Impact of yeast strain on ester levels and fruity aroma persistence during aging of Bordeaux red wines

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ABSTRACT

The impact of yeast and lactic acid bacteria strains on the fruity aroma of red wines was investigated by sensory and analytical strategies. The ester composition of four different Bordeaux red wines was quantified by HS-SPME-GC/MS. These wines, made with selected yeast and bacteria strains were investigated at the end of alcoholic fermentation and regularly until 12 months of aging, during 2011 and 2012 vintages. Sensory analyses of wines after 3 and 12 months of aging revealed significant differences with regard to yeast strains. Bacteria seemed to have only a slight impact on changes in aromatic profile. Ester levels were strongly influenced by yeast strain and very little affected by malolactic fermentation and aging. Differences and similarities between sensory data and ester profile are discussed. This study highlights the importance of yeast strains in red winemaking. Their sensory impact remains despite the other vinification steps after alcoholic fermentation.

KEYWORDS

Wine, fruity aroma, yeast, esters

INTRODUCTION

Winemaking involves various agricultural, mechanical, chemical, and microbiological processes to enable the best expression of the typical characteristics of the *terroir*. Microbiological processes, such as alcoholic fermentation (AF) and malolactic fermentation (MLF), involve respectively yeast and lactic acid bacteria (LAB). *Saccharomyces cerevisiae* is the main yeast species responsible for AF and *Oenococus oeni* is the main lactic acid bacteria responsible for MLF. Both of these microorganisms play a central role in red winemaking and for the expression of organoleptic qualities. Their metabolism is involved in vinification processes and has a varying impact on wine composition. Consequently, any change in wine composition modulates the taste and flavors[1] of young wines and aged wines.

Even though the musts of many "simple-flavored" grape varieties such as Merlot, Cabernet Sauvignon or Pinot noir are quite odorless, the red wines produced from them present characteristic aromas such as fruity notes. These fruity notes are more or less dependent on the grape variety but also on pedological and climatic characteristics and local traditional processes (the "terroir" or soil). For example, Bordeaux red wines are partly characterized by notes referring to red and black berries.[2] However, no key compounds of these fruity notes have ever been proposed to date. Recent studies suggest that fruity notes could result from perceptive interactions between several of odorous molecule families[3,4] such as varietal compounds like C-13 norisoprenoids[5,6] and lactones[7] or sulfur-containing compounds such as thiols[8,9] or dimethyl sulfide.[10] Many studies have also highlighted the impact of fermentative compounds on fruity aroma especially esters that are considered as one of the most important families of fruity compound in wines.[11–14]

From a qualitative point of view, all red wines contain the same set of ester compounds but their concentrations vary from one wine to another,[15] which could impact the fruity notes

perception. These volatile compounds are mainly produced by yeast during AF. Average ester levels and their relative proportions are highly influenced during fermentation by various parameters such as fermentation temperature, oxygen levels and yeast assimilable nitrogen levels.[16] Some studies have also highlighted a yeast strain-specific effect on ester concentrations.[17,18] Moreover, MLF could impact the ester profile of the finished wine, but no real consensus has been established[19], although there might be a bacterial strain effect.[20–22] The composition of wine after AF partly depends on the substrates released by yeast which in turn will influence bacterial metabolism. Thus, fruity note variations in red wines seem to be more complex than a simple strain effect, involving interactions between the matrix, yeast and LAB strains.

Studies investigating the effect of specific yeast species or strains on wine aroma and flavor have generally focused on white wines such as Riesling,[23] Chardonnay[24] and Sauvignon Blanc[25] while few have been performed on red wine. Yeast species or strains effects on sensory and chemical composition have been reported for Pinot Noir,[26] Shiraz[27] and Prieto Picudo wines.[28] However, their impact on fruity notes in Bordeaux red wines is relatively unknown. Similarly, no consensus has been established regarding the impact of MLF or bacteria strains on the intensity of the fruity aroma. The lack of fundamental data on aromatic markers of red wine fruity notes and the dearth of concomitant biochemical, chemical and sensory studies are probably two of the reasons why the impact of yeast and bacteria strains on fruity flavor in Bordeaux red wines has remained elusive.

This study aims at evaluating the formation and evolution of yeast- and LAB-derived volatile compounds during wine aging. In particular, we assessed the influence of three active dry *Saccharomyces cerevisiae* yeast strains and two commercial *Oenococcus oeni* strains on ester levels and organoleptic characteristics from the end of AF until 12 months of aging. Ester levels were quantified using a HS-SPME-GC/MS method developed by Antalick et al.[15] in our

laboratory. Sensory analyses were also carried out to highlight the influence of the interaction of these microorganisms on the aroma profile of wines produced in conditions of "macro-vinification" and in industrial-scale winemaking conditions. To our knowledge, this is the first report evaluating the effect of both yeast and LAB strains on ester levels as assessed by sensory analysis in aged Bordeaux red wines.

MATERIALS AND METHODS

Yeast and Bacteria Strains and nutrient composition. The yeasts strains *Saccharomyces cerevisiae,* Actiflore cerevisiae (522D), Zymaflore FX10 (Laffort Oenologie, Floirac, France) and Excellence XR (Lamothe-Abiet, Canéjan, France) and the bacterial strains *Oenococcus oeni,* Lactoenos 450 PreAc and Lactoenos B28 PreAc (Laffort Oenologie, Floirac, France) used were commercially dried preparations. Controls of the implantation of yeast commercial starter cultures were performed by PCR at the SARCO laboratory (Laffort Oenologie, Floirac, France) (data not shown). Controls of the implantation of bacterial commercial starter cultures were performed by the Microflora laboratory (Univ. Bordeaux, France) based on a method developed by Claisse and Lonvaud-Funel[29] (data not shown).

Yeast nutrient Superstart/Dynastart (Laffort Oenologie, Floirac, France) is approximately composed of 45% proteins, 35% carbohydrates, 7% total nitrogen, and 6% minerals. Energizer bacterial nutrient (Laffort Oenologie, Floirac, France) contains approximately 55% proteins, 20% carbohydrates, 9% total nitrogen, and 7% lipids.

Winemaking. Complete experimentation on the 2011 Bordeaux appellation vintage was performed in the Laffort Wine Experimental Center (WEC) located in the "Graves de Vayres" area. Cabernet Sauvignon grapes were harvested by hand, destemmed, crushed, and homogeneously distributed into nine 2 hL stainless steel tanks (150 kg of grapes in each). A

sample of each batch was analyzed before AF for sugar and nitrogen content, total acidity and pH (Table 1). Grape must was treated by addition of Lafase Fruit enzyme (Laffort Oenologie, Floirac, 3 g/100 kg) containing pectinase (6700 PGNU/g). Yeast assimilable nitrogen in musts was corrected to around 210 mg N/L. AF was conducted at 19−22°C and initiated by inoculation with rehydrated dry yeast. The yeast nutrient Superstart/Dynastart was added during rehydration of active dried yeast, according to the manufacturer's recommendations. AF was performed using three different yeast strains in triplicate. Implantation controls were performed for each tank at the middle of AF (density close to 1.040). At completion of AF (< 0.2 g/L glucose/fructose), wine composition was analyzed (sugar and malic acid content, total acidity, total SO_2 content, pH, and alcohol content) (Table 1). Samples were collected for volatile compound analysis in a 0.75 L glass bottle and stored at 10 °C for 1 week after addition of 5 g/hL SO_2. Wines were then decanted and frozen at −18 °C before being analyzed.

Each 2 hL tank was divided into two stainless steel barrels of 30 L for MLF. Bacterial cells were rehydrated with Energizer bacterial nutrient according to the manufacturer's instructions and inoculated into wines at the recommended rate. For the entire duration of MLF, the malic acid concentration was measured once a week to monitor the bacterial metabolism. At the end of MLF (< 0.1 g/L malic acid), 50 g/hL of SO_2 were added. Samples were collected like those after AF to assess chemical composition (Table 1) and volatile compounds. Wines were drained into 20 L stainless steel barrels for 3 months of aging. After 3 months, they were sampled in a 0.75 L glass bottle for chemical and volatile analysis, and were decanted and frozen like those after AF and MLF. Sub-batches (3 × 20 L) of each wine were racked and mixed to ensure homogeneity. Wines were bottled in two 0.75 L glass bottles for sensory analysis and then evaluated. The rest of the wine was stored in a 30 L stainless steel barrel for 3 more months of aging. SO_2 content was measured and adjusted if necessary. After the sixth month of aging, the wines were bottled and frozen for those destined for analysis or analyzed

immediately for the purpose of sensory analysis. Some of the wines were also bottled in 1.5 L glass bottles and stored at 10 °C for one year of aging. These wines were then analyzed like the others with chemical and sensory analysis.

The same experimentation was also performed during the 2012 vintage. Cabernet Sauvignon grapes harvested for the WEC 2012 experimentation were thermovinified, (i.e., heated at 70 °C for 6 h). The complete experimental design is shown Figure 1.

Two other experimentations were also performed in 2012 on two other Bordeaux region sites, Vineyard A (V-A) with Cabernet Sauvignon grapes and Vineyard B (V-B) with Merlot grapes in order to observe yeast/LAB interactions at industrial-scale winemaking during 2012. AF in Vineyard A was performed in one 65 hL stainless steel tank with 522D or FX10 strains and was performed in one 120 hL stainless steel tank with 522D or XR strains in Vineyard B (Table 2). For both experimentations, MLF and storage were achieved in plastic food barrels of 30 L and 20L respectively. Only MLF and the first three months of aging were performed in triplicate. These experimentations were stopped after six months of aging.

