Research Article

Received: 15 November 2016 Revised: 25 January 2017 Accepted article published: 15 February 2017 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.8272

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

Marine Gammacurta,[a,b,c*] Stéphanie Marchand,[a,b] Virginie Moine[b,c] and Gilles de Revel[a,b]

Abstract

BACKGROUND: The typical fruity aroma of red Bordeaux wines depends on the grape variety but also on microbiological processes, such as alcoholic and malolactic fermentations. These transformations involve respectively the yeast *Saccharomyces cerevisiae* and the lactic acid bacterium *Oenococcus oeni*. Both species play a central role in red winemaking but their quantitative and qualitative contribution to the revelation of the organoleptic qualities of wine has not yet been fully described. The aim of this study was to elucidate the influence of sequential inoculation of different yeast and bacteria strains on the aromatic profile of red Bordeaux wine.

RESULTS: All microorganisms completed fermentations and no significant difference was observed between tanks regarding the main oenological parameters until 3 months' aging. Regardless of the yeast strain, B28 bacteria required the shortest period to completely degrade the malic acid, compared to the other strain. Quantification of 73 major components highlighted a specific volatile profile corresponding to each microorganism combination. However, the yeast strain appeared to have a predominant effect on aromatic compound levels, as well as on fruity aroma perception.

CONCLUSION: Yeasts had a greater impact on wine quality and have more influence on the aromatic style of red wine than bacteria.

Keywords: red wine; yeast; lactic acid bacteria; aromatic compounds

INTRODUCTION

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

During AF, yeasts such as *Saccharomyces cerevisiae* play a significant role in the formation and modulation of wine taste and aroma[3−5] by releasing varietal aromatic compounds from grape precursors,[6,7] as well as synthesizing *de novo* volatile compounds.[8,9] In contrast, the influence of MLF and lactic acid bacteria (LAB), such as *O. oeni*, on red wine fruity aroma is not as clear. MLF is often empirically associated with a decrease in the intensity of fruity notes. However, according to the literature, LAB enhance the fruity aroma of red wines in some cases, attenuate it in others, and sometimes have no influence on it at all.[10] These diverging results may be explained either by the use of different LAB strains in these studies or by a matrix effect involving the cultivar and the yeast strain used to carry out AF as well as the LAB. Indeed, it is well known that yeasts influence LAB growth during winemaking.[11,12] Therefore, it would not be surprising that they also influence LAB metabolism and thus the aromatic compounds in the wine. The few studies investigating these effects demonstrated significant differences in the aroma of Chardonnay[13] and Chancellor wines[14] fermented with several yeast/LAB strain combinations, at different temperatures.

The lack of fundamental data on the aromatic markers responsible for the fruity aroma of red wines is probably another reason for the lack of consensus. Recent studies suggested that these fruity notes were due to perceptive interactions

* *Correspondence to: M Gammacurta, Unité de recherche Œnologie, Université de Bordeaux, ISVV, EA 4577 210 chemin de Leysotte CS 50008, 33882 Villenave d'Ornon Cedex, France. E-mail: marine.gammacurta@u-bordeaux.fr*

a *Unité de recherche Œnologie, Université de Bordeaux, Villenave d'Ornon, France*

b *INRA, ISVV, Villenave d'Ornon, France*

c *Biolaffort, Bordeaux, France*

between different families of aromatic compounds, rather than individual compounds.[15,16] Varietal compounds, such as C_{13}-norisoprenoids,[17] lactones,[18] thiols,[19] sulfur-containing compounds such as dimethyl sulfide,[20] and yeast- and LAB-derived compounds, including higher alcohols,[21] esters,[22] volatile fatty acids[23] and diacetyl[24] are examples of aromatic molecules that have a negative or positive impact on red wine aroma.

This uncertainty surrounding the influence of fermentative microorganisms on wine quality is problematic for winemakers. From a practical point of view, it would be useful to know whether the influence of LAB strains on red wine quality is affected by some winemaking variables, particularly the yeast strain used for AF. Thus the aim of this study was to analyze the impact of different yeast/LAB combinations on the pool of aromatic markers potentially responsible for the perception of fruity notes in red wines. Several combinations of yeast and LAB were studied, using three commercial *Saccharomyces cerevisiae* strains and two commercial *O. oeni* strains in sequential inoculation. Seventy-three compounds known to contribute to the fruity notes of red wines were quantified using methods previously developed in our laboratory.

MATERIAL AND METHODS

Yeast and bacteria strains

The three commercial *Saccharomyces cerevisiae* strains used in this work were Actiflore cerevisiae® (522D), Zymaflore FX10® (Laffort, Floirac, France) and Excellence XR (Lamothe-Abiet, Canéjan, France). Yeast implantation was verified by polymerase chain reaction (PCR) at the SARCO laboratory (Laffort, Floirac, France) (data not shown). Two commercial *O. oeni* strains – Lactoenos 450 PreAc® and Lactoenos B28 PreAc® (Laffort, Floirac, France) – were used as MLF starters in this study. Bacteria implantation (data not shown) was verified by the Microflora® laboratory (University of Bordeaux, France), using a method developed by Claisse and Lonvaud-Funel.[25]

Winemaking

Cabernet Sauvignon grapes from the Bordeaux appellation in the 2011 vintage were manually harvested, destemmed, crushed and homogeneously distributed into nine 2 hL stainless-steel tanks (150 kg grapes per tank). Grape must was treated by adding pectolytic enzyme (Lafase® Fruit, 0.03 µg g^{-1}, Laffort, Floirac, France) and yeast assimilable nitrogen was corrected to around 210 mg N L^{-1} by adding ammonium sulfate (Laffort, Floirac, France). Alcoholic fermentation was conducted at 19–22 °C and initiated by inoculation with rehydrated dried yeasts according to the manufacturer's recommendations. AF was performed in triplicate for each yeast strain. Implantation in each tank was verified in the middle of AF (density close to 1.040). On completion of AF (<0.2 g L^{-1} glucose/fructose), each 2 hL tank was divided into two 30 L stainless steel barrels for MLF. Bacterial cells were rehydrated with bacterial nutrient (Energizer®, Laffort, Floirac, France) 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 per week to monitor the bacterial metabolism. At the end of MLF (<0.1 g L^{-1} malic acid), 50 g hL^{-1} SO_2 was added. Wines were drained into 20 L stainless steel barrels for 3 months' aging. After 3 months, wine composition was analyzed (total and volatile acidity, total and free SO_2 content, pH, alcohol content) (Table 1). Samples were collected for volatile compound analysis in 0.75 L glass bottles and stored at 10 °C for 1 week. SO_2 content was measured and adjusted, if necessary. Wines were then decanted and frozen at −18 °C until analysis.

Standard chemical analyses

The standard chemical parameters of the wines (total acidity, sugar, malic acid, yeast assimilable nitrogen, SO_2 content, pH and alcohol) were analyzed by SARCO laboratory (Laffort, 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 (OIV)(26).

Volatile compound analyses

Each wine sample was analyzed simultaneously after defrosting, which did not affect the aroma compound concentrations in the racked wine. Eighty molecules were analyzed, using eight different methods developed and validated in the laboratory.

Chemicals

Commercial compounds were used as internal standards: butan-1,4-diol was obtained from Merck (Darmstadt, Germany); 4-methylpentan-2-ol (99%), octan-3-ol (99%), thiophene (>99%), hexan-2,3-dione (97%) and ethyl-2-hydroxyisobutyrate (98%) were supplied by Sigma-Aldrich (Steinheim, Germany), as well as 1,2-diaminobenzene (98%), used for derivatization. Methanol (>99.9%), dichloromethane (>99%), phosphoric acid (85%), sodium hydroxide (98%), sulfuric acid (98%) and sodium chloride (norma pure) were purchased from VWR Chemicals (Fontenay-sous-Bois, France). Diethyl ether (>99%) and isohexane (>99%) were obtained from Carbo Erba Reactif-SDS (Val de Reuil, France) and ethanol (≥99.9%) from Merck (Damstadt, Germany). Anhydrous sodium sulfate (99%) was supplied by Scharlau Chemie (Sentmenat, Spain).

