	NS	NS	NS
C2C12	NS	NS	NS	NS	NS
higher alcohols acetates					
C3C2	NS	NS	NS	NS	NS
iC4C2	NS	NS	NS	NS	NS
C4C2	NS	NS	NS	NS	NS
iC5C2	NS	NS	NS	NS	*
C6C2	NS	NS	NS	NS	NS
C8C2	NS	NS	*	NS	NS
2-PhC2C2	NS	NS	NS	NS	NS
ethyl esters with odd number of carbon					
C2C3	NS	NS	NS	NS	NS
C2C5	NS	NS	NS	NS	*
C2C7	NS	NS	NS	NS	NS
C2C9	NS	NS	*	NS	NS
methyl esters					
C1C4	NS	*	NS	NS	NS
C1C6	NS	NS	NS	NS	NS
C1C8	NS	NS	NS	NS	NS
C1C10	NS	NS	NS	NS	NS
isoamyl esters					
iC5C4	NS	NS	NS	NS	NS
iC5C6	NS	NS	*	NS	NS
iC5C8	NS	*	NS	NS	NS
minor esters					
C2hex	NS	NS	NS	NS	NS
iC4C6	NS	NS	NS	NS	NS
C1ger	NS	NS	NS	NS	NS
C2dhcinn	NS	NS	NS	NS	NS
C2cin	NS	NS	NS	NS	NS

ethyl branched acid esters					
C2 2-mC3	NS	NS	NS	NS	NS
C2 2-mC4	NS	NS	NS	NS	NS
C2 3-mC4	NS	NS	NS	NS	NS
C2PhC2	NS	NS	NS	NS	NS
ethyl branched hydroxylated esters					
E2H3MB	NQ	***	***	***	***
E2H4MP	NQ	***	***	***	***

*, significant at $p < 0.05$; **, significant at $p < 0.01$; ***, significant at $p < 0.001$; NS, not significant; NQ: not quantified.

Figures

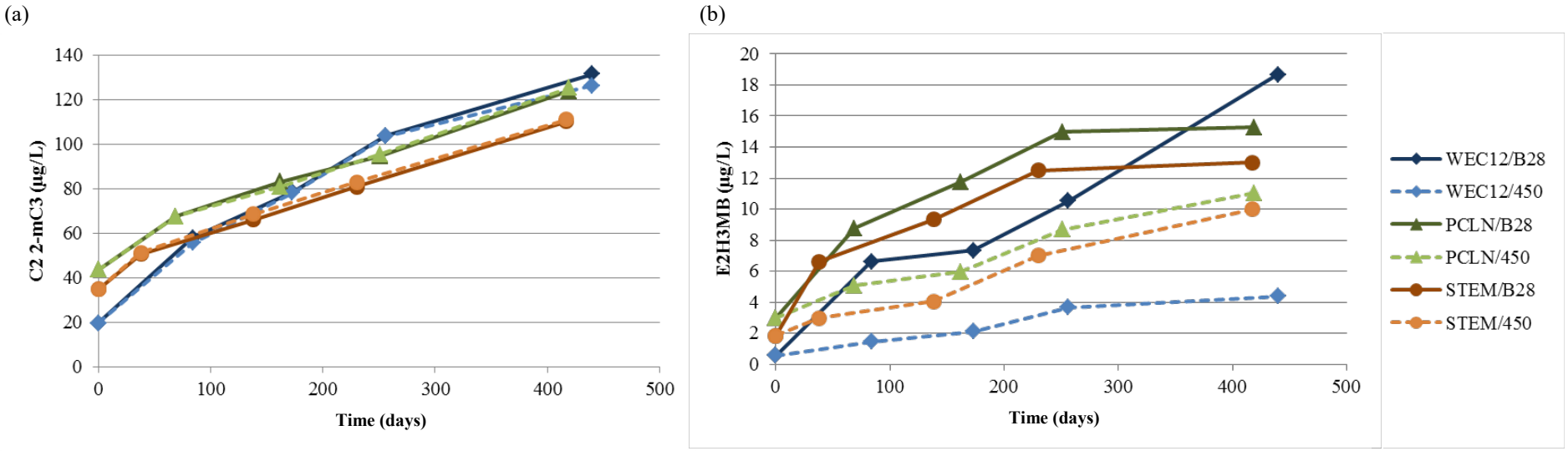
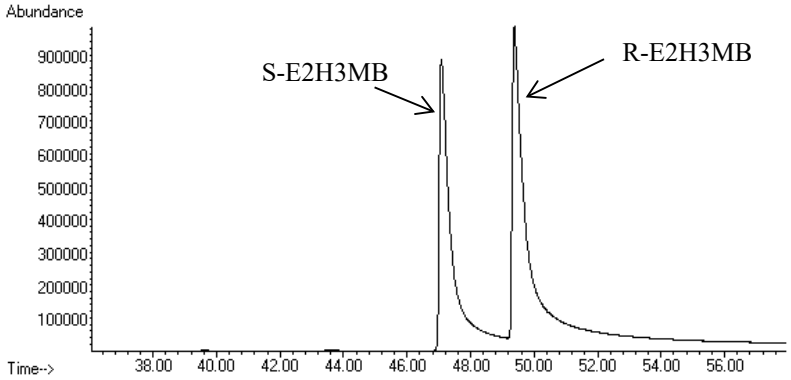


Figure 1. Mean concentrations (in µg/L, n = 3) of ethyl 2-methylpropanoate (a, C2 2-mC3) and ethyl 2-hydroxy-3-methylbutanoate (b, E2H3MB) in WEC 2012, PCLN, and STEM wines fermented with the same yeast and two different bacteria.

(a)



(b)

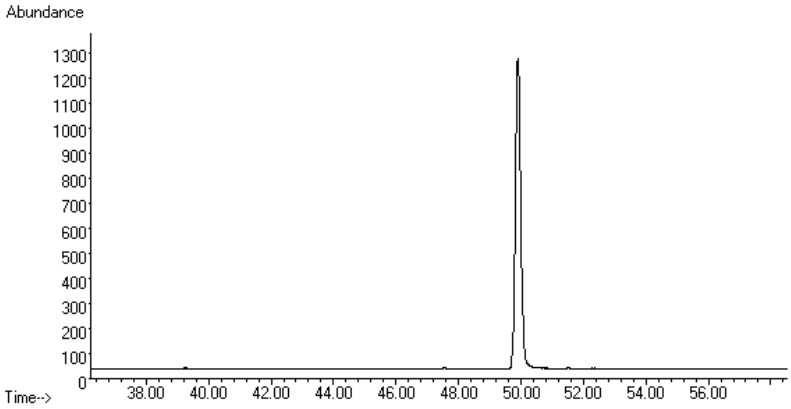


Figure 2. Chromatograms obtained after chiral column analysis of: (a) E2H3MB commercial racemic mixture and (b) a post-MLF PCLN wine extract.