Chemicals. Deuterated compounds 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). Ethanol (≥ 99.9 %) was obtained from Merck (Damstadt, Germany) and sodium chloride (norma pure) from VWR (Fontenay-sous-Bois, France).

Standard Chemical Analysis. The standard chemical parameters of wines (as total acidity, sugar, malic acid, yeast assimilable nitrogen, SO_2 contents, pH, and alcohol) were analyzed by SARCO laboratory (Laffort Oenologie, Floirac, France) which has been accredited by COFRAC since 1995 (NF EN ISO 17025, accreditation no. 1-0588). Analyses were carried out using the official methods or those recommended by the International Organization of Viticulture and Wine.

Sensory Analysis. Sensory analyses were performed as described by Martin and de Revel (1999).[30] Samples (about 50 mL) were poured into clear INAO wine glasses (NF V09-110, 1971), labeled with random three-digit codes and covered with half of a plastic Petri dish. Evaluations were performed in a dedicated room (ISO 8589: 2007) equipped with individual booths to prevent communication between assessors, under normal daylight, and at room temperature (around 20 °C). All the 20 panelists were from research laboratory staff at ISVV, Bordeaux University, or from the Laffort Company and had previous experience with the sensory evaluation of wines. Analyses were carried out by orthonasal and gustative evaluations.

Napping positioning and ultraflash profiling[31] were used to evaluate WEC wines for the 2011 vintage after 3, 6 and 12 months of aging. The six wines were simultaneously presented to each judge in random order. They had to position the six glasses on a sheet of paper (40 × 60 cm) in such a way that two wines were very near if they were globally perceived similar and that two wines were distant from one another if they seemed different, on the basis of their own criteria. After Napping, judges were asked to enrich their tablecloth by adding a few terms to describe the wines or groups of wine. They were encouraged to choose specific descriptors of each wine or groups of wine they had previously separated.

Ranking tests (ISO 8587: 2006) were used for 2012 vintage wines. To evaluate the influence of an experimental factor on the perceived fruitiness in wine, the samples were presented simultaneously to the panel. The members were asked to order each of the 4 (V-A or V-B wines) or 6 samples (WEC wines) according to its fruitiness, from the least to the most. Equal ranking was not allowed.

Finally, comparison profiles (ISO 13299: 2003) were used on both 2011 and 2012 vintages, to compare the aroma profiles of wines of the same age (3 months, 6 months and 1 year of aging). A list of five odorant descriptive terms was previously proposed. The odorant terms chosen were based on the fruity aroma (“fermentation aroma”, “fresh fruit”, and “cooked

fruit") and overall aroma potentially having a masking impact on the fruity aroma ("vegetal" and "smoked/toasted"). The panelists evaluated the intensity of the five attributes on a discontinuous scale from 0 to 7.

Statistical Analyses applied to sensory analysis. Results obtained from comparison profiles and ranking tests were statistically interpreted according to the norms published by the international organization for standardization (ISO).

Results from Napping were processed by Multiple Factor Analysis (MFA) as by Pagès.[31] Vocabulary generated with ultraflash profiling was treated as described by Perrin et al.[32]

The second test implemented here consisted in a ranking test with no preordained order, so the Friedman test was applied. For each assessor, a value between 1 and 4 was attributed to each sample, depending on the response of the assessor (1 for samples designated as the least intense, 4 for the most intense). The sums of the ranks were obtained for each sample, then parameter F was calculated using Friedman test specifications and compared with a χ^2 value in order to determine whether the result of the test was significant ($F \geq \chi^2$) or not ($F < \chi^2$).

Statistical significance of data from the comparison profile was evaluated with a three-way analysis of variance (yeast, bacteria, and yeast/bacteria interaction). To compensate for idiosyncratic scale usage, attribute ratings were normalized to obtain equal means and standard deviations for each subject. ANOVA was analyzed by a Duncan *post hoc* test to confirm differences between groups.

Statistical analyses (ANOVA and Duncan's *post hoc* tests) were performed using XL-STAT (Addinsoft, Paris, France), whereas graphical representations of MFA were performed using R v2.15.0 (R Development Core Team 2009, Vienna, Austria, R Foundation for Statistical Computing).

Esters quantification by HS-SPME-GC/MS analyses. Each wine sample from the same winemaking step was analyzed at the same time after defrosting. Analyses were performed in triplicate.

The method developed and validated by Antalick et al.[15] was used to quantify 32 esters: ethyl fatty acid esters, acetates of higher alcohol, ethyl branched acid esters, isoamyl esters, methyl esters, ethyl cinnamates, and some other esters (Table 3). A mixture of ethyl butyrate-4,4,4-d_3, ethyl hexanoate-d_{11}, ethyl octanoate-d_{15}, and ethyl trans-cinnamate-d_5 (phenyl-d_5) at about 200 mg/L in ethanol was used as internal standard. In accordance with this method, 20 µL of internal standard solution was added to 25 mL of wine. An aliquot of 10 mL of this wine was introduced into a 20 mL standard headspace vial filled with 3.5 g of sodium chloride. The samples were extracted by HS-SPME and analyzed by GC/MS. The fiber used was polydimethylsiloxane 100 µm (PDMS-100) (Supelco, Bellefonte, PA, U.S.A.). They were conditioned before use, as recommended by the manufacturer. Quantification was performed with calibration curves built in red wines.

Statistical analysis for esters quantifications. Volatile compound concentrations (micrograms per liter) were expressed as mean ± standard deviation. The effects of yeast and LAB strains on each variable were tested by two-way analysis of variance (yeast strain, LAB strain and yeast*LAB strain interactions). ANOVA was followed by a Duncan *post hoc* test to identify differences between groups using a 95% confidence interval.

To obtain a general overview of the data, a PCA was performed from ester concentrations (32 esters). We sought whether the different factors (sampling time, vintage, yeast/LAB couples) allowed clear clustering of the data. Since the matrix effect was considerable, the data were standardized independently, i.e., the data were mean-centered and scaled for each vintage in order to reveal the impact of the other factors. PCA was run using the ade4, car, and plotrix packages from the R program.

Statistical analyses (ANOVA and Duncan's *post hoc* tests) and graphical representations were performed using R v2.15.0 (R Development Core Team 2009, Vienna, Austria, R Foundation for Statistical Computing).

RESULTS AND DISCUSSION

First approach for characterizing the impact of yeast/bacteria couples on red wine aromatic profiles. Napping is one of the best sensory tests to evaluate global aroma and taste.[32] It permits the global characterization of wines and allows each judge to build his/her representation based on sensory dimensions he/she considers important. Representations of attributes and wines from the 2011 vintage after 3 and 12 months of aging are presented in Figure 2. The most frequently used descriptors to define the aroma of these wines belonged to the lexical field of fruit (fruity, fresh fruits, cooked fruits, red fruits, black fruits, fermentation aroma), as expected for young Bordeaux red wines. Other descriptors used to qualify these wines were "smoked", "toasted", and "vegetal".

Figure 2b for three months aged wines and figure 2d for twelve months aged wines show the projection of the sensory variables on the plane formed by Principal Components 1 and 2 (58% of the total variance) and by PC 2 and 3 (45% of the total variance) respectively. For wines after three months of aging, the first component (36%) is defined by the smoked/toasted descriptor, whereas the second component (22%) is mainly characterized by fruity descriptors (fruity, red fruits, fermentation aroma, cooked fruits). The second component (24%) of Figure 2d (wines aged 12 months) highlights the contrast between the fresh fruits on one side and the smoked and toasted descriptor on the other, positioning the latter aromas as a potential mask of fruity aroma. The third axis (21%) opposes "fruity" (red and cooked fruits, fermentation aroma, fruity) and "pleasant mouthfeel" terms to the vegetal attribute.

242 Projections of wines after 3 and 12 months of aging in the bidimensional plot are
243 presented in Figure 2a,c. As shown, samples were scattered over the map, principally according
244 to the "fruity" vector: in both cases, the trained panel perceived FX10s and XRs wines as being
245 fruitier than 522D fermented wines. Moreover, wines were also separated according to the
246 bacteria strain: samples fermented with the 450 *O. oeni* strain appeared more smoked and
247 toasted in wines after 3 months of aging than the wines where the B28 strain performed MLF.
248 After 12 months of aging, a separation of wines regarding to the bacteria strain were also
249 observed (axis 1, 24.5%, data not shown), but no correlation with none descriptors could be
250 established to confirm 3 months aging results.

251 According to these initial results obtained with wines at two different steps of aging, the
252 differences observed between wines seemed to be correlated with yeast strain. Most descriptors
253 used for discriminating wines refer to fruity notes. This suggests that yeast strains could be the
254 predominant factor involved in the modulation of fruity aroma, which is not surprising in young
255 wines (after 3 and 12 months of aging). Nevertheless, a bacterial strain effect was also observed,
256 to a lesser extent, and seemed to be linked to smoked/toasted descriptors. The development of
257 these notes during MLF has already been observed by Antalick et al.[19] and could play a role in
258 the masking of the perception of fruity aroma. They also hypothesized that interactions between
259 yeast and LAB could play a key role in the modification of aroma in wine during MLF, but at
260 this point, the hypothesis of an effect of the yeast/LAB association on the fruity aroma of red
261 wines seemed less probable.

262 **Impact of yeast and bacterial strain on fruity aroma perception.** Descriptors
263 associated with fruity notes were proposed by each judge during the Napping tests to
264 characterize a wine or group of wines. Thus, a ranking test and comparison profile were
265 performed with wines from the 2012 vintage to investigate the impact of the choice of yeast
266 and LAB strains on fruity aroma perception.