Higher alcohols and ethyl acetate (direct injection and gas chromatography–flame ionization detection (GC-FID) analysis)

Propan-1-ol, 2-methylpropanol, 2-methylbutan-1-ol, 3-methylbutan-1-ol and ethyl acetate were quantified using a modified version of the official OIV method (OIV-MA-AS315-02A).[26] According to this method, 5 mL wine was spiked with 50 µL internal standard solution (4-methylpentan-2-ol at 14.062 g L^{-1} in 50% hydroalcoholic solution). The vials were filled with this solution for direct injection into an HP 5890 gas chromatograph coupled to a flame ionization detector. The column was a CP-WAX 57 CB (50 m × 0.25 mm × 0.2 µm, Varian). Quantification was performed using a calibration curve obtained from 12% hydroalcoholic solution.

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

The method developed by de Revel *et al.*[27] was used to quantify acetoin, D-butan-2,3-diol and meso-butan-2,3-diol. As specified in this method, 1 mL wine was spiked with 50 µL internal standard solution (butan-1,4-diol at 1 g L^{-1} in 40% hydroalcoholic solution) and diluted with 2 mL methanol. The vials were filled with this solution for direct injection into an Agilent 6890 N gas chromatograph coupled to a flame ionization detector. The column was an FFAP type (BP21, 50 m × 0.25 mm × 0.2 µm, SGE). Quantification was performed using a calibration curve obtained from 12% hydroalcoholic solution.

Table 1. Mean concentration with standard deviation of oenological parameters of wines after 3 months' aging

	XR/B28	522D/B28	FX10/B28	XR/450	522D/450	FX10/450
Fermentation duration (days)	47	47	47	40	42	35
Alcoholic degree (% v/v)	13.2 ± 0.2	13.2 ± 0.3	13.2 ± 0.2	13.2 ± 0.1	13.2 ± 0.3	13.1 ± 0.2
pH	3.68 ± 0.03	3.67 ± 0.01	3.60 ± 0.02	3.64 ± 0.03	3.64 ± 0.01	3.59 ± 0.02
Total acidity (g L^{-1} H_2SO_4)	3.4 ± 0.04 cd	3.43 ± 0.08 cd	3.56 ± 0.03b	3.5 ± 0.1c	3.54 ± 0.04bc	3.67 ± 0.02a
Volatile acidity (g L^{-1} acetic acid)	0.29 ± 0.02a	0.23 ± 0.03ab	0.30 ± 0.02a	0.13 ± 0.02c	0.09 ± 0.01d	0.16 ± 0.01c
Total sulfur dioxide (mg L^{-1})	41 ± 2	43 ± 6	42 ± 4	33 ± 9	39 ± 13	29 ± 1
Free sulfur dioxide (mg L^{-1})	30 ± 4	28 ± 2	30 ± 2	20 ± 5	25 ± 7	21 ± 1

Values with different superscript roman letter (a-d) in the same row are significantly different according to Tukey's post hoc test ($P < 0.05$).

Volatile fatty acids (liquid–liquid extraction and GC/FID analysis)

Butyric, hexanoic, octanoic, decanoic and dodecanoic acids were quantified using the method developed by Bertrand.[28] In accordance with this method, 50 mL wine was spiked with 200 μL internal standard solution (octan-3-ol at 400 mg L^{-1} in 40% hydroalcoholic solution) and 0.3 mL phosphoric acid (diluted 1/3). Samples were successively extracted with 4 mL, 2 mL and 2 mL of a diethyl ether–isohexane mix (1:1, v/v). The organic phases were collected, dried with anhydrous sodium sulfate and injected into an HP5890 gas chromatograph coupled to a flame ionization detector. The column was an FFAP type (BP 21, 50 m × 0.25 mm × 0.2 µm, SGE). Quantification was performed with calibration curves obtained from red wines.

Volatile sulfur compounds (headspace–gas chromatography–flame photometric detection (HS-GC-FPD analysis))

Dimethyl sulfide (DMS) and hydrogen sulfide (H_2S) were quantified using the method developed and validated by Anocibar-Beloqui *et al.*[20] According to this method, 100 mL wine was spiked with 10 μL internal standard solution (thiophene at 300 mg L^{-1} in ethanol) in a 125 mL headspace vial. After 24 h at 22 °C, 1 mL of the gas phase was taken from the headspace and injected into an HP5890 gas chromatograph coupled to a flame photometric detector. The column was an HP5 (30 m × 0.25 mm × 0.25 µm, Agilent). Quantification was performed using a calibration curve obtained from 12% hydroalcoholic solution.

Diacetyl (liquid–liquid extraction after derivatization and gas chromatography–mass spectrometry (GC-MS) analysis)

The method developed by de Revel *et al.*[27] was used for direct quantification of diacetyl, glyoxal, methylglyoxal and pentan-2, 3-dione. In accordance with this method, 50 mL wine was spiked with 100 μL internal standard solution (hexan-2,3-dione at 3.80 g L^{-1} in 50% hydroalcoholic solution). Then, 5 mL 1,2-diaminobenzene was added and pH was adjusted to 8 with NaOH (10 mol L^{-1}). After a 3 h derivatization reaction at 60 °C, the pH of the mixture was adjusted to 2 with sulfuric acid (2 mol L^{-1}) and it was extracted twice with 5 mL dichloromethane. The organic phases were collected, dried with anhydrous sodium sulfate and injected into an Agilent 6890 N gas chromatograph coupled to a mass spectrometer (Agilent 5973). GC-MS analysis conditions were as previously described.[27] Quantification was performed with a calibration curve obtained from 12% hydroalcoholic solution.

C_{13}-Norisoprenoids and lactones (stir bar sorptive extraction GC-MS)

This method, developed and validated by Antalick *et al.*,[29] was used to quantify four C_{13}-norisoprenoids (β-damascenone, β-damascone, β-ionone and α-ionone) and six lactones (γ-octalactone, γ-nonalactone, γ-decalactone, γ-undecalactone, γ-dodecalactone and δ-decalactone). According to the method, 25 mL wine was spiked with 25 μL internal standard solution (ethyl-d5 cinnamate at 1.74 g L^{-1} in ethanol) and a 20 mL sample was introduced into a 25 mL vial. A 20 mm × 1 mm (length × film thickness) polydimethylsiloxane (PDMS) stir bar (Twister®, 126 μL coating) (Gerstel, Müllheim an der Ruhr, Germany) was dropped into the vial, which was capped with a PTFE-faced rubber stopper. The closed vial was stirred at 900 rpm for 1 h at room temperature. At the end of the extraction time, the Twister® was removed from the vial, washed quickly with Milli-Q water and dried with lint-free tissue. Each Twister® was then transferred into a glass tube for thermal desorption (Gerstel) and GC-MS analysis, under the conditions described previously.[29] Quantification was performed using calibration curves obtained from red wines. Ethyl-d5 cinnamate was synthesized using the method described by Antalick *et al.*[30]

Apolar esters (HS-SPME-GC-MS)

The method developed and validated by Antalick *et al.*[30] was used to quantify 32 esters: six ethyl fatty acid esters, seven higher alcohol acetates, four ethyl branched acid esters, four methyl esters, three isoamyl esters, three ethyl esters with odd numbers of carbon atoms, two ethyl cinnamates and some other minor esters. A mixture of ethyl-d_5 butyrate, ethyl-d_5 hexanoate, ethyl-d_5 octanoate and ethyl-d_5 cinnamate at about 200 mg L^{-1} in ethanol was used as internal standard. Deuterated esters were synthesized as described by Antalick *et al.*[30] In accordance with this method, 20 μL internal standard solution was added to 25 mL wine. An aliquot of 10 mL was introduced into a 20 mL standard headspace vial containing 3.5 g sodium chloride. The samples were extracted by HS-SPME and analyzed by GC-MS. The fiber used was 100 µm polydimethylsiloxane (PDMS-100) (Supelco, Bellefonte, PA, USA), conditioned before use as recommended by the manufacturer. Quantification was performed with calibration curves obtained from red wines.

