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Characterization of Fruity Aroma Modifications in Red Wines during Malolactic Fermentation

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ABSTRACT: The role of malolactic fermentation (MLF) in the fruity aroma of red wines was investigated by an analytical study on more than 60 volatile compounds in 48 red wines made in varied conditions and supplemented by a sensory study. Lactic acid bacteria (LAB) modify the fruity notes of red wines but without a specific trend. The absence, in the short term, of a lactic mask was emphasized, whereas the existence of a smoked/toasted reduction-like mask note was evoked but not characterized. Variations in the composition of the fruity aroma markers were predominant. Although LAB β-glycosidase activities were not very involved, on the other hand, esterase seemed to play a central role that was sometimes associated with the metabolism of the sulfur-containing compounds. New insights in ester metabolism in enological LAB and the importance of wine composition on bacterial variations in metabolites and aromatic alterations were emphasized.

KEYWORDS: *malolactic fermentation, wine, aroma, volatile compounds, quantification, sensory, esters, metabolic interactions*

INTRODUCTION

The vinification process brings into play different microbiological processes, among which alcoholic fermentation (AF), carried out by the yeast *Saccharomyces cerevisae*, and malolactic fermentation (MLF), carried out by lactic acid bacteria (LAB), play a central role. MLF mainly carried out by the species *Oenococcus oeni* is an essential step to improve the quality of red wines.[1] LAB activities convert L-malic acid to L-lactic acid and carbon dioxide, resulting in better balanced wines due to a decrease in acidity and an increase in pH.

Furthermore, MLF significantly influences the aromatic complexity of wine by the production of odor-active compounds and the transformation of both grape- and yeast-derived volatile compounds and flavor precursors. Some compounds and metabolisms are known. MLF mainly concerns molecules derived from the metabolism of organic acids, residual sugars, and certain amino acids such as methionine.[1−3] All of these metabolites are involved in the generation of lactic, buttery, sulfured, and toasted notes.

On the other hand, our understanding of the impact of LAB on the fruity aroma of red wines is not as sophisticated and is more controversial. MLF is often empirically associated with decreases in the intensity of fruity notes due to an aroma mask generated by the development of lactic notes. However, the data found in the literature are not as evident, and LAB seem to be able to either decrease or increase the fruity aroma of red wines and sometimes have no influence on it at all.[4−8] Very few data about the metabolism of LAB involved in these variations are available. The absence of fundamental data on the aromatic markers found in red wines is probably one reason why it is difficult to come to a consensus. The lack of concomitant biochemical, analytical, and sensorial studies dealing with this topic has hindered progress on this question. This disagreement demonstrates the complexity of this subject and the need for additional work, especially given that the preservation of fruity aromatic expression in wines is a major concern for winemakers.

The aim of this study was to establish a sensorial and analytical state of the impact of MLF on the composition of the aromatic markers potentially involved in the perception of fruity notes in red wines. Recent studies have shown that fruity attributes in red wines were mainly due to synergistic effects generated by the presence of markers such as esters, C13-norisoprenoids, lactones, or sulfur-containing compounds.[9,10] Thus, 55 compounds known to contribute to the fruity notes of red wines and diacetyl, which is mainly responsible for the lactic note, were quantified using adapted methods previously developed in our laboratory.[11,12]

Moreover, one of the difficulties with regard to finding a consensus is that the previous works dealing with this topic have mainly focused on a few cases of bacteria strains or wines, whereas many enological parameters can affect wine aroma. Thus, it was essential to increase the conditions of the studied cases and to vary them as much as possible to obtain more relevant trends.

MATERIALS AND METHODS

Wines. Forty-eight single-varietal red wines from three vintages (2007, 2008, and 2009) were sampled before and after MLF in eight different wineries. The winemaking conditions varied following the samples as summarized in Figure 1. Wines made in winery conditions were sampled for analyses in wineries at the end of AF (sugar $\leq$ 2 g/L) and after completion of MLF (malic acid $\leq$ 0.2 g/L). In the case of MLF carried out in laboratory conditions, the wines were sampled in wineries after the completion of AF and before the beginning of MLF after controlling the samples through L-lactic acid measurement and Epifluorescence analysis (Chemunex, Ivry-sur-Seine, France). In the