First, each taster had to assign 1 point to the least fruity wine and 4 (V-A, V-B) or 6 points (WEC) to the fruitiest one. The sum of the ranks was calculated for each modality from each experiment. Statistical analysis was significant at 1% (Table 4) with wines in the WEC experiment, which means that the tasters were able to distinguish different strains according to their fruity perception. Moreover, the highest sum of ranks was observed for the associations FX10/B28 and FX10/450, followed by 522D/B28 and 522D/450, and XR/B28 and XR/450. Results were in agreement with those of the Napping tests. Furthermore, they were similar to those obtained in Vineyard A and in Vineyard B in the Bordeaux region with two different grape cultivars in real conditions of winemaking. The Friedman test was significant at 0.1% with wines from the V-A essay and significant at 5% with V-B wines (Table 4). The highest sum of ranks for V-A wines was observed with the associations FX10/B28 and FX10/450, followed by 522D/450, and finally 522D/B28. Results with wines from V-B confirmed that wines inoculated with the 522D yeast strain appeared less fruity, since the highest sum of ranks was observed for XR/B28, XR/450, 522D/B28, and then 522D/450. These results observed in 3 different wines, made in "macro-vinification" or in real winemaking conditions confirmed the impact of the yeast strain on fruity aroma in Bordeaux red wines, despite a potential matrix effect (different region, grape cultivars or winemaking process).

Next, each judge had to attribute a score for each modality from 0 to 7 for 5 descriptors potentially involved in the modulation of fruity aroma (Figure 3). Statistical analyses confirmed that the judges differentiated wines inoculated with yeast strain FX10 from the others. Indeed, for WEC wines (Figure 3a), the FX10/450 association appeared significantly different from the others with "fermentation aroma" notes ($P < 0.01$). Wines fermented with yeast strain FX10 also seemed to be different from the other wines and were described as having more "fresh fruit" notes (yeast effect: $P < 0.01$). Alternatively, wines inoculated with yeasts XR or 522D were described as less fruity and more vegetal (yeast effect: $P < 0.05$) than FX10 wines. A

bacterial strain effect was also observed for "fresh fruit" notes, indicating that wines fermented with B28 LAB seemed more fruity than those with the 450 strain (LAB effect: $P < 0.01$). Similar results were observed in wineries, especially in Vineyard A (Figure 3b), where both FX10/450 and FX10/B28 wines were described as fruitier than 522D/450 and 522D/B28 wines, thereby confirming the results from the ranking test. In contrast, the two latter wines appeared more smoked and toasted than the others (yeast effect: $P < 0.01$). Finally, results obtained with Vineyard B wines (Figure 3c) were less clear, perhaps indicating a less significant difference between yeasts XR and 522D. This is consistent with observations made with wines from WEC, where differences were perceived only between FX10 and XR wines and FX10 and 522D wines, but where no significant difference was observed between wines inoculated with XR or 522D.

In this study, the yeast strain appears 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. These findings highlight the persistence of a yeast strain effect on fruity aroma over time. The impact of bacterial strains is not as clear.

There is known to be a decrease in fruity notes further to an intensification of the lactic aroma after MLF,[33] mainly due to the increase in diacetyl levels. However, no reference was made to lactic or buttery notes during the Napping test to characterize the differences between wines. It seems unlikely that diacetyl is responsible for the modulation of fruity aroma. Recent studies highlight the difficulty to perceive lactic notes in very young wines,[19,22] probably because of interactions between diacetyl and sulfur dioxide.[33] The olfactory mask of smoked notes over the fruity aroma described by Antalick et al.[19] could also explain differences observed between wines. The bacterial effect observed mostly with the Napping test could be due to sulfur compounds such as hydrogen sulfide or dimethyl sulfide synthesized by certain

317 LAB strains with toasted notes.[34,35] However, the link between bacterial strain and these notes
318 is not clear and other compounds with reduction notes might be involved. Finally, the question
319 is which aromatic compounds could be responsible for the differences observed. Esters are
320 considered to be the primary source of fruity aroma.[36] They are mainly synthesized by yeast
321 during alcoholic fermentation but LAB can modulate their concentration during MLF close to
322 their perception threshold. These variations could explain the differences observed during
323 sensory tests.

324 **Overview of changes in ester composition due to yeast/bacteria associations over**
325 **time.** The PCA scores plot and corresponding loadings plot in Figure 4 provide an overview of
326 ester profiles associated with the metabolic activity of the six associations from the end of AF
327 to 12 months of aging in WEC 2011 and 2012 wines.

328 Separation along the first axis (48.3% of the total variance) was due to an effect of
329 sampling time. Samples taken at the same time (AF11 and AF12, MLF1 and MLF12, and so
330 on) overlapped and were situated toward the first axis in chronological order, with clear
331 separations between AF, MLF samples, and those collected during wine aging. Indeed, samples
332 after AF for both vintages are positioned to the left of the scores plot and are strongly correlated
333 with esters with long carbon chains such as ethyl decanoate, ethyl octanoate, ethyl hexanoate,
334 octyl acetate, hexyl acetate, and methyl decanoate. This indicates that samples after AF have
335 high levels of these esters. MLF11 and MLF12 samples are positioned toward the left of the
336 scores plot and associated with a lesser extent with long carbon chain esters, indicating lower
337 concentrations in *post* MFL wines than in *post* AF wines. Samples after 3, 6 and 12 months of
338 aging are positioned toward the right of the scores plot. Separation between the three aging
339 times is less prominent but the chronology still seems to be respected. They are inversely
340 correlated to long carbon chain esters, indicating lower concentrations than *post* MLF and *post*
341 AF wines. This is in accordance with other studies that highlighting the decrease in EFAE and

AHA concentrations during wine aging.[37,38] Wine aging samples (12m12, 6m12, 3m12, and to a lesser extent 6m11 and 3m11) are correlated with ethyl branched acid esters (C2iC4, C2-2mC4 and C2iC5), indicating higher levels in these samples than in MLF11, MLF12, AF11, and AF12, and therefore an increase in the concentration of EBAE during wine aging.[39,40]

The second axis (11.6% of the total variance) seems to separate samples with regard to the yeast strain. Samples inoculated with strain 522D (represented with circles in Figure 4) are positioned toward the bottom of the scores plot and are associated with propyl acetate, 2-phenylethyl acetate, ethyl propanoate, ethyl phenylacetate, and methyl butanoate. Wines fermented with strain FX10 (represented by squares) tend to cluster in the middle of the scores plot, whereas XR samples (denoted with diamonds) are positioned toward the top of the scores plot and are inversely correlated to almost all esters, indicating that the levels of esters in these samples are lower than in FX10 and 522D samples. Interestingly, samples inoculated with the same yeast overlap in the scores plot regardless of bacteria strain. The synthesis of esters by yeast has been known for decades, whereas the impact of bacteria is still controversial. Evidence exists of esterase activity in *O. oeni*[41,42] and several reports have shown changes in ester concentrations in wines after MLF with *O. oeni*. However, no consensus has been established, since these variations differ between studies.[19,43,44] In the present study, the impact of bacterial metabolism on ester concentrations seems very limited compared to that of yeast, which seems to be the predominant factor.

Ester profiles and link with sensory analysis. Significance levels of a yeast effect calculated with the Analyses of Variance on the different wines are presented Table 5. Significant effect of yeast strain was observed for all esters in all wines at almost each step of winemaking. Quantitative data of the 32 esters analyzed in the experimental wines are summarized in Figure 5 while the entire data collected are available in the Supporting Information (SI, Table S1−S4). In terms of valuable contribution to the fruity aroma of red

367 wines, three subfamilies of esters can be highlighted: ethyl fatty acid esters (EFAE), acetates of
368 higher alcohols (AHA), and ethyl branched acid esters (EBAE).[3,19] All esters from the same
369 family generally presented the same pattern, so we assumed that total concentration in esters in
370 each family would be a good representation.

371 Changes in ethyl fatty acid esters depended on both matrix and yeast strain. FX10 yeast
372 strain tended to produce significantly higher EFAE concentrations in WEC 2012 and Vineyard
373 A wines, in accordance with the sensory results. However, Napping tests in the WEC on 2011
374 wines clearly differentiated 522D wines from FX10 and XR wines, which were described as
375 fruitier, whereas chemical analysis demonstrated higher EFAE levels in 522D wines after 3 and
376 12 months. Similar results were observed with Vineyard B wines, fermented with Merlot
377 grapes, which were described as fruitier when inoculated with XR than with 522D, even though
378 the latter synthesized more EFAE. A recent study suggested that precursor availability rather
379 than the expression level of genes responsible of ethyl ester synthesis is the limiting factor in
380 esters production.[45] In other words, matrix effect could be the predominant factor responsible
381 for the modulation of ethyl ester levels, and it appears that EFAE might not explain yeast
382 differences observed during sensory analyses. Similar observations were made with acetates of
383 higher alcohols, which were found in comparable concentrations in wines inoculated with 522D
384 and FX10 as in wines from WEC 2011, WEC 2012 and V-A, while their aromatic profiles were
385 significantly different. Finally, 522D also significantly synthesized more ethyl branched acid
386 esters than XR and FX10 in all wines analyzed in this study. Overall, wines fermented with
387 yeast strains FX10 and 522D had roughly the same profile in terms of ester levels, but 522D
388 wines appeared less fruity. These observations are not as surprising and confirm results from
389 other studies investigating fruity aroma in red wine over the past decade. In fact, wine consists
390 of a highly complex mixture of volatiles but only a few of these compounds are known to
391 contribute directly to wine aroma.[46,47] However, the perception of flavors is not the result of a

392 single dominant compound, but rather stems from the result of interactions between a multitude
393 of volatiles.[48] Thus, no key aromatic compounds responsible for fruity aroma in Bordeaux red
394 wines have yet been identified, although several studies highlight the indirect potential role of
395 a certain number of compounds. Moreover, Ferreira et al.[49] suggested that the concentrations
396 of compounds from the same chemical family had a cumulative effect, resulting in a perception
397 of their overall fruity character. Pineau et al.[3] highlighted the importance of esters and acetates
398 in the red- and blackberry aroma, which was confirmed subsequently.[4,14] They also
399 demonstrated in dearomatized wines that very small variations in ester levels can be perceived
400 by a panelist, even at concentrations far below their olfactory threshold, and that this can affect
401 the perception of fruity aroma. Ester profiles of 522D and FX10 strains may look quite similar,
402 but small variations in the concentration of only a few esters could cause a flavor profile to be
403 perceived completely differently. However, the study of minor esters as isoamyl esters, methyl
404 esters, or ethyl cinnamates also failed to establish a link with fruity aroma perception (available
405 in SI Table S5).