Additional volatile compounds (liquid–liquid extraction and GC-MS analysis)

The method developed and validated by Antalick *et al.*[29] was used to quantify seven polar esters (ethyl lactate, ethyl leucate, ethyl succinates and hydroxylated ethyl esters), three branched acids

(isobutyric acid, isovaleric acid and 2-methylbutyric acid), frambinone and linalol. According to this method, 50 mL wine was spiked with 10 μL internal standard solution (ethyl-2-hydroxyisobutyrate at 0.96 g L^{-1} in ethanol). The mixture was successively extracted with 4 mL, 2 mL and 2 mL dichloromethane. The organic phases were combined, dried with anhydrous sodium sulfate and then analyzed by GC-MS, under the conditions described elsewhere.[29] Quantification was performed with calibration curves obtained with red wines.

Statistical analyses

Volatile compound concentrations and oenological parameters (milligrams or micrograms per liter) were expressed as mean value ± standard deviation. The effects of yeast/LAB combinations were tested using one-way and two-way analysis of variance. Principal component analysis (PCA) was also carried out on the concentrations quantified for certain compounds. Statistical analyses were performed using XL-STAT (Addinsoft, Paris, France), whereas graphical representations of PCA were obtained using R v2.15.0 (R Development Core Team 2009, Vienna, Austria; R Foundation for Statistical Computing).

RESULTS AND DISCUSSION

Fermentation conditions and chemical composition of wines

Six combinations of yeast/LAB starter cultures (three yeasts, two bacteria) were tested in Cabernet Sauvignon wines made under micro-vinification conditions (2 hL). The whole winemaking process, including AF and MLF, highlighting the kinetic performance of the microorganisms, is presented in Fig. 1. Since no significant difference was observed between the triplicate experiments, one representative fermentation curve is presented for each modality.

As shown in Fig. 1(a), all AF followed the same pattern and were completed in 7 days (170 h). Total reducing sugar in the must was around 218 g L^{-1} and no differences were found between wines after AF (<1 g L^{-1}). There was a negligible difference in the ethanol concentrations of the wines, with an average of 13.2% (v/v). The pH value of the must was 3.48, which had increased slightly after AF (around 3.51). No significant differences in total or volatile acidity were found between wines. Finally, concentrations of L-malic acid in musts fermented with the 522D and XR yeast strains decreased during AF (0.29 and 0.18 g L^{-1} respectively). This suggested that these two strains had the ability to metabolize malic acid in the presence of glucose or other assimilable carbon sources.[31]

After alcoholic fermentation was completed, LAB were inoculated. As shown in Fig. 1(b), MLF was completed in every case, irrespective of the bacteria strain used. However, the degradation kinetics of L-malic acid during the course of MLF varied depending on the LAB strain. All *O. oeni* 450 samples completed MLF in 26 days (L-malic acid < 0.1 g L^{-1}), irrespective of the yeast strain. In contrast, all B28 samples required much longer to complete MLF: 31 days for XR/B28 and 522D/B28 and 33 days for FX10/B28. It is important to note that dissimilarities in the kinetics of these two bacteria strains were not due to a difference in the L-malic acid degradation rate. The latency phase of B28 strain was longer than that of the 450 (5 days), suggesting a differential adaptation to growth in wine.[32]

After 3 months' aging, differences between most of the oenological parameters of the various modalities were negligible. Only volatile acidity, expressed in grams of acetic acid per liter, was significantly affected by the LAB cultures. The largest increase was measured in 522D/B28 (0.23 g L^{-1}), XR/B28 (0.29 g L^{-1}) and FX10/B28 (0.30 g L^{-1}) samples, with statistically significant differences depending on the LAB strain used. The influence of bacterial strains on volatile acidity has already been reported.[33,34] Acetic acid is produced from citric acid by some genera of LAB,[10] and the statistically significant differences in acetic acid content observed may be due to degradation of larger quantities of citric acid by *O. oeni* B28.

Influence of yeast/LAB combination on wine aromatic compounds

Seventy-three major volatile compounds were quantified, including eight acids, six alcohols, six aldehydes and ketones, six lactones, four C_{13}-norisoprenoids, two sulfur-containing compounds, one terpene and 40 esters, using analytical methods that were previously developed and validated in our laboratory. Concentrations measured in the different modalities are presented in Tables 2 and 3. First, a one-way analysis of variance (ANOVA) was used to study the yeast/LAB combination parameter. Results revealed a significant effect of the microorganism combination on the concentrations of 51 volatile compounds, mainly alcohols, acids and esters. Varietal compounds and α-dicarbonyl compounds were less affected. Concentrations of aldehydes (glyoxal, methylglyoxal) and volatile sulfur compounds (DMS, H_2S) did not vary according to the microorganism combination.

Larger quantities of higher alcohols are present in alcoholic beverages than any other group of aroma compounds. Their concentrations were significantly affected by the yeast/bacteria combination used in winemaking. The total amount of higher alcohols was strongly associated with the concentration of 3-methylbutan-1-ol, which constituted over 60% of the total alcohol for each modality. However, this was the only higher alcohol not affected by the yeast/LAB combination, while concentrations of other alcohols, such as propan-1-ol, 2-methypropan-1-ol and 2-methylbutan-1-ol, differed significantly according to the yeast/LAB combination (0.1%, 5% and 0.1%, respectively).

Eleven varietal compounds known to contribute to the fruity aroma of red wines, including C_{13}-norisoprenoids, lactones and terpene, were quantified. For C_{13}-norisoprenoids, differences between the six combinations were low or non-existent and only α-ionone presented small, but significant, variations (1%). Lactones were mainly represented by γ-octalactone, with significant variations in concentration (5%) according to the yeast/LAB combination. The concentrations of other lactones were not significantly affected by the microorganism combinations.

Eight volatile acids known to contribute to the balance of fruity aroma were assayed. Concentrations of branched acids (isobutyric, isovaleric and 2-methylbutyric acids) were significantly modulated by the yeast/LAB combination (0.1%). Similarly, levels of linear acids (butyric, hexanoic, decanoic and dodecanoic) all varied depending on the microorganism combinations (0.1%, except for decanoic acid, significant at 5%).

Finally, esters are considered one of the most important families of aromatic compounds for modulating red wine fruity aromas. Among the 40 esters quantified, only seven were not affected by the yeast/LAB combination. Concentrations of over half of the compounds (33 esters) differed significantly according to the microorganisms used.