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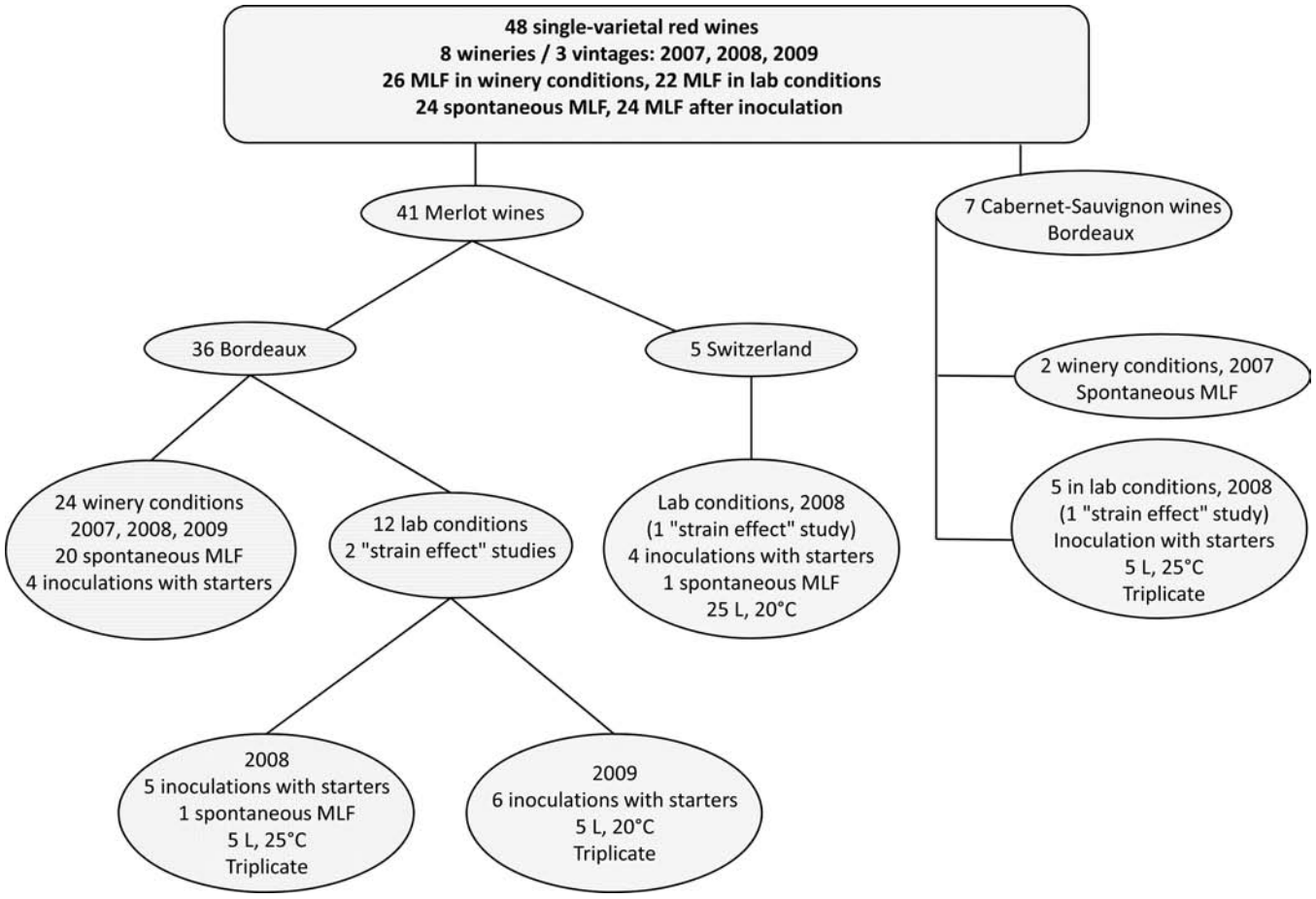

Figure 1. Origin of samples.

samples taken after alcoholic fermentation (control), 50 mg/L of SO_2 was added to inhibit MLF, and the wines were racked and stored at 12 °C for the duration of MLF. Samples of control wines corresponding to laboratory conditions were stored, after the addition of sulfite, under the same condition as the treatment samples for the duration of MLF, regularly checking that the fermentation did not degrade the malic acid. In the case of wines with bacterial inoculation, starters were introduced at a rate of 10^6 CFU/mL following instructions from the provider without nutrient supplementation. Finally, 11 different malolactic starter cultures from four different providers were tested (Table 1), among which some strains were inoculated in different wines to study the impact of the wine's composition after AF (matrix effect). All of the starters were used in laboratory conditions and three of them in winery conditions. Implantation controls were performed with the starter strains when the malic acid contents were decreased by half.[13] For the entire duration of MLF, the malic acid concentration was measured twice per week to monitor the bacterial metabolism. At the end of MLF, 50 mg/L of SO_2 was added and the wines were racked and sampled for analyses. For the volatile compound analyses, all of the wines were sampled and frozen before being analyzed. Wines submitted to sensory analyses were bottled in conventional 0.75 L glass bottles and quickly evaluated.