406 Other interactions involving volatile compounds from other families could also be
407 responsible for the modulation of fruity aroma. Empirical observations have shown that the
408 addition of copper sulfate in Cabernet Sauvignon or Merlot wines leads to a significant decrease
409 in the perception of fruity flavor. This suggests the contribution of sulfur-derivative compounds
410 and especially odorous thiols in the development of the aromatic complexity of red wines.
411 Varietal compounds revealed by yeast during AF such as mercaptans present in Cabernet
412 Sauvignon and Merlot wines may participate in the toasted and blackcurrant nuances of these
413 wine aromas.[50,51] Small variations in the release of these powerful odoriferous volatile
414 compounds due to yeast strain during AF may be sufficient to modulate the aromatic expression
415 of wines.

In summary, sensory analyses of wines from different Bordeaux appellations at different fermentation and aging times allowed the impact of both yeast and bacteria strain on fruity aroma to be investigated. The perception of fruity notes in Bordeaux red wines was particularly impacted by the yeast strain, while bacterial strain had little impact on the modulation of fruity aroma. Ester profiles alone cannot account for the differences in the perception of fruity notes despite their important role. Yeast strains also had a large impact on ester composition in wines, to such an extent that significant differences detectable at the end of alcoholic fermentation were unaffected by MLF and aging. The impact of these microorganisms on the concentration of other compounds involved in the modulation of fruity notes should lead to better correlation between sensory and chemical analyses. Henceforth, winemakers have a sensory parameter choosing industrial yeast strains and no longer need to rely only on kinetic and technical specifications.

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ASSOCIATED CONTENT

Supporting information available:

Mean concentrations with standard deviations (micrograms per liter) after AF, MLF, 3, 6 and 12 months of aging in WEC 2011, WEC 2012, Vineyard A and Vineyard B wines for each yeast strain are presented in Tables S1, S2, S3 and S4 respectively.

Results of Duncan *post hoc* tests for yeast effect on the different esters quantified are presented in Table S5. Indicated groups represent yeasts which had synthesized the most esters after AF, 3 months and 12 months (only for WEC 2011 wines) of aging.

This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Peynaud, E. In *Connaissance et travail du vin*. Dunod Ed. Paris, France, 1981.
2. Pineau, B., Barbe, J.-C., Leeuwen, C. V. & Dubourdieu, D. Olfactory specificity of red-and black-berry fruit aromas in red wines and contribution to the red Bordeaux wine concept. *J. Int. Sci. Vigne Vin* **44,** 39–49 (2010).
3. Pineau, B., Barbe, J.-C., Van Leeuwen, C. & Dubourdieu, D. Examples of perceptive interactions involved in specific 'red-' and 'black-berry' aromas in red wines. *J. Agric. Food Chem.* **57,** 3702–3708 (2009).
4. Lytra, G., Tempere, S., De Revel, G. & Barbe, J.-C. Impact of perceptive interactions on red wine fruity aroma. *J. Agric. Food Chem.* **60,** 12260–12269 (2012).
5. Escudero, A., Campo, E., Fariña, L., Cacho, J. & Ferreira, V. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *J. Agric. Food Chem.* **55,** 4501–4510 (2007).
6. Pineau, B., Barbe, J.-C., Van Leeuwen, C. & Dubourdieu, D. Which impact for β-damascenone on red wines aroma? *J. Agric. Food Chem.* **55,** 4103–4108 (2007).
7. Loscos, N., Hernandez-Orte, P., Cacho, J. & Ferreira, V. Release and formation of varietal aroma compounds during alcoholic fermentation from nonfloral grape odorless flavor precursors fractions. *J. Agric. Food Chem.* **55,** 6674–6684 (2007).
8. Tominaga, T., Blanchard, L., Darriet, P. & Dubourdieu, D. A powerful aromatic volatile thiol, 2-furanmethanethiol, exhibiting roast coffee aroma in wines made from several Vitis vinifera grape varieties. *J. Agric. Food Chem.* **48,** 1799–1802 (2000).
9. Blanchard, L., Tominaga, T. & Dubourdieu, D. Formation of furfurylthiol exhibiting a strong coffee aroma during oak barrel fermentation from furfural released by toasted staves. *J. Agric. Food Chem.* **49,** 4833–4835 (2001).
10. Anocibar Beloqui, A., Kotseridis, Y. & Bertrand, A. Determination of the content of dimethyl sulphide in some red wines. *J. Int. Sci. Vigne Vin* **30,** 167–170 (1996).
11. Ebeler, S. E. Analytical chemistry: Unlocking the secrets of wine flavor. *Food Rev. Int.* **17,** 45–64 (2001).
12. Ferreira, V., López, R. & Cacho, J. F. Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agric.* **80,** 1659–1667 (2000).
13. Jiang, B., Xi, Z., Luo, M. & Zhang, Z. Comparison on aroma compounds in Cabernet Sauvignon and Merlot wines from four wine grape-growing regions in China. *Food Res. Int.* **51,** 482–489 (2013).
14. Falcao, L. D., Lytra, G., Darriet, P. & Barbe, J.-C. Identification of ethyl 2-hydroxy-4-methylpentanoate in red wines, a compound involved in blackberry aroma. *Food Chem.* **132,** 230–236 (2012).
15. Antalick, G., Perello, M. C. & de Revel, G. Development, validation and application of a specific method for the quantitative determination of wine esters by headspace-solid-phase microextraction-gas chromatography–mass spectrometry. *Food Chem.* **121,** 1236–1245 (2010).

16. Vilanova, M. *et al.* Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by Saccharomyces cerevisiae wine yeasts. *Appl. Microbiol. Biotechnol.* **77,** 145–157 (2007).
17. Mateo, J. J., Jiminez, M., Huerta, T. & Pastor, A. Contribution of different yeasts isolated from musts of Monastrell grapes to the aroma of wine. *Int. J. Food Microbiol.* **14,** 153–160 (1991).
18. Molina, A. M. *et al.* Differential synthesis of fermentative aroma compounds of two related commercial wine yeast strains. *Food Chem.* **117,** 189–195 (2009).
19. Antalick, G., Perello, M. C. & de Revel, G. Characterization of fruity aroma modifications in red wines during malolactic fermentation. *J. Agric. Food Chem.* **60,** 12371–12383 (2012).
20. Malherbe, S., Tredoux, A. G. J., Nieuwoudt, H. H. & Toit, M. Comparative metabolic profiling to investigate the contribution of *O. oeni* MLF starter cultures to red wine composition. *J. Ind. Microbiol. Biotechnol.* **39,** 477–494 (2011).
21. Vestner, J. *et al.* Investigation of the volatile composition of pinotage wines fermented with different malolactic starter cultures using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOF-MS). *J. Agric. Food Chem.* **59,** 12732–12744 (2011).
22. Ruiz, P., Izquierdo, P. M., Seseña, S., García, E. & Palop, M. L. Malolactic Fermentation and secondary metabolite production by *Oenoccocus oeni* strains in low pH wines. *J. Food Sci.* **77,** M579–M585 (2012).
23. Henick-Kling, T., Edinger, W., Daniel, P. & Monk, P. Selective effects of sulfur dioxide and yeast starter culture addition on indigenous yeast populations and sensory characteristics of wine. *J. Appl. Microbiol.* **84,** 865–876 (1998).
24. Egli, C. M., Edinger, W. D., Mitrakul, C. M. & Henick-Kling, T. Dynamics of indigenous and inoculated yeast populations and their effect on the sensory character of Riesling and Chardonnay wines. *J. Appl. Microbiol.* **85,** 779–789 (1998).
25. Dubourdieu, D., Tominaga, T., Masneuf, I., des Gachons, C. P. & Murat, M. L. The role of yeasts in grape flavor development during fermentation: the example of Sauvignon blanc. *Am. J. Enol. Vitic.* **57,** 81–88 (2006).
26. Takush, D. G. & Osborne, J. P. Impact of yeast on the aroma and flavour of Oregon Pinot Noir wine: Yeast and wine aroma. *Aust. J. Grape Wine Res.* **18,** 131–137 (2012).
27. Holt, H. *et al.* Influence of yeast strain on Shiraz wine quality indicators. *Int. J. Food Microbiol.* **165,** 302–311 (2013).
28. Álvarez-Pérez, J. M. *et al.* Sensory and chemical characterisation of the aroma of Prieto Picudo rosé wines: The differential role of autochthonous yeast strains on aroma profiles. *Food Chem.* **133,** 284–292 (2012).
29. Claisse, O. & Lonvaud-Funel, A. Development of a multilocus variable number of tandem repeat typing method for *Oenococcus oeni*. *Food Microbiol.* **30,** 340–347 (2012).
30. Martin, N. & de Revel, G. Sensory evaluation: scientific bases and oenological applications. *J. Int. Sci. Vigne Vin* **33,** 81–93 (1999).
31. Pagès, J. Collection and analysis of perceived product inter-distances using multiple factor analysis: Application to the study of 10 white wines from the Loire Valley. *Food Qual. Prefer.* **16,** 642–649 (2005).
32. Perrin, L. *et al.* Comparison of three sensory methods for use with the Napping® procedure: Case of ten wines from Loire valley. *Food Qual. Prefer.* **19,** 1–11 (2008).
33. Bartowsky, E. J. & Henschke, P. A. The 'buttery' attribute of wine - Diacetyl - Desirability, spoilage and beyond. *Int. J. Food Microbiol.* **96,** 235–252 (2004).
34. Pripis-Nicolau, L., De Revel, G., Bertrand, A. & Lonvaud-Funel, A. Methionine catabolism and production of volatile sulphur compounds by *Œnococcus œni*. *J. Appl. Microbiol.* **96,** 1176–1184 (2004).
35. Vallet, A., Lucas, P., Lonvaud-Funel, A. & De Revel, G. Pathways that produce volatile sulphur compounds from methionine in *Oenococcus oeni*. *J. Appl. Microbiol.* **104,** 1833–1840 (2008).
36. Sumby, K. M., Grbin, P. R. & Jiranek, V. Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. *Food Chem.* **121,** 1–16 (2010).
37. Marais, J. & Pool, H. J. Effect of storage time and temperature on the volatile composition and quality of dry white table wines. *Vitis* 151–154 (1980).