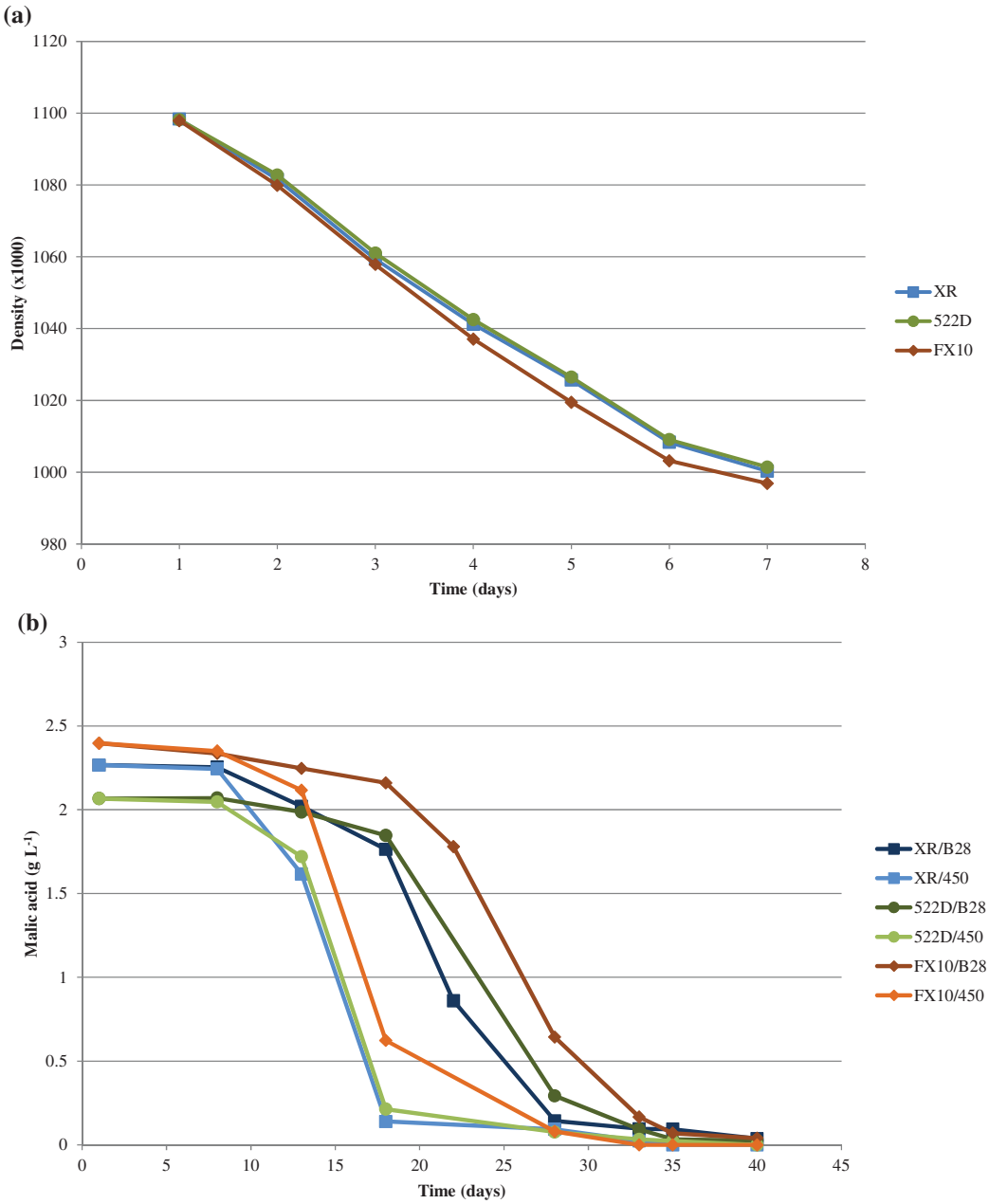

Figure 1. Kinetics of alcoholic (a) and malolactic (b) fermentations in wines fermented with different yeast/LAB combinations.

Predominant impact of yeast on concentrations of aromatic compounds

Principal component analysis (PCA) was used to refine these observations. Among 73 molecules quantified, 22 did not exhibit any significant combination effect and were not included in the PCA. Using 51 analytical variables (volatile data) and 18 objects (3 yeasts × 2 bacteria in triplicate), PCA explained over 65% of the total variance on the first two axes (Fig. 2). Triplicates of each modality were all represented close to each other, indicating good reproducibility of the experiment. According to this PCA, the yeast strain alone had a greater impact on volatile compound levels than the yeast/LAB combination. Indeed, triplicate samples fermented with FX10/450 and FX10/B28 were separated from the other wines along axis 1. Samples inoculated with XR/450 and XR/B28 combinations were at the bottom of the two-dimensional plot, whereas the 522D/B28 and 522D/450 samples were higher on axis 2. Ethyl lactate and diacetyl were the only compounds strongly represented on axis 3 (10.37% of total variance; data not shown), which separated the wines according to the LAB strain, as expected. In contrast, no yeast/LAB combination effect was revealed.

These observations were confirmed with a two-way ANOVA (yeast/bacteria/yeast × bacteria interaction) (Table 4). Among the 51 compounds previously highlighted, only eight were actually affected by the yeast/LAB interaction, while a yeast strain effect was observed for 48 of these aromatic compounds.

The concentration of higher alcohols was only modulated by the yeast strain. Wines fermented by the 522D strain contained significantly more 2-methylpropan-1-ol, propan-1-ol and

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

Compound	XR/B28	XR/450	522D/B28	552D/450	FX10/B28	FX10/450	One-way ANOVA
Alcohols							
Propan-1-ol	35 ± 5	37 ± 3	55 ± 5	55 ± 5	38 ± 2	40 ± 2	***
2-Methylpropan-1-ol	54 ± 2	56 ± 2	61 ± 3	62 ± 3	57 ± 1	58.4 ± 0.4	*
2-Methylbutan-1-ol	88 ± 5	90 ± 4	115 ± 4	114 ± 8	79 ± 1	80 ± 3	***
3-Methylbutan-1-ol	319 ± 17	330 ± 10	346 ± 13	341 ± 21	326 ± 8	334 ± 7	NS
Sum of higher alcohols	496	514	577	570	499	513	
Butan-2,3-diol (D)	127 ± 31	99 ± 16	140 ± 17	98 ± 16	189 ± 66	116 ± 9	*
Butan-2,3-diol (M)	49 ± 17	56 ± 9	54 ± 8	52 ± 11	66 ± 21	52 ± 4	NS
Aldehydes and ketones							
Glyoxal	0.14 ± 0.03	0.1 ± 0.01	0.16 ± 0.05	0.19 ± 0.05	0.15 ± 0.02	0.13 ± 0.03	NS
Methylglyoxal	0.4 ± 0.06	0.42 ± 0.07	0.46 ± 0.06	0.45 ± 0.06	0.55 ± 0.08	0.43 ± 0.03	NS
Acetoin	19 ± 4	24 ± 3	24 ± 5	19 ± 2	30 ± 10	21 ± 2	NS
Diacetyl	11 ± 1	7.4 ± 0.6	10 ± 1	7 ± 2	10 ± 1	5.6 ± 0.5	***
Pentan-2,3-dione	1.5 ± 0.1	1.68 ± 0.09	1.47 ± 0.01	1.9 ± 0.2	1.1 ± 0.2	1.4 ± 0.2	***
Frambinone (μg L^{-1})	15 ± 5	14 ± 3	14 ± 3	13 ± 3	12 ± 2	11.1 ± 0.7	NS
Sulfur-containing compounds							
Hydrogen sulfide	0.8 ± 0.1	0.7 ± 0.2	0.9 ± 0.3	1.0 ± 0.4	1.2 ± 0.6	1.2 ± 0.2	NS
Dimethyl sulfide	3.7 ± 0.2	3.7 ± 0.2	4.0 ± 0.5	3.6 ± 0.7	4.1 ± 0.3	4.0 ± 0.4	NS
Acids							
Butyric acid	7.5 ± 1.0	5.5 ± 0.5	6.7 ± 0.6	4.6 ± 0.4	5.2 ± 0.3	3.7 ± 0.2	***
Isobutyric acid	1.22 ± 0.09	1.2 ± 0.1	1.22 ± 0.03	1.22 ± 0.03	1.01 ± 0.01	1.00 ± 0.02	***
Isovaleric acid	1.11 ± 0.07	1.1 ± 0.07	1.11 ± 0.04	1.12 ± 0.01	0.76 ± 0.01	0.78 ± 0.02	***
2-Methylbutyric acid	0.82 ± 0.04	0.8 ± 0.05	0.94 ± 0.06	0.93 ± 0.03	0.51 ± 0.02	0.51 ± 0.01	***
Hexanoic acid	7.8 ± 0.4	8.0 ± 0.4	8.7 ± 0.4	9.4 ± 0.3	8.4 ± 0.5	9.2 ± 0.5	***
Octanoic acid	2.6 ± 0.1	3.0 ± 0.3	2.9 ± 0.1	3.4 ± 0.2	3.3 ± 0.2	3.62 ± 0.05	***
Decanoic acid	0.7 ± 0.04	0.76 ± 0.06	0.75 ± 0.05	0.88 ± 0.09	0.72 ± 0.04	0.82 ± 0.07	*
Dodecanoic acid (μg L^{-1})	9 ± 3	6 ± 1	6.7 ± 0.8	11 ± 1	6.8 ± 0.7	12.1 ± 0.8	***
C_{13}-Norisoprenoids, lactones and terpene							
β-Damascone (μg L^{-1})	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.04	0.02 ± 0.01	NS
β-Damascenone (μg L^{-1})	6.6 ± 0.2	6.0 ± 0.4	5.5 ± 0.6	6.3 ± 0.9	6.1 ± 0.5	6.5 ± 0.5	NS
α-Ionone (μg L^{-1})	0.22 ± 0.02	0.12 ± 0.02	0.15 ± 0.02	0.12 ± 0.03	0.14 ± 0.04	0.11 ± 0.02	**
β-Ionone (μg L^{-1})	0.09 ± 0.02	0.09 ± 0.01	0.08 ± 0.01	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.01	NS
γ-Octalactone (μg L^{-1})	16 ± 3	17 ± 3	23 ± 2	21 ± 2	19 ± 3	18 ± 2	*
γ-Nonalactone (μg L^{-1})	7.7 ± 0.4	7.1 ± 0.5	8 ± 1	8 ± 1	8 ± 2	7.1 ± 0.5	NS
γ-Decalactone (μg L^{-1})	0.91 ± 0.01	0.8 ± 0.2	0.63 ± 0.08	0.62 ± 0.06	0.8 ± 0.3	0.7 ± 0.01	NS
δ-Decalactone (μg L^{-1})	1.06 ± 0.04	1.3 ± 0.3	1.2 ± 0.1	1.3 ± 0.1	1.9 ± 0.5	1.57 ± 0.09	*
γ-Undecalactone (μg L^{-1})	0.07 ± 0.01	0.09 ± 0.02	0.08 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	NS
γ-Dodecalactone (μg L^{-1})	0.05 ± 0.01	0.05 ± 0.02	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	NS
Linalol (μg L^{-1})	12 ± 2	9.7 ± 0.7	13 ± 2	12 ± 1	8 ± 2	8.0 ± 0.4	**