Table 1. LAB Starter Cultures Used in the Study

LAB starter culture	manufacturer
350 Preac	Laffort Oenologie (Floirac, France)
450 Preac	Laffort Oenologie (Floirac, France)
SB3	Laffort Oenologie (Floirac, France)
Enoferm β	Lallemand Ltd. (Toulouse, France)
VP41	Lallemand Ltd. (Toulouse, France)
Lalvin 31	Lallemand Ltd. (Toulouse, France)
Lallemand D	Lallemand Ltd. (Toulouse, France)
CH16	Chr. Hansen (Horsohlm, Denmark)
Viniflora Oenos	Chr. Hansen (Horsohlm, Denmark)
Viniflora Ciné	Chr. Hansen (Horsohlm, Denmark)
Expertise S	Oenofrance (Bordeaux, France)

Standard Chemical Analyses. The standard chemical parameters of the wines were determined according to the methods outlined by the International Organisation of Vine and Wine.[14]

Volatile Compound Analyses. Each wine sample was analyzed simultaneously with its control after defrosting, which did not affect the content of the aroma compounds in the racked wine. Sixty molecules were analyzed using six different methods that were developed and validated in the laboratory, among which four were used in this work.

Diacetyl (Liquid–Liquid Extraction after Derivatization and GC-MS Analysis). The diacetyl contents were measured using the method developed by de Revel et al.[15] In accordance with this method, 5 mL of 1,2-diaminobenzene was added to 50 mL of wine that was previously spiked with 50 μL of internal standard solution, and the pH was adjusted to 8 with NaOH (10 N). After a derivatization reaction of 3 h at 60 °C, the pH of the mixture was adjusted to 2 with sulfuric acid (2 M) and was extracted twice with 5 mL of dichloromethane. The extract was then analyzed by GC/MS using the conditions described elsewhere.[15] Quantification was performed with a calibration curve built in 12% hydroalcoholic solution. Hexan-2,3-dione at 1 g/L in 50% hydroalcoholic solution was used as an internal standard.

Volatile Sulfur Compounds (HS-GC/FPD). Dimethyl sulfide (DMS) and hydrogen sulfide (H_2S) were analyzed according to the method proposed by Anocibar-Beloqui.[16] In accordance with this method, 100 mL of wine, previously spiked with 100 μL of internal standard

Table 2. Mean Concentrations with Standard Deviations (Micrograms per Liter) after AF and after MLF and Distribution of the Concentrations Variations (Micrograms per Liter) Measured during MLF Represented by Key Values of Box-Plot Graphics: Minimum, Maximum, Median, First Quartile (Q1), and Third Quartile (Q2)