38. Ramey, D. & Ough, C. S. Volatile ester hydrolisys or formation during storage of model solutions of wine. *J. Agric. Food Chem.* 928–934 (1980).
39. Ferreira, V., Aznar, M., López, R. & Cacho, J. Quantitative gas chromatography−olfactometry carried out at different dilutions of an extract. Key differences in the odor profiles of four high-quality Spanish aged red wines. *J. Agric. Food Chem.* **49,** 4818–4824 (2001).
40. Díaz-Maroto, M. C., Schneider, R. & Baumes, R. Formation pathways of ethyl esters of branched short-chain fatty acids during wine aging. *J. Agric. Food Chem.* **53,** 3503–3509 (2005).
41. Sumby, K. M., Matthews, A. H., Grbin, P. R. & Jiranek, V. Cloning and characterization of an intracellular esterase from the wine-associated lactic acid bacterium *Oenococcus oeni*. *Appl. Environ. Microbiol.* **75,** 6729–6735 (2009).
42. Sumby, K. M., Grbin, P. R. & Jiranek, V. Characterization of EstCOo8 and EstC34, intracellular esterases, from the wine-associated lactic acid bacteria *Oenococcus oeni* and *Lactobacillus hilgardii*. *J. Appl. Microbiol.* **114,** 413–422 (2013).
43. Boido, E. *et al.* The effect of bacterial strain and aging on the secondary volatile metabolites produced during malolactic fermentation of Tannat red wine. *J. Agric. Food Chem.* **57,** 6271–6278 (2009).
44. Costello, P. J., Francis, I. L. & Bartowsky, E. J. Variations in the effect of malolactic fermentation on the chemical and sensory properties of Cabernet Sauvignon wine: interactive influences of *Oenococcus oeni* strain and wine matrix composition: Malolactic fermentation in Cabernet Sauvignon wine. *Aust. J. Grape Wine Res.* **18,** 287–301 (2012).
45. Saerens, S. M. G. *et al.* The *Saccharomyces cerevisiae* EHT1 and EEB1 genes encode novel enzymes with medium-chain fatty acid ethyl ester synthesis and hydrolysis capacity. *J. Biol. Chem.* **281,** 4446–4456 (2006).
46. Mestres, M., Busto, O. & Guasch, J. Analysis of organic sulfur compounds in wine aroma. *J. Chromatogr. A* **881,** 569–581 (2000).
47. Ortega-Heras, M., González-SanJosé, M. L. & Beltrán, S. Aroma composition of wine studied by different extraction methods. *Anal. Chim. Acta* **458,** 85–93 (2002).
48. Francis, I. L. & Newton, J. L. Determining wine aroma from compositional data. *Aust. J. Grape Wine Res.* **11,** 114–126 (2005).
49. Ferreira, V., López, R., Escudero, A. & Cacho, J. F. The aroma of Grenache red wine: Hierarchy and nature of its main odorants. *J. Sci. Food Agric.* **77,** 259–267 (1998).
50. Bouchilloux, P., Darriet, P., Henry, R., Lavigne-Cruège, V. & Dubourdieu, D. Identification of volatile and powerful odorous thiols in Bordeaux red wine varieties. *J. Agric. Food Chem.* **46,** 3095–3099 (1998).
51. Rigou, P., Triay, A. & Razungles, A. Influence of volatile thiols in the development of blackcurrant aroma in red wine. *Food Chem.* **142,** 242–248 (2014).

TABLES

Table 1. Main technological parameters of the different musts and wines after alcoholic and malolactic fermentations.

Wine	Yeast strain	Bacteria strain	Reducing sugar (g/L)	D-glucose + D-fructose (g/L)	alcoholic degree (% v/v)	pH	Total acidity (g/L H_2SO_4)	Volatile acidity (g/L acetic acid)	YAN (mg N/L)[a]	Total sulfur dioxide (mg/L)	Free sulfur dioxide (mg/L)	malic acid (g/L)
Must												
WEC 2011	NA[b]	NA	218	NQ[c]	NQ	3.48	3.38	NQ	100	55	NQ	NQ
WEC 2012	NA	NA	207	NQ	NQ	3.59	4.31	NQ	129	42	NQ	NQ
V-A 2012	NA	NA	216	NQ	NQ	3.60	2.87	NQ	94	56	NQ	NQ
V-B 2012	NA	NA	199	NQ	NQ	3.50	3.66	NQ	95	58	NQ	NQ
After alcoholic fermentation												
WEC 2011	XR	NA	1.1	nd[d]	12,9	3,53	4,81	0,07	NQ	27	4	2,27
WEC 2011	522D	NA	1.0	nd	12,8	3,53	4,77	0,07	NQ	29	4	2,07
WEC 2011	FX10	NA	1.1	nd	13,1	3,50	4,92	0,09	NQ	30	5	2,22
WEC 2012	XR	NA	1.0	0.01	11,9	3,64	4,93	0,19	NQ	23	5	3,25
WEC 2012	522D	NA	0.7	0.01	11,9	3,65	4,85	0,23	NQ	22	4	3,08
WEC 2012	FX10	NA	0.8	0.01	11,9	3,66	5,00	0,29	NQ	23	4	3,41
V-A 2012	522D	NA	1.0	0.09	13,1	3,59	4,47	0,15	NQ	8	nd	1,66
V-A 2012	FX10	NA	1.1	0.12	13,0	3,60	4,75	0,22	NQ	7	nd	2,16
V-B 2012	XR	NA	1.2	0.06	13,0	3,64	4,26	0,20	NQ	6	nd	2,19
V-B 2012	522D	NA	1.2	0.07	13,1	3,57	4,27	0,16	NQ	3	nd	2,04
After malolactic fermentation												
WEC 2011	XR	B28	1.0	nd	13,2	3,68	3,40	0,29	NQ	41	30	0,01
WEC 2011	522D	B28	1.0	nd	13,2	3,67	3,43	0,23	NQ	43	28	0,01
WEC 2011	FX10	B28	0.9	nd	13,2	3,60	3,56	0,30	NQ	42	30	0,01
WEC 2011	XR	450	1.1	nd	13,2	3,64	3,51	0,13	NQ	33	20	0,01
WEC 2011	522D	450	1.0	nd	13,2	3,64	3,54	0,09	NQ	39	25	0,01
WEC 2011	FX10	450	1.0	nd	13,1	3,59	3,67	0,16	NQ	29	21	0,01
WEC 2012	XR	B28	0.8	0.09	12,1	3,81	2,83	0,37	NQ	52	31	0,01
WEC 2012	522D	B28	0.8	0.09	12,1	3,82	2,84	0,44	NQ	46	29	0,01
WEC 2012	FX10	B28	0.9	0.14	12,2	3,81	2,95	0,50	NQ	45	29	0,01
WEC 2012	XR	450	0.8	0.11	12,1	3,79	2,86	0,28	NQ	69	37	0,01
WEC 2012	522D	450	0.8	0.12	12,1	3,81	2,84	0,35	NQ	61	34	0,01
WEC 2012	FX10	450	0.9	0.13	12,1	3,79	2,95	0,39	NQ	60	35	0,01
V-A 2012	522D	B28	1.3	0.12	13,2	3,70	3,42	0,23	NQ	32	18	0,01
V-A 2012	FX10	B28	1.3	0.14	13,1	3,71	3,53	0,35	NQ	32	17	0,01
V-A 2012	522D	450	1.3	0.10	13,2	3,70	3,60	0,20	NQ	31	18	0,01
V-A 2012	FX10	450	1.3	0.14	13,1	3,71	3,57	0,28	NQ	29	18	0,01
V-B 2012	XR	B28	1.1	0.01	13,0	3,69	3,25	0,31	NQ	60	33	0,01
V-B 2012	522D	B28	1.1	0.01	13,1	3,68	3,25	0,30	NQ	70	36	0,01
V-B 2012	XR	450	1.2	0.04	13,0	3,67	3,27	0,23	NQ	39	20	0,01
V-B 2012	522D	450	1.2	0.04	13,1	3,67	3,26	0,21	NQ	34	17	0,01

[a] YAN : Yeast Assimilable Nitrogen ; [b] NA : Not Applicable ; [c] NQ : Not Quantified ; [d] nd : not detectable

Table 2. Experimentations implemented.