Significant effect: NS, not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

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

Yeast strains also influenced the C_{13}-norisoprenoid and lactone concentrations, but their impact was not as clear (Table 4). Indeed, only small variations were measured in lactone concentrations. Among these compounds, γ-octalactone was the most representative, with levels ranging from 15.86 μg L^{-1} (XR/B28 wine) to 22.50 μg L^{-1} (522D/B28 wine), but it is unlikely to have had any aromatic impact in view of its perception threshold (35 μg L^{-1}).[35] Little information is available concerning lactone formation pathways in wine, but they are assumed to be mainly synthesized from hydroxylated fatty acids or esters via an enzymatic or chemical pathway.[18,36] The results of this study were consistent with previous observations that yeasts were capable of enzymatic esterification but not, apparently, LAB.[37] However, lactones are mainly synthesized during wine aging[38] and some differences in concentrations may occur depending on the LAB strain used during MLF. Indeed, some studies have indicated the possibility of a late synthesis of these compounds, related to bacterial β-glycosidase and oxidase activities.[36,39] Among the C_{13}-norisoprenoids, only α-ionone presented small variations in concentration with different yeast or LAB strains, as well as yeast/LAB interactions (from 0.11 μg L^{-1} for FX10/450 to 0.22 μg L^{-1} for XR/B28). Although levels found in this study were below the perception threshold (2.6 μg L^{-1}),[40] which is highly dependent on the matrix, some studies have highlighted the potential implication of these compounds in modulating fruity aroma via perceptive interactions.[17] These results are in accordance with numerous data presented in the literature, demonstrating the ability

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

Compound	XR/B28	XR/450	522D/B28	552D/450	FX10/B28	FX10/450	One-way ANOVA
Major polar esters							
Ethyl lactate (mg L^{-1})	56 ± 5	31 ± 1	55 ± 2	31 ± 2	55 ± 1	38 ± 4	***
Monoethyl succinate (mg L^{-1})	22 ± 1	20 ± 2	22 ± 3	20 ± 1	21 ± 2	20 ± 1	NS
Diethyl succinate	683 ± 29	586 ± 38	793 ± 105	697 ± 115	621 ± 81	588 ± 50	*
Polar esters							
Ethyl leucate	70 ± 5	58.2 ± 0.3	94 ± 6	96 ± 13	85 ± 13	80 ± 16	**
Ethyl 3-hydroxybutyrate	333 ± 10	323 ± 12	384 ± 29	387 ± 18	454 ± 26	454 ± 21	***
Ethyl 2-hydroxyhexanoate	0.9 ± 0.3	1.6 ± 0.6	1.27 ± 0.04	1.2 ± 0.2	1.2 ± 0.2	1.0 ± 0.1	NS
Ethyl 6-hydroxyhexanoate	3.12 ± 0.06	3.4 ± 0.8	3.1 ± 0.7	4 ± 1	3.9 ± 0.4	3.9 ± 0.7	NS
Ethyl fatty acid esters							
Ethyl butyrate	185 ± 17	179 ± 8	218 ± 22	218 ± 28	198 ± 9	194 ± 17	*
Ethyl hexanoate	286 ± 11	294 ± 5	319 ± 13	320 ± 23	313 ± 18	324 ± 29	NS
Ethyl octanoate	289 ± 20	282 ± 25	307 ± 27	302 ± 34	330 ± 21	334 ± 15	NS
Ethyl decanoate	71 ± 6	94 ± 3	91 ± 10	103 ± 8	87 ± 6	115 ± 7	***
Ethyl dodecanoate	4.8 ± 0.4	6.8 ± 0.4	7.4 ± 0.6	9.9 ± 0.9	8 ± 1	13 ± 2	***
Ethyl branched acid esters							
Ethyl isobutyrate	62 ± 6	58 ± 6	63.5 ± 0.9	49.0 ± 15.0	61 ± 4	59 ± 6	NS
Ethyl 2-methylbutyrate	11.7 ± 0.7	12 ± 1	13.7 ± 0.3	13.8 ± 0.8	7.4 ± 0.2	7.1 ± 0.3	***
Ethyl isovalerate	18.4 ± 0.4	17 ± 1	18 ± 1	19 ± 1	11.6 ± 0.4	11.7 ± 0.6	***
Ethyl phenylacetate	3.22 ± 0.02	3.2 ± 0.4	5.0 ± 0.6	5.5 ± 0.5	3.0 ± 0.3	3.1 ± 0.1 μ	***
Acetate of higher alcohols							
Ethyl acetate (mg L^{-1})	90 ± 2	78 ± 3	72 ± 1	71 ± 4	88 ± 4	80 ± 3	***
Propyl acetate	17.1 ± 0.5	15.5 ± 0.5	23 ± 2	21 ± 1	21 ± 2	19.3 ± 0.3	***
Isobutyl acetate	41 ± 1	37 ± 2	53 ± 4	51 ± 5	50 ± 2	51 ± 1	***
Butyl acetate	0.8 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	1.2 ± 0.2	1.26 ± 0.07	0.8 ± 0.2	**
Isoamyl acetate	1105 ± 61	1064 ± 37	1484 ± 181	1339 ± 196	1367 ± 143	1317 ± 73	**
Hexyl acetate	6 ± 1	9 ± 2	7 ± 1	9 ± 2	8.1 ± 0.9	8.7 ± 0.3	NS
Octyl acetate	0.08 ± 0.01	0.12 ± 0.02	0.1 ± 0.01	0.15 ± 0.04	0.15 ± 0.03	0.18 ± 0.01	***
2-Phenylethyl acetate	87 ± 8	89 ± 3	144 ± 24	145 ± 17	117 ± 10	120 ± 9	***
Methyl esters							
Methyl butyrate	0.86 ± 0.05	0.78 ± 0.02	1.22 ± 0.01	1.09 ± 0.08	1.0 ± 0.1	0.84 ± 0.04	***
Methyl hexanoate	1.9 ± 0.1	1.71 ± 0.06	2.2 ± 0.2	2.16 ± 0.09	1.9 ± 0.2	2.0 ± 0.1	**
Methyl octanoate	1.26 ± 0.02	1.34 ± 0.05	1.4 ± 0.1	1.5 ± 0.1	1.44 ± 0.09	1.5 ± 0.1	**
Methyl decanoate	0.33 ± 0.01	0.42 ± 0.03	0.42 ± 0.03	0.48 ± 0.03	0.4 ± 0.01	0.48 ± 0.01	***
Ethyl esters with odd number of carbon atoms							
Ethyl propanoate	306 ± 12	292 ± 8	425 ± 25	384 ± 58	281 ± 22	258 ± 16	***
Ethyl valerate	0.67 ± 0.03	0.54 ± 0.04	1.0 ± 0.1	0.87 ± 0.03	0.84 ± 0.06	0.9 ± 0.2	***
Ethyl heptanoate	0.9 ± 0.1	0.9 ± 0.1	0.92 ± 0.06	0.95 ± 0.06	0.64 ± 0.01	0.66 ± 0.03	***
Ethyl nonanoate	0.61 ± 0.01	0.69 ± 0.03	0.89 ± 0.03	0.9 ± 0.04	0.89 ± 0.08	1.07 ± 0.09	***
Isoamyl esters							
Isoamyl butyrate	0.66 ± 0.06	0.67 ± 0.05	0.75 ± 0.02	0.8 ± 0.1	0.71 ± 0.05	0.57 ± 0.03	***
Isoamyl hexanoate	1.9 ± 0.1	1.88 ± 0.06	2.0 ± 0.1	1.9 ± 0.2	2.1 ± 0.1	2.18 ± 0.07	*
Isoamyl octanoate	2.8 ± 0.3	2.9 ± 0.2	3.1 ± 0.3	3.2 ± 0.2	3.4 ± 0.1	3.48 ± 0.07	**
Cinnamates							
Ethyl cinnamate	2.55 ± 0.02	2.57 ± 0.05	2.4 ± 0.1	2.4 ± 0.2	2.56 ± 0.07	2.71 ± 0.06	**
Ethyl dihydrocinnamate	1.7 ± 0.1	1.69 ± 0.05	1.5 ± 0.1	1.52 ± 0.06	1.51 ± 0.07	1.57 ± 0.05	**
Minor esters							
Ethyl hexenoate	1.8 ± 0.2	1.6 ± 0.1	1.41 ± 0.07	1.51 ± 0.09	1.3 ± 0.1	1.68 ± 0.09	***
Isobutyl hexanoate	0.16 ± 0.00	0.17 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.2 ± 0.01	0.21 ± 0.01	***
Methyl *trans*-geranate	0.15 ± 0.02	0.21 ± 0.01	0.19 ± 0.02	0.22 ± 0.00	0.24 ± 0.01	0.27 ± 0.02	***