	mean of concentrations		variation					
compound	after AF	after MLF	min	Q1	median	Q3	max	significant difference[a]
diacetyl[b]	4.5 ± 2.8	9.9 ± 6.0	−1.6	2.1	4.1	7.1	17.0	*** (n = 43)
linalool	4.8 ± 2.5	6.3 ± 4.4	−0.9	0.2	0.5	1.1	10.9	* (n = 45)
β-damascone	0.016 ± 0.012	0.015 ± 0.009	−0.019	−0.003	0.000	0.002	0.013	NS (n = 37)
β-damascenone	1.99 ± 0.68	1.87 ± 0.63	−0.76	−0.17	−0.07	0.006	0.28	NS (n = 37)
β-ionone	0.12 ± 0.03	0.11 ± 0.03	−0.03	−0.007	−0.004	0.000	0.011	NS (n = 37)
α-ionone	0.10 ± 0.05	0.09 ± 0.6	−0.110	−0.024	−0.005	0.012	0.049	NS (n = 37)
γ-octalactone	1.3 ± 0.5	1.3 ± 7.0	−0.51	−0.049	0.02	0.093	0.846	NS (n = 37)
γ-nonalactone	11.4 ± 7.1	11.4 ± 7.0	−2.03	−0.44	−0.11	0.32	2.29	NS (n = 37)
γ-decalactone	0.82 ± 0.29	0.82 ± 0.31	−0.2	−0.07	−0.01	0.04	0.78	NS (n = 37)
γ-undecalactone	0.11 ± 0.04	0.10 ± 0.03	−0.08	−0.01	0	0.01	0.03	NS (n = 37)
γ-dodecalactone	0.12 ± 0.04	0.12 ± 0.29	−0.058	−0.01	0	0.01	0.07	NS (n = 37)
δ-decalactone	3.6 ± 1.1	3.4 ± 1.0	−1.07	−0.48	−0.21	−0.04	0.42	NS (n = 37)
3-sulfanylhexanol	6.2 ± 1.7	5.8 ± 2.8	−4.2	−2.12	−0.88	0.78	4.68	NS (n = 20)
dimethyl sulfide	5.7 ± 3.4	6.4 ± 4.0	−0.63	0.25	0.58	1.03	4.2	NS (n = 48)
hydrogen sulfide	2.4 ± 1.1	2.3 ± 1.1	−3.7	−0.3	0.13	0.68	3.6	NS (n = 48)
ethyl fatty acid esters								
ethyl propanoate	60 ± 37	60 ± 32	−25	−3.9	1.1	5.3	24	NS (n = 48)
ethyl butyrate	147 ± 47	147 ± 44	−52	−10	−1.6	8.3	53	NS (n = 48)
ethyl valerate	0.54 ± 0.51	0.70 ± 0.36	−0.86	−0.12	0	0.41	1.14	NS (n = 48)
ethyl hexanoate	320 ± 79	322 ± 80	−83	−13	5.8	23	116	NS (n = 48)
ethyl heptanoate	0.9 ± 0.5	0.95 ± 0.53	−0.27	−0.013	0.07	0.117	0.474	NS (n = 48)
ehyl octanoate	412 ± 92	445 ± 114	−112	−3.3	27	71	224	NS (n = 48)
ethyl nonanoate	0.83 ± 0.41	0.96 ± 0.56	−0.73	−0.05	0.09	0.34	1.82	NS (n = 48)
ethyl decanoate	147 ± 44	165 ± 61	−80	−5	16	35	133	NS (n = 48)
ethyl dodecanoate	9.4 ± 4	9.92 ± 4.17	−7.21	−0.84	0.70	1.84	6.29	NS (n = 48)
acetates								
ethyl acetate[b]	38 ± 18	40 ± 15	−28	−5.4	0.4	4.7	22	NS (n = 48)
propyl acetate	6.6 ± 4.2	6.9 ± 3.4	−3.2	−0.5	0.3	1.4	2.7	NS (n = 48)
isobutyl acetate	45 ± 29	46 ± 22	−37	−1.1	1.7	6.5	38	NS (n = 48)
butyl acetate	0.8 ± 0.8	0.9 ± 1.3	−1.1	−0.3	−0.04	0.02	0.6	NS (n = 30)
isoamyl acetate	572 ± 577	522 ± 431	−683	−34	7.6	52	162	NS (n = 48)
hexyl acetate	2.73 ± 4.01	2.37 ± 2.94	−6.04	−0.21	0.03	0.18	1.14	NS (n = 48)
octyl acetate	0.02 ± 0.03	0.03 ± 0.03	−0.025	0	0.006	0.014	0.078	NS (n = 33)
phenylethyl acetate	37 ± 49	32 ± 32	−122	−2.00	−0.45	0.91	2.63	NS (n = 48)
ethyl branched acid esters								
ethyl isobutyrate	41 ± 14	44 ± 17	−10	−4.6	−0.6	6.8	34	NS (n = 48)
ethyl 2-methylbutyrate	8.6 ± 2.2	9.5 ± 2.9	−2.2	−0.7	0.3	1.4	4	NS (n = 48)
ethyl isovalerate	10.5 ± 3	11.6 ± 4	−2.2	−0.5	0.5	1.8	7.2	NS (n = 48)
ethyl phenylacetate	4.21 ± 2	1.85 ± 0.8	−6.94	−1.69	−0.76	0	1.42	*** (n = 48)
cinnamates								
ethyl cinnamate	1.39 ± 0.29	1.39 ± 0.29	−0.64	−0.11	−0.01	0.14	0.54	NS (n = 48)
ethyl dihydrocinnamate	0.46 ± 0.32	0.51 ± 0.3	−0.34	−0.03	0.02	0.18	0.36	NS (n = 48)
methyl fatty acid esters								
methyl butyrate	0.72 ± 0.23	0.70 ± 0.21	−0.5	−0.04	0	0.2	0.3	NS (n = 35)
methyl hexanoate	1.17 ± 0.39	1.14 ± 0.45	−0.63	−0.11	−0.05	0.04	0.57	NS (n = 48)
methyl octanoate	1.35 ± 0.65	1.56 ± 0.85	−0.38	0	0.18	0.28	1.33	NS (n = 48)
methyl decanoate	0.36 ± 0.19	0.42 ± 0.3	−0.16	−0.02	0.02	0.06	0.73	NS (n = 48)
isoamyl esters of fatty acid								
isoamyl butyrate	0.37 ± 0.13	0.38 ± 0.14	−0.25	−0.03	0.01	0.04	0.15	NS (n = 48)
isoamyl hexanoate	1.00 ± 0.41	1.05 ± 0.51	−0.57	−0.05	0.02	0.13	0.93	NS (n = 48)
isoamyl octanoate	1.74 ± 0.77	2.01 ± 1.08	−1.02	0.01	0.21	0.36	4.1	NS (n = 48)
minor polar esters								
ethyl 3-hydroxybutyrate	236 ± 111	304 ± 133	−209	−6.3	4.8	24	342	NS (n = 44)
ethyl levulinate	1.2 ± 1.1	1.3 ± 0.9	−1.6	−0.2	0.05	0.3	1.5	NS (n = 40)
ethyl 3-hydroxyhexanoate	1.57 ± 1.7	2.36 ± 1.7	−1.52	0.12	0.57	1.31	4.9	** (n = 40)
ethyl 6-hydroxyhexanoate	2.3 ± 1.9	3.58 ± 1.3	−2.06	−0.22	1.34	2.28	4.97	*** (n = 40)
major polar esters								