Sites	Vintage	Volume* (hL)	Grape cultivars	Yeast	Bacteria
WEC	2011	2	Cabernet Sauvignon	522D, FX10, XR	450, B28
WEC	2012	2	Cabernet Sauvignon**	522D, FX10, XR	450, B28
Vineyard A (V-A)	2012	65	Cabernet Sauvignon	522D, FX10	450, B28
Vineyard B (V-B)	2012	120	Merlot	522D, XR	450, B28

Volume* correspond to the volume of stainless steel tank used for AF; ** thermovinification

Table 3. Esters quantified.

Aroma compound	Abbreviation	Aroma compound	Abbreviation
Ethyl fatty acid esters (EFAE)		*Methyl fatty acid esters*	
ethyl propanoate	C2C3	methyl butyrate	C1C4
ethyl butyrate	C2C4	methyl hexanoate	C1C6
ethyl hexanoate	C2C6	methyl octanoate	C1C8
ethyl octanoate	C2C8	methyl decanoate	C1C10
ethyl decanoate	C2C10	*Isoamyl esters of fatty acid*	
ethyl dodecanoate	C2C12	isoamyl butyrate	iC5C4
Ethyl branched acid esters (EBAE)		isoamyl hexanoate	iC5C6
ethyl isobutyrate	C2iC4	isoamyl octanoate	iC5C8
ethyl 2-methylbutyrate	C2 2-mC4	*Ethyl acid esters with odd number of carbon*	
ethyl isovalerate	C2iC5	ethyl valerate	C2C5
ethyl phenylacetate	C2PhC2	ethyl heptanoate	C2C7
Acetates of higher alcohols (AHA)		ethyl nonanoate	C2C9
propyl acetate	C3C2	*Cinnamates and minor esters*	
isobutyl actetate	iC4C2	ethyl cinnamate	C2Cin
butyl acetate	C4C2	ethyl dihydrocinnamate	C2dhCinn
isoamyl acetate	iC5C2	ethyl *trans* 2-hexanoate	C2hex
hexyl acetate	C6C2	isobutyl hexanoate	iC4C6
octyl acetate	C8C2	methyl *trans*-geranate	C1ger
2-phenylethyl acetate	2-PhC2C2		

Table 4. Ranking of perceived fruitiness in wines after 3 months of aging in vintage 2012.

Wine	n	Strains and sums of ranks						F†	Results ‡
		XR/B28	XR/450	FX10/B28	FX10/450	522D/B28	522D/450		
WEC	22	72	53	95	94	76	72	16.10	Significant at 1%
Vineyard A	24	-	-	75	72	39	54	21.15	Significant at 0.1%
Vineyard B	18	59	44	-	-	42	35	10.2	Significant at 5%

†F was calculated as described in ISO 8587:2006 for Friedman test. $F = \frac{12 \times \sum_{i=1}^{p} Ri^2}{n \times p \times (p+1)} - 3 \times n \times (p+1)$ with n represents the number of tasters, p represents the number of modalities and Ri represents the sum of the ranks for the modality i. ‡With six modalities (WEC), the test was significant at 5% when F > 11.07, at 1% when F > 15.09 and at 0.1% when F > 20.52. With four modalities (V-A, V-B), the test was significant at 5% when F > 7.81, at 1% when F > 11.34 and at 0.1% when F > 16.27.

Table 5. Significance level in the analysis of variance for yeast effect on the different esters quantified.

	WEC 2011					WEC 2012				Vineyard A				Vineyard B		
	after AF	after MLF	after 3 months	after 6 months	after 12 months	after AF	after MLF	after 3 months	after 6 months	after AF	after MLF	after 3 months	after 6 months	after AF	after MLF	after 3 months
Ethyl fatty acid esters (EFAE)																
C2C3	***	***	***	***	***	***	***	***	***	***	***	***	***	**	***	***
C2C4	NS	***	**	***	NS	*	***	***	***	**	**	***	*	NS	***	***
C2C6	**	*	*	NS	NS	***	***	***	***	***	***	***	***	***	***	***
C2C8	NS	***	**	*	*	***	***	***	***	***	***	***	***	***	***	***
C2C10	NS	NS	**	**	**	***	NS	***	***	**	***	***	***	***	***	***
C2C12	***	NS	***	**	***	***	*	***	**	***	NS	NS	***	***	***	**
EFAE sum	**	***	***	**	**	***	**	***	***	***	*	*	*	**	***	***
Ethyl branched acid esters (EBAE)																
C2iC4	**	***	NS	NS	NS	***	***	***	***	**	***	***	***	*	**	NS
C2 2-mC4	***	***	***	***	***	***	***	***	***	***	***	***	***	**	***	***
C2iC5	***	**	***	***	*	***	***	***	***	***	***	***	***	*	NS	NS
C2PhC2	***	***	***	***	***	***	***	***	***	**	***	***	***	***	***	***
EBAE sum	***	***	*	**	**	**	***	***	***	***	***	***	***	**NS**	**	*
Acetate of higher alcohols (AHA)																
C3C2	**	***	***	***	***	***	***	***	***	NS	**	***	NS	***	***	***
iC4C2	***	***	***	**	***	***	***	***	***	NS	*	***	NS	***	***	**
C4C2	***	***	*	**	NS	***	***	***	***	NS	**	***	NS	***	***	***
iC5C2	**	***	**	**	***	***	***	***	***	NS	NS	*	*	***	***	***
C6C2	***	NS	NS	*	*	**	***	***	***	**	***	***	***	***	***	***
C8C2	*	**	***	**	NS	***	***	***	***	NS	***	NS	NS	NS	NS	*
2-PhC2C2	***	***	***	**	***	***	***	***	***	***	***	***	***	***	***	***
AHA sum	**	***	***	***	***	**	***	***	***	**NS**	**NS**	**NS**	**NS**	**	***	***

Table 5. Continued.

	WEC 2011					WEC 2012				Vineyard A				Vineyard B		
	after AF	after MLF	after 3 months	after 6 months	after 12 months	after AF	after MLF	after 3 months	after 6 months	after AF	after MLF	after 3 months	after 6 months	after AF	after MLF	after 3 months
Methyl fatty acid esters																
C1C4	***	***	***	***	***	NS	NS	NS	***	NS	NS	*	*	***	*	***
C1C6	***	***	***	*	**	***	***	***	***	NS	NS	*	*	***	***	***
C1C8	*	***	**	**	**	***	***	***	***	**	***	***	NS	***	***	***
C1C10	NS	**	***	***	***	***	*	***	*	**	***	***	NS	***	***	*
Ethyl acid esters with odd number of carbon																
C2C5	***	*	***	***	**	NS	***	***	**	**	***	***	*	***	*	***
C2C7	*	***	***	***	***	***	***	***	***	**	***	***	***	***	***	***
C2C9	***	NS	***	*	**	***	***	***	*	***	**	***	*	***	***	***
Isoamyl esters of fatty acid																
iC5C4	NS	***	***	**	**	***	***	***	***	*	NS	**	NS	***	***	***
iC5C6	NS	*	**	*	***	*	***	***	***	NS	NS	NS	***	***	***	***
iC5C8	NS	NS	**	**	**	**	***	**	NS	**	***	***	***	***	***	***
Cinnamates and minor esters																
C2hex	NS	NS	**	**	*	***	***	***	***	NS	NS	NS	NS	***	***	***
iC4C6	**	**	***	**	NS	NS	NS	***	***	NS	***	***	**	*	NS	NS
C1ger	***	*	***	NS	***	***	***	***	***	NS	NS	**	**	*	**	NS
C2dhCinn	*	NS	**	NS	*	***	***	***	***	*	***	***	***	***	***	***
C2Cin	*	NS	***	**	**	***	NS	*	***	NS	***	***	**	***	***	***

*, **, *** indicate significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ respectively; NS : non significant differences.