of both yeast and LAB to hydrolyze glycosidic precursors of C_{13}-norisoprenoids.[36,41]

Two groups were identified among the 40 esters quantified in this study. Major esters, including ethyl acetate, ethyl lactate and monoethyl succinate, were present at higher concentrations (mg L^{-1}) compared to other esters, which are nevertheless considered 'odorant esters', due to their lower perception threshold in wine.

In the major ester group, diethyl succinate and ethyl acetate concentrations were slightly impacted by yeast strains. However, in view of its perception threshold (154 mg L^{-1})[40] and the variations measured in this study (<20 mg L^{-1}), ethyl acetate probably

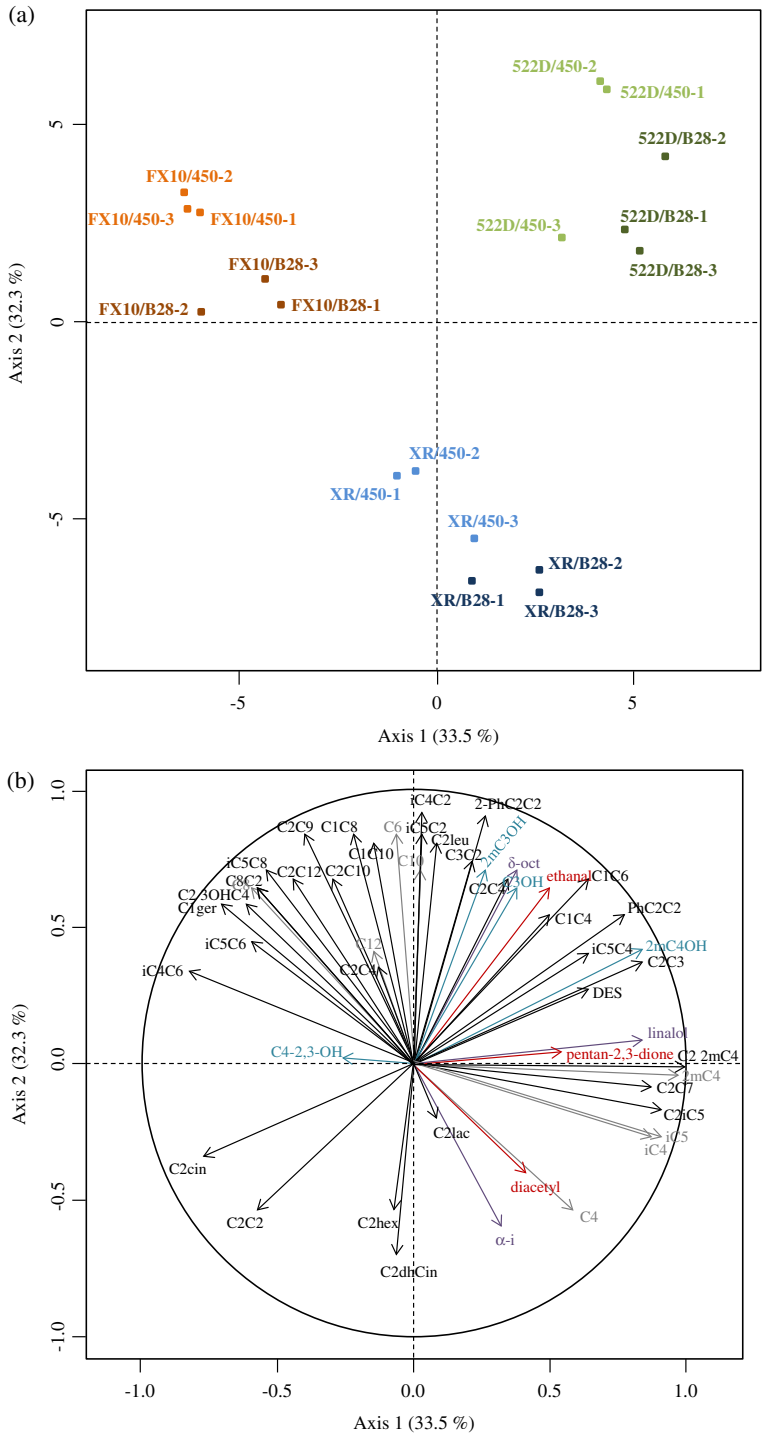

Figure 2. Principal component analysis represented as a scatter point plot (a) and 51 parameters (b) on axes 1 × 2. Abbreviations for the various parameters are presented in Table 4.

did not affect wine aroma. Ethyl lactate levels varied significantly among the different modalities, reaching higher concentrations in wines inoculated with LAB strain B28 (0.1%), confirming the literature reporting the capacity of LAB to synthesize this compound during MLF.[42,43]

Concentrations of other esters, known as 'odorant esters', were also mainly influenced by the yeast strain. Three groups may be identified in terms of their contribution to fruity aroma. Fatty acid ethyl esters were the least influenced by the yeast/LAB combination. Ethyl butyrate, decanoate and dodecanoate, as well as their corresponding acids, were mainly synthesized by FX10 and 522D yeasts. Higher concentrations of most acetates were found in wines fermented with 522D and FX10 (except hexyl acetate). Higher concentrations of branched esters, such as ethyl 2-methylbutyrate and ethyl isovalerate, were found in wines fermented with 522D or XR (significant at 0.1%). Similarly, significantly higher levels of the corresponding acids, such as isobutyric, isovaleric and 2-methylbutyric acids, were also found