Table 2. continued

compound	mean of concentrations		variation					significant difference[a]
	after AF	after MLF	min	Q1	median	Q3	max	
ethyl lactate[b]	16.9 ± 10.1	44 ± 10.4	7.0	21.2	28.7	33.0	46.5	*** (n = 48)
diethyl succinate	949 ± 892	1819 ± 909	−427	208	584	1343	3390	*** (n = 48)
monoethyl succinate[b]	62.3 ± 73	144 ± 75	−37.7	25	66.7	128	275	*** (n = 48)
miscellaneous esters								
ethyl *trans*-2-hexenoate	1.73 ± 0.82	1.73 ± 0.8	−0.51	−0.12	−0.01	0.14	0.48	NS (n = 48)
isobutyl hexanoate	0.3 ± 0.42	0.33 ± 0.47	−0.09	−0.02	0.06	0.03	0.3	NS (n = 48)
methyl geranate	0.2 ± 0.14	0.2 ± 0.14	−0.07	−0.01	0	0.01	0.07	NS (n = 30)
branched acids								
isobutyric acid	1047 ± 454	1081 ± 426	−659	−62	31	153	475	NS (n = 48)
2-methylbutyric acid	232 ± 119	313 ± 176	−118	13	65	120	622	* (n = 48)
isovaleric acid	550 ± 191	579 ± 210	−84	−13	30	75	196	NS (n = 48)

[a]Significant differences: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. [b]Milligrams per liter.

solution, was added to a 125 mL headspace vial. After 24 h at 22 °C in the dark, 1 mL of the gas phase was taken from the headspace and injected into the GC for GC/FPD analysis under the conditions described elsewhere.[17] Quantification was performed with calibration curves built in 12% hydroalcoholic solution. Thiophene at 100 mg/L in 50% hydroalcoholic solution was used as an internal standard.

C13-Norisoprenoids and Lactones (SBSE-GC/MS). This method, which was recently developed and validated by Antalick,[12] allowed us to quantify four C13-norisoprenoids (β-damascenone, β-damascone, β-ionone, α-ionone) and six lactones (γ-octalactone, γ-nonalactone, γ-decalactone, γ-undecalactone, γ-dodecalactone, δ-decalactone). In accordance with the method, 25 μL of internal standard solution was added to an exact volume of 25 mL of wine. An aliquot of 20 mL of this wine was introduced into a 25 mL adapted vial. A 20 mm × 1 mm (length × film thickness) PDMS stir bar (Twister, 126 μL coating) (Gerstel, Müllheim an der Ruhr, Germany) was dropped into the vial, and the latter was capped with a PTFE-faced rubber stopper. The closed vials were stirred for 1 h at 900 rpm and room temperature. At the end of the extraction time, the Twisters were removed from the vials, quickly washed with Milli-Q quality water, and dried with lint-free tissue. Each Twister was then transferred into a glass tube for thermal desorption (Gerstel) and GC/MS analysis with the conditions described elsewhere.[12] Quantification was performed using calibration curves built in red wines. Ethyl-d_5 cinnamate at 20 mg/L in ethanol was used as an internal standard. The ethyl-d_5 cinnamate was synthesized according to the method described by Antalick et al.[11]