FIGURES GRAPHICS

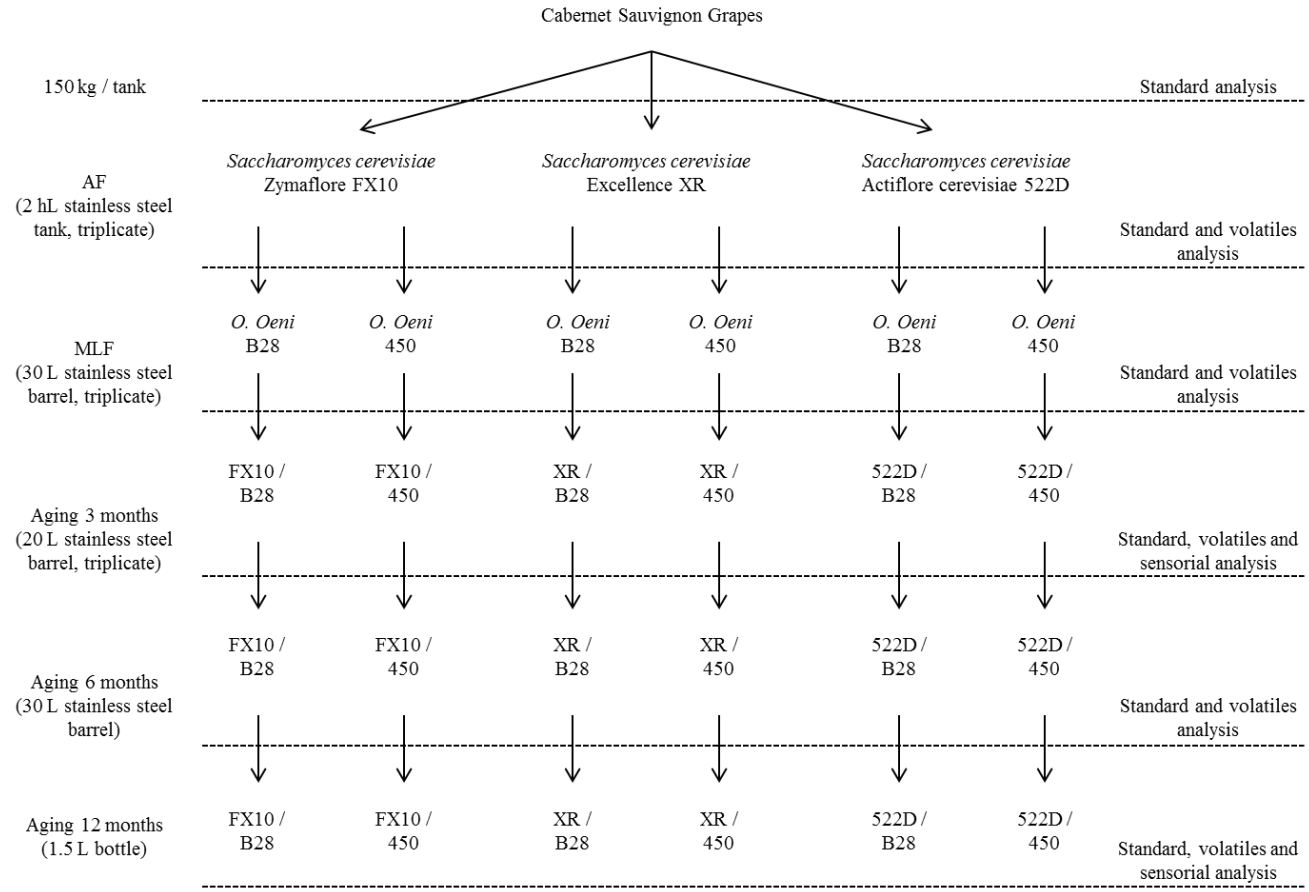

Figure 1. Experimental design in wine experimental center (WEC) for vintages 2011 and 2012.

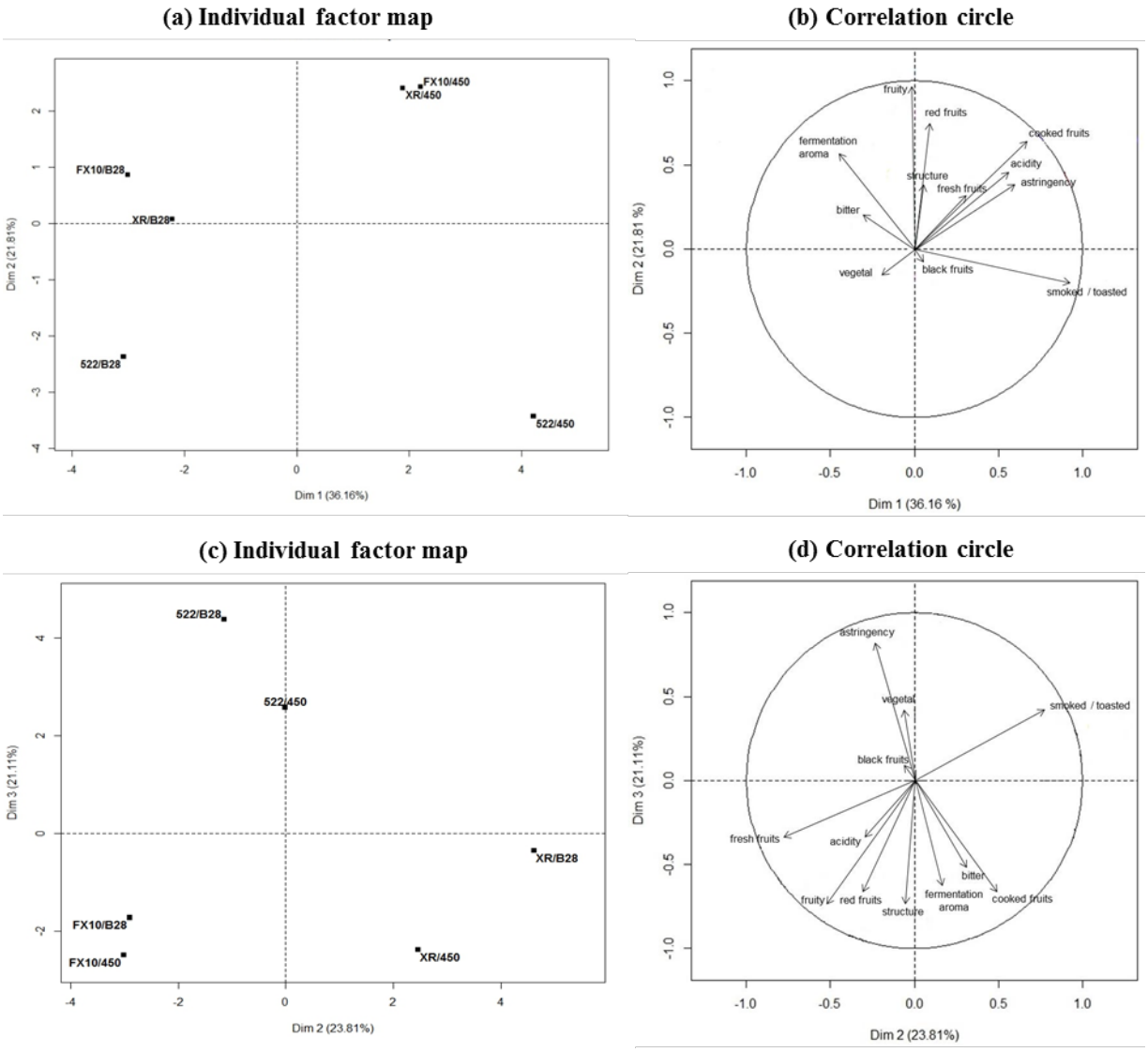

Figure 2. Wines representation (a, c) and characterization (b, d) emerging from Napping combined with Ultra-Flash Profiling (MFA, plane 1−2 and 2−3), realized with wines after 3 months (a, b) and 12 months (c, d) of aging, 2011 vintage.

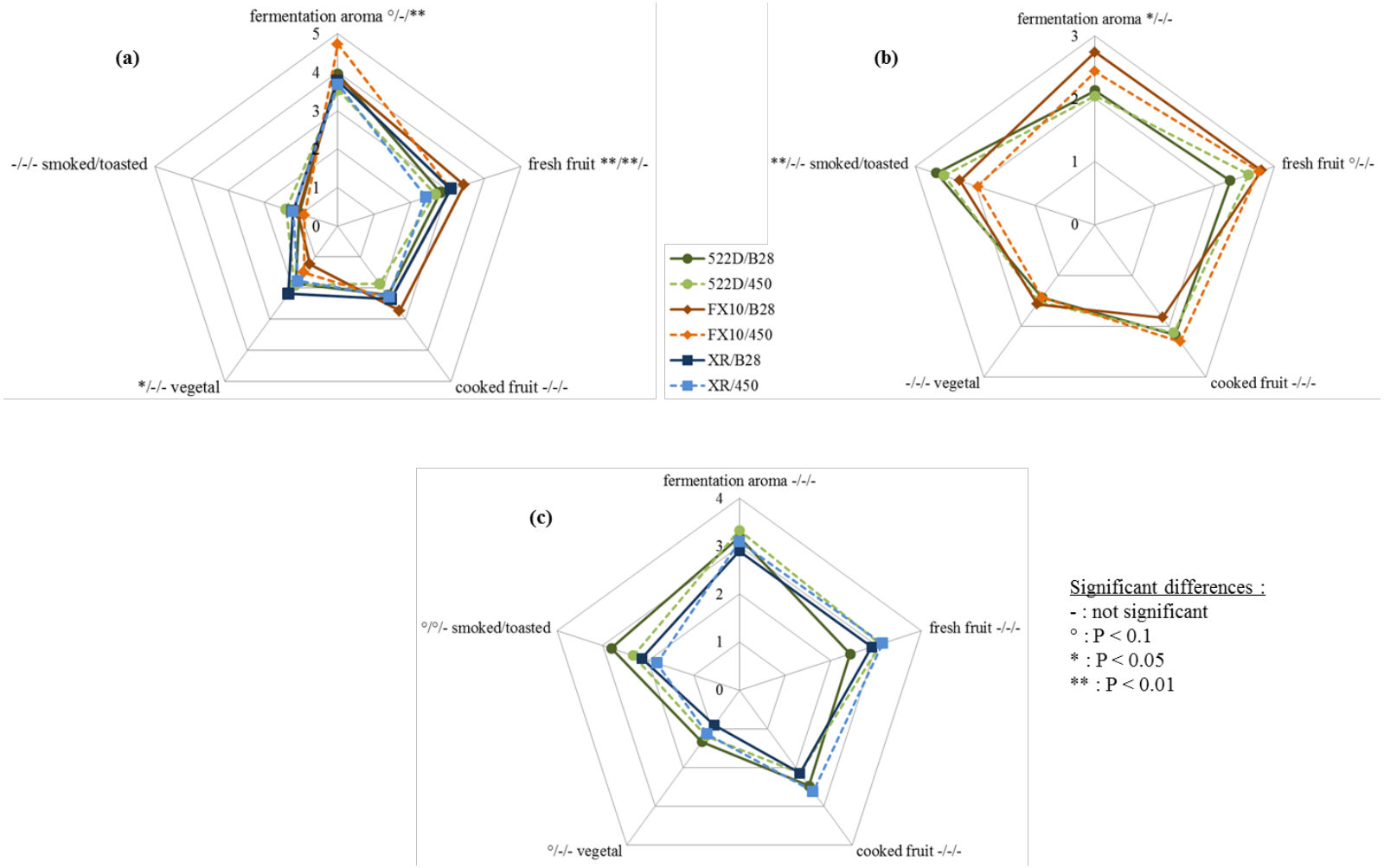

Figure 3. Mean sensory descriptor values for WEC (a), Vineyard A (b) and Vineyard B (c) red wines after 3 months of aging made in 2012 vintage with six yeast/bacteria couples. Significant differences are indicated with asterisks (yeast strain effect / LAB strain effect / yeast x LAB interaction effect).