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

Compound	PCA abbreviation	Two-way ANOVA		
		Yeast	Bacteria	Yeast × Bacteria
Alcohols				
Propan-1-ol	C3OH	***	NS	NS
2-Methylpropan-1-ol	2mC3OH	**	NS	NS
2-Methylbutan-1-ol	2mC4OH	***	NS	NS
Butan-2,3-diol (D)	C4-2,3OH	NS	**	NS
Aldehydes and ketones				
Diacetyl	diacetyl	NS	***	NS
Pentan-2,3-dione	pentan-2,3-dione	***	***	NS
Acids				
Butyric acid	C4	***	***	NS
Isobutyric acid	iC4	***	NS	NS
Isovaleric acid	iC5	***	NS	NS
2-Methylbutyric acid	2mC4	***	NS	NS
Hexanoic acid	C6	***	**	NS
Octanoic acid	C8	***	***	NS
Decanoic acid	C10	*	**	NS
Dodecanoic acid	C12	*	**	***
C_{13}-Norisoprenoids, lactones and terpene				
α-Ionone	α-i	*	***	*
γ-Octalactone	γ-oct	**	NS	NS
δ-Decalactone	δ-dec	**	NS	NS
Linalol	linalol	**	NS	NS
Major polar esters				
Ethyl lactate	C2lac	NS	***	NS
Diethyl succinate	DES	*	NS	NS
Polar esters				
Ethyl leucate	C2leu	**	NS	NS
Ethyl 3-hydroxybutyrate	C2 3OHC2	***	NS	NS
Ethyl fatty acid esters				
Ethyl butyrate	C2C4	**	NS	NS
Ethyl decanoate	C2C10	**	***	NS
Ethyl dodecanoate	C2C12	***	***	*
Ethyl branched acid esters				
Ethyl 2-methylbutyrate	C2 2mC4	***	NS	NS
Ethyl isovalerate	C2iC5	***	NS	NS
Ethyl phenylacetate	C2PhC2	***	NS	NS
Acetate of higher alcohols				
Ethyl acetate	C2C2	***	***	**
Propyl acetate	C3C2	***	**	NS
Isobutyl acetate	iC4C2	***	NS	NS
Butyl acetate	C4C2	*	NS	**
Isoamyl acetate	iC5C2	**	NS	NS
Octyl acetate	C8C2	***	**	NS
2-Phenylethyl acetate	2-PhC2C2	***	NS	NS
Methyl esters				
Methyl butyrate	C1C4	***	**	NS
Methyl hexanoate	C1C6	***	NS	NS
Methyl octanoate	C1C8	**	*	NS
Methyl decanoate	C1C10	***	***	NS

Table 4. Continued

Compound	PCA abbreviation	Two-way ANOVA		
		Yeast	Bacteria	Yeast × Bacteria
Ethyl esters with odd number of carbon atoms				
Ethyl propanoate	C2C3	***	*	NS
Ethyl valerate	C2C5	***	NS	NS
Ethyl heptanoate	C2C7	***	NS	NS
Ethyl nonanoate	C2C9	***	**	*
Isoamyl esters				
Isoamyl butyrate	iC5C4	***	NS	**
Isoamyl hexanoate	iC5C6	**	NS	NS
Isoamyl octanoate	iC5C8	**	NS	NS
Cinnamates				
Ethyl cinnamate	C2cin	**	NS	NS
Ethyl dihydrocinnamate	C2dhicin	***	NS	NS
Minor esters				
Ethyl hexenoate	C2hex	**	*	**
Isobutyl hexanoate	iC4C6	***	NS	NS
Methyl *trans*-geranate	C1ger	***	***	NS

Significant effect: NS, not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

in these last two wines (also at 0.1%). The concentrations of the other esters (esters with an odd number of carbon atoms, methyl esters, isoamyl esters, cinnamates and minor esters) were also affected by the different combinations used, particularly the yeast strain, as reported in previous studies.[9,44] Although the variations measured for these esters were below the perception threshold, some studies have demonstrated that they may still be perceived by a trained panelist.[15,45]

While the majority of these compounds were synthesized by yeast during AF, the esterase activity of wine LAB has also been reported.[46] Besides diacetyl, known to be synthesized during MFL by LAB,[24] these results suggest that microorganisms may be capable of modulating the concentrations of esters and their corresponding acids. The carbon chain length seemed to be an important parameter in the synthesis of these compounds by LAB. Indeed, the longer the carbon chain, the more the esters and acids were affected by the LAB strain (Table 4). Hexanoic, octanoic, decanoic and dodecanoic acids were all found in significantly higher concentrations in wines inoculated with LAB strain 450 (1%). This was also true of the corresponding esters, ethyl decanoate and ethyl dodecanoate (significant at 0.1%). These results contradicted some data in the literature. Matthews *et al.*[47] reported that the hydrolytic activity of esterases in different species or genera (*O. oeni*, *Lactobacillus*, *Pediococcus*) had greater specificity for substrates with short carbon chains (C2, C4). In particular, the esterase activity of *O. oeni* was reported to be greater for substrates in C4. In contrast, other recent studies reported the ability of LAB to synthesize ethyl esters and acetates with long carbon chains (C8, C10, C12).[29] In all cases, these long carbon chain esters play a minor role in red wine fruity aroma. It is therefore unlikely that the small variations in concentration observed between samples inoculated with different LAB strains would be perceived by a tasting panel.

This study examined the influence of *S. cerevisiae* and *O. oeni* strains on the production of Bordeaux red wines using six different yeast/LAB combinations. Results obtained for standard chemical parameters revealed that the level of volatile acidity varied significantly according to the LAB strain. For aromatic compounds, each microorganism combination resulted in a specific volatile profile. However, the yeast strain was apparently the predominant component in the yeast/LAB combination in modulating aromatic compound levels. In particular, the 522D and FX10 strains exhibited a similar capacity to produce esters, acids and higher alcohols. These results showed that yeasts had a more significant effect on wine quality and are thus likely to have a greater impact on wine style than the LAB used. A previous study had already demonstrated the predominant impact of yeast strain rather than yeast/LAB combination on cherry wines.[48]

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

If we consider the volatile composition of these wines, samples fermented with the yeast FX10 had higher values for the attributes referring to 'fruity', due to their large quantities of fruity ethyl esters. Surprisingly, 522D wines, described as fruitless, also contained important levels of these aromatic compounds, as well as high amounts of higher alcohols. These compounds, recognized

by their strong, pungent smell, influence the taste and character of wine depending on their concentration: below 300 mg L^{-1}, they contribute to the desirable complexity of wine, but at concentrations exceeding 400 mg L^{-1} they are regarded as a negative influence on wine quality.[21] The high alcohol levels found in this study, particularly in 522D/B28 and 522D/450 samples (577 and 570 mg L^{-1}, respectively), may have had a negative effect on fruity aroma perception in these wines.

While these experiments offer new insights into the organoleptic effect of fermentation, the chemistry underlying the sensory interactions is highly complex. Further investigations are necessary to elucidate the influence of yeast- and LAB-derivative compounds on fruity aroma. Moreover, in light of recent articles dealing with the interactions between volatile and non-volatile compounds,[50] the impact of both microorganisms on the non-volatile matrix should also be investigated as a potential modulating factor of wine aroma.

REFERENCES

1 Pineau B, Barbe JC, Leeuwen CV and 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).
2 Peynaud E, *Connaissance et Travail du Vin*. Bordas, Paris (1981).
3 Henick-Kling T, Edinger W, Daniel P and 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).
4 Ugliano M, Travis B, Francis IL and Henschke PA, Volatile composition and sensory properties of Shiraz wines as affected by nitrogen supplementation and yeast species: Rationalizing nitrogen modulation of wine aroma. *J Agric Food Chem* **58**:12417–12425 (2010).
5 Marchal A, Marullo P, Durand C, Moine V and Dubourdieu D, Erratum: Fermentative conditions modulating sweetness in dry wines: genetics and environmental factors influencing the expression level of the *Saccharomyces cerevisiae* HSP12 gene. *J Agric Food Chem* **63**:2364 (2015).
6 Dubourdieu D, Tominaga T, Masneuf I, des Gachons CP and Murat ML, The role of yeasts in grape flavor development during fermentation: the example of Sauvignon blanc. *Am J Enol Vitic* **57**:81–88 (2006).
7 Belda I, Ruiz J, Navascués E, Marquina D and Santos A, Improvement of aromatic thiol release through the selection of yeasts with increased β-lyase activity. *Int J Food Microbiol* **225**:1–8 (2016).
8 Rankine BC, Influence of yeast strain and malo-lactic fermentation on composition and quality of table wines. *Am J Enol Vitic* **23**:152–158 (1972).
9 Saerens SMG, Verstrepen KJ, Van Laere SDM, Voet ARD, Van Dijck P, Delvaux FR *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).
10 Cappello MS, Zapparoli G, Logrieco A and Bartowsky EJ, Linking wine lactic acid bacteria diversity with wine aroma and flavour. *Int J Food Microbiol* **243**:16–27 (2017).
11 Lonvaud-Funel A, Joyeux A and Desens C, Inhibition of malolactic fermentation of wines by products of yeast metabolism. *J Sci Food Agric* **44**:183–191 (1988).
12 Arnink K and Henick-Kling T, Influence of *Saccharomyces cerevisiae* and *Oenococcus oeni* strains on successful malolactic conversion in wine. *Am J Enol Vitic* **56**:228–237 (2005).
13 Avedovech RM, Mcdaniel MR, Watson BT and Sandine WE, An evaluation of combinations of wine yeast and *Leuconostoc oenos* strains in malolactic fermentation of Chardonnay wine. *Am J Enol Vitic* **43**:253–260 (1992).
14 Delaquis P, Cliff M, King M, Girard B, Hall J and Reynolds A, Effect of two commercial malolactic cultures on the chemical and sensory properties of chancellor wines vinified with different yeasts and fermentation temperatures. *Am J Enol Vitic* **51**:42–48 (2000).
15 Pineau B, Barbe JC, Van Leeuwen C and 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).
16 Lytra G, Tempere S, de Revel G and Barbe JC, Impact of perceptive interactions on red wine fruity aroma. *J Agric Food Chem* **60**:12260–12269 (2012).
17 Escudero A, Campo E, Fariña L, Cacho J and 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).
18 Loscos N, Hernandez-Orte P, Cacho J and 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).
19 Tominaga T, Blanchard L, Darriet P and 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).
20 Anocibar Beloqui A, Kotseridis Y and Bertrand A, Determination of the content of dimethyl sulphide in some red wines. *J Int Sci Vigne Vin* **30**:167–170 (1996).
21 Rapp A and Mandery H, Wine aroma. *Experientia* **42**:873–884 (1986).
22 Ebeler SE, Analytical chemistry: unlocking the secrets of wine flavor. *Food Rev Int* **17**:45–64 (2001).
23 Rodríguez-Bencomo JJ, Cabrera-Valido HM, Pérez-Trujillo JP and Cacho J, Bound aroma compounds of Gual and Listán blanco grape varieties and their influence in the elaborated wines. *Food Chem* **127**:1153–1162 (2011).
24 Bertrand A, Zmirou-Bonnamour C and Lonvaud-Funel A, Aroma compounds formed by malolactic bacteria, in *Flavour Research of Alcoholic Beverages*, ed. by Nykänen L and Lehtonen P. Foundation for Biotechnical and Industrial Fermentation Research, Helsinki, pp. 39–49 (1984).
25 Claisse O and Lonvaud-Funel A, Development of a multilocus variable number of tandem repeat typing method for *Oenococcus oeni*. *Food Microbiol* **30**:340–347 (2012).
26 International Organisation of Vine and Wine, *Compendium of International Methods of Wine and Must Analysis*. OIV, Paris (2016).
27 de Revel G, Pripis-Nicolau L, Barbe JC and Bertrand A, The detection of α-dicarbonyl compounds in wine by formation of quinoxaline derivatives. *J Sci Food Agric* **80**:102–108 (2000).
28 Bertrand A, Formation de substances volatiles au cours de la fermentation alcoolique, in *Colloque de la Société Française de Microbiologie*, Reims; pp. 251–267 (1981).
29 Antalick G, Perello MC and de Revel G, Characterization of fruity aroma modifications in red wines during malolactic fermentation. *J Agric Food Chem* **60**:12371–12383 (2012).
30 Antalick G, Perello MC and 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).
31 Benito Á, Jeffares D, Palomero F, Calderón F, Bai F-Y, Bähler J *et al.*, Selected *Schizosaccharomyces pombe* strains have characteristics that are beneficial for winemaking. *PLOS ONE* **11**:e0151102 (2016).
32 Garbay S and Lonvaud-Funel A, Response of *Leuconostoc nosto* environmental changes. *J Appl Bacteriol* **81**:619–625 (1996).
33 Ruiz P, Izquierdo PM, Seseña S, García E and Palop ML, Malolactic fermentation and secondary metabolite production by *Oenoccocus oeni* strains in low pH wines. *J Food Sci* **77**:M579–M585 (2012).
34 Pérez-Martín F, Izquierdo-Cañas PM, Seseña S, García-Romero E and Palop ML, Aromatic compounds released from natural precursors by selected *Oenococcus oeni* strains during malolactic fermentation. *Eur Food Res Technol* **240**:609–618 (2014).
35 Cutzach I, Chatonnet P, Henry R, Pons M and Dubourdieu D, Study in aroma of sweet natural non muscat wines. 2. Quantitative analysis of volatile compounds taking part in aroma of sweet natural wines during ageing. *J Int Sci Vigne Vin* **32**:211–221 (1998).
36 Segurel MA, Baumes RL, Langlois D, Riou C and Razungles AJ, Role of glycosidic aroma precursors on the odorant profiles of Grenache noir and Syrah wines from the Rhone valley. 2. Characterisation of derived compounds. *J Int Sci Vigne Vin* **43**:213–223 (2009).
37 Abeijón Mukdsi MC, Medina RB, Alvarez M de F and González SN, Ester synthesis by lactic acid bacteria isolated from goat's and ewe's milk and cheeses. *Food Chem* **117**:241–247 (2009).
38 López R, Ezpeleta E, Sánchez I, Cacho J and Ferreira V, Analysis of the aroma intensities of volatile compounds released from mild acid hydrolysates of odourless precursors extracted from Tempranillo

and Grenache grapes using gas chromatography–olfactometry. *Food Chem* **88**:95–103 (2004).
39 Wanikawa A, Hosoi K and Kato T, Conversion of unsaturated fatty acids to precursors of γ-lactones by lactic acid bacteria during the production of malt whisky. *J Am Soc Brew Chem* **58**:51–56 (2000).
40 Etiévant PX, Wine, in *Volatile Compounds in Foods and Beverages*, ed. by Maarse, H. CRC Press, Boca Raton, FL, pp. 483–586 (1991).
41 Boido E, Lloret A, Medina K, Carrau F and Dellacassa E, Effect of β-glycosidase activity of *Oenococcus oeni* on the glycosylated flavor precursors of Tannat wine during malolactic fermentation. *J Agric Food Chem* **50**:2344–2349 (2002).
42 Costello PJ, Francis IL and Bartowsky EJ, 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).
43 Malherbe S, Tredoux AGJ, Nieuwoudt HH and 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).
44 Saerens SMG, Delvaux F, Verstrepen KJ, Van Dijck P, Thevelein JM and Delvaux FR, Parameters affecting ethyl ester production by *Saccharomyces cerevisiae* during fermentation. *Appl Environ Microbiol* **74**:454–461 (2008).
45 Ferreira V, López R, Escudero A and Cacho JF, The aroma of Grenache red wine: hierarchy and nature of its main odorants. *J Sci Food Agric* **77**:259–267 (1998).
46 Sumby KM, Grbin PR and 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).
47 Matthews A, Grbin PR and Jiranek V, Biochemical characterisation of the esterase activities of wine lactic acid bacteria. *Appl Microbiol Biotechnol* **77**:329–337 (2007).
48 Sun SY, Che CY, Sun TF, Lv ZZ, He SX, Gu HN *et al.*, Evaluation of sequential inoculation of *Saccharomyces cerevisiae* and *Oenococcus oeni* strains on the chemical and aromatic profiles of cherry wines. *Food Chem* **138**:2233–2241 (2013).
49 Gammacurta M, Marchand S, Albertin W, Moine V and de Revel G, Impact of yeast strain on ester levels and fruity aroma persistence during aging of Bordeaux red wines. *J Agric Food Chem* **62**:5378–5389 (2014).
50 Muñoz-González C, Martín-Álvarez PJ, Moreno-Arribas MV and Pozo-Bayón MÁ, Impact of the nonvolatile wine matrix composition on the in vivo aroma release from wines. *J Agric Food Chem* **62**:66–73 (2014).