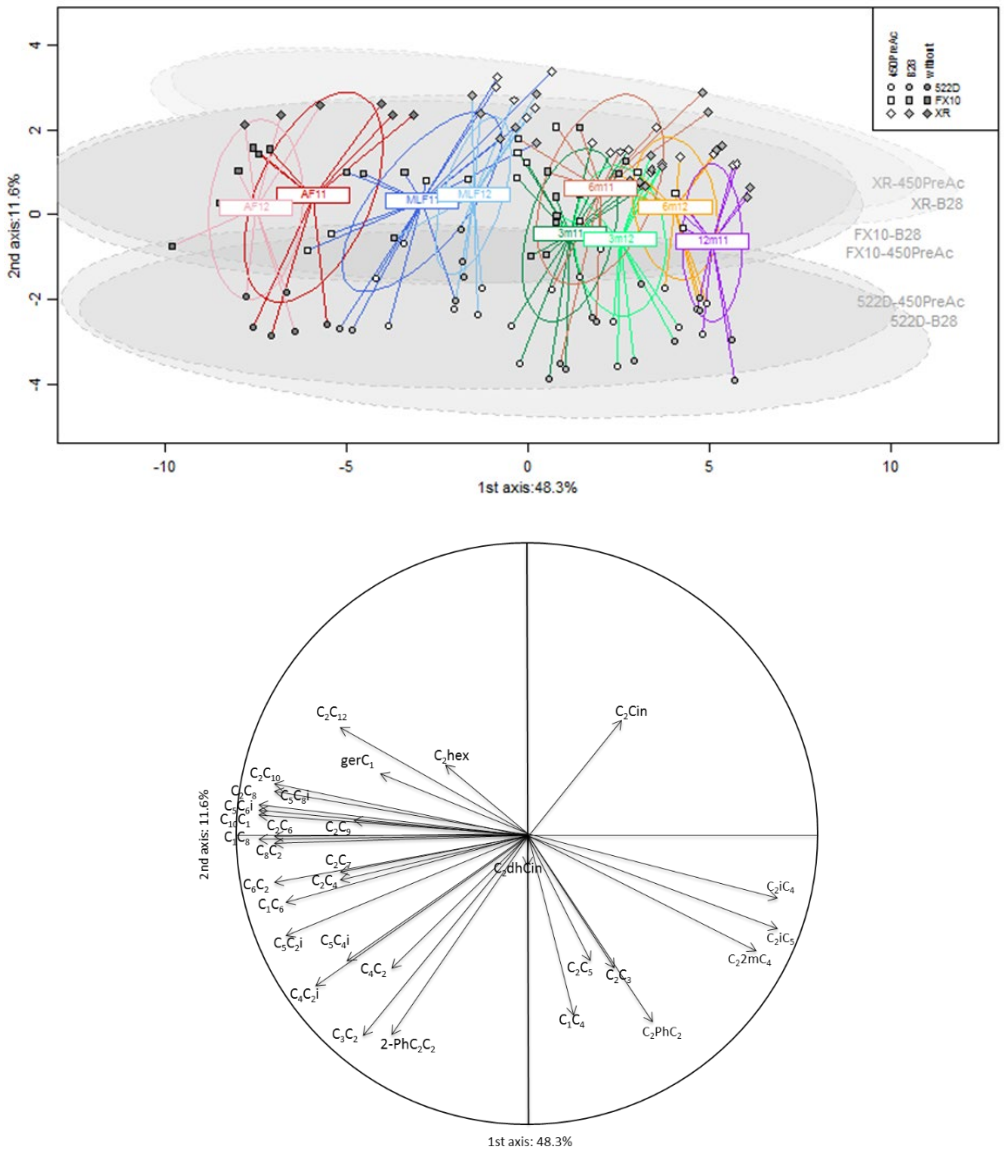

Figure 4. Principal Component Analysis (PCA) providing a visual overview of changes in the esters composition over the time in WEC wines from 2011 (AF11 to 12m11) and 2012 (AF12 to 6m12) vintages: after Alcoholic Fermentation (AF), Malolactic Fermentation (MLF), 3, 6 and 12 months of aging (3m, 6m, 12m). The data were mean-centered and scaled for each vintage in order to decrease the matrix impact and to reveal the impact of the other factors. Yeasts strains are represented by circle (522D), square (FX10) and diamond (XR); empty geometric shapes represent 450 LAB strain and full ones are for B28 LAB strain. Abbreviations of esters in the correlation circle are listed in Table 3.

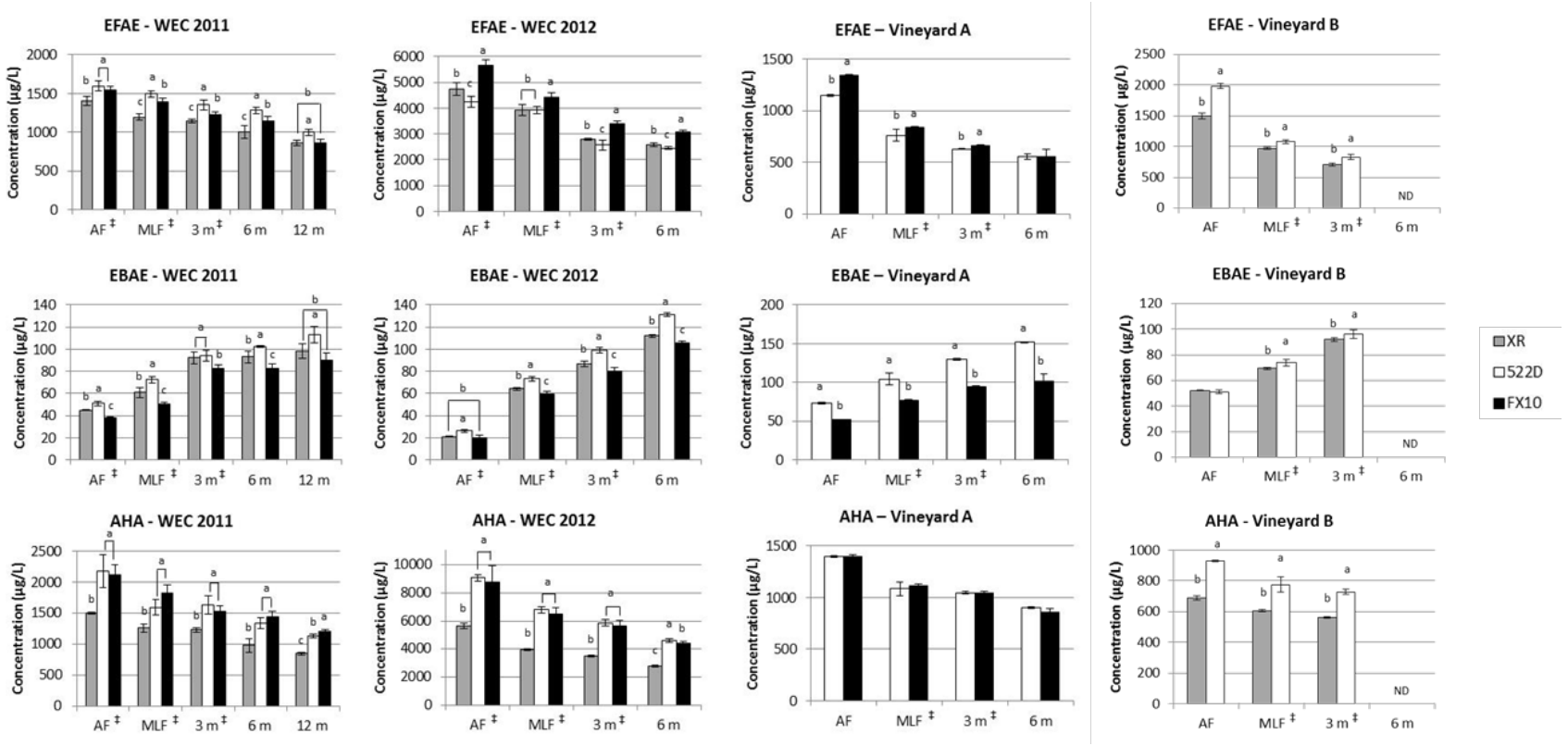

Figure 5. Sum of mean concentrations with standard deviations (micrograms per liter) of esters after alcoholic fermentation (AF), malolactic fermentation (MLF), 3, 6 and 12 months of aging (3m, 6m, 12m) in wines from WEC 2011, WEC 2012, Vineyard A (V−A) and Vineyard B (V−B). Esters were grouped in families described in Table 3 regarding yeast strains. Samples with biological triplicates were injected twice each. For samples which were not triplicated, three injections were carried out.
Different alphabetical letters indicate significant differences.
EFAE: Ethyl Fatty Acid Esters; EBAE: Ethyl Branched Acid Esters; AHA: Acetates of Higher Alcohols
ND: No Data; ‡ indicates biological triplicates

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