Thiols (SPE-SBSE-GC/MS). The method proposed by Antalick[12] was adapted from the previous works of Mateo-Vivaracho et al.[17] and Rodriguez-Bencomo et al.[18] It allowed us to measure the content of 3-sulfanylhexanol (3SH). In accordance with this method, 50 μL of internal standard solution was added to 50 mL of wine. The wine sample was passed through a 500 mg Bond-Elut-ENV cartridge (Varian, Walnut Creek, CA, USA) that was previously conditioned with 5 mL of dichloromethane, methanol, and water using the Vitisprep SPE vacuum manifold (Supelco, Bellefonte, PA, USA). A derivatization reaction was carried out directly in the cartridge by passing 5 mL of DBU aqueous solution (6.7%) and 1 mL of a 400 mg/L solution of PFBBr in isohexane and letting the cartridge become imbibed with the reagent for 20 min at room temperature. A volume of 1 mL of mercaptoglycerol solution in 6.7% DBU aqueous solution was then loaded and left in the cartridge again for 20 min at room temperature before the cartridge was rinsed with 20 mL of a 40% methanol−water solution 0.2 M in H_3PO_4 and 5 mL of water. Derivatized 3SH was then eluted with 4 mL isohexane/diethyl ether (1:3). This extract was then evaporated and, finally, a small volume of solvent was introduced into a 25 mL vial and diluted with 20 mL of water for SBSE-GC/MS analysis with the same parameters as the C13-norisoprenoids and lactones. Quantification was performed for 3SH from a calibration curve built in red wines. 2-Methyl-3-tetrahydrofuranthiol at 400 mg/L in ethanol was used as an internal standard.

Apolar Esters (HS-SPME-GC/MS). This method, developed and validated by Antalick et al.,[11] allowed us to quantify 32 apolar esters: fatty acid ethyl esters, higher alcohol acetates, branched acid ethyl esters, isoamyl esters, methyl esters, ethyl cinnamates, and minor esters (Table 2). In accordance with this method, 20 μL of internal standard solution was added to an exact volume of 25 mL of wine. An aliquot of 10 mL of this wine was introduced into a 20 mL standard headspace vial previously filled with 3.5 g of sodium chloride. The vial was tightly sealed with a PTFE-lined cap, and after the solution was homogenized using a vortex shaker, it was loaded onto a Gerstel autosampler. The samples were extracted by HS/SPME and analyzed by GC/MS according to the conditions described elsewhere.[11] Quantification was performed with calibration curves built in red wines. A mixture of ethyl-d_5 butyrate, ethyl-d_5 hexanoate, ethyl-d_5 octanoate, and ethyl-d_5 cinnamate at 20 mg/L in ethanol was used as an internal standard. Deuterated esters were synthesized according to the method described by Antalick et al.[11]

Ethyl Acetate (Direct Injection and GC/FID Analysis). Ethyl acetate was quantified using a modified version of the official OIV method (OIV-MA-AS315-02A). In accordance with this method, 5 mL of wine was spiked with 50 μL of internal standard solution. The vials were filled with this solution for direct injection and analysis by GC/FID. Quantification was performed using a calibration curve built in 12% hydroalcoholic solution. 4-Methylpentan-2-ol at 10 g/L in 50% hydroalcoholic solution was used as an internal standard.

Additional Volatile Compounds (Liquid−Liquid Extraction and GC/MS Analysis). The method developed and validated by Antalick[12] allowed us to quantify seven polar esters (ethyl lactate, ethyl succinates, hydroxylated ethyl esters), three branched acids (isobutyric, isovaleric, 2-methylbutyric acids), and linalool. In accordance with this method, 50 mL of wine previously spiked with 10 μL of internal standard solution was successively extracted with 4 mL and twice with 2 mL of dichloromethane. The extract was then analyzed by GC/MS using the conditions described elsewhere.[12] Quantification was performed with calibration curves built in red wines. Octan-2-one at 1 g/L in 50% hydroalcoholic solution was used as an internal standard.

Sensory Analysis. The sensory analyses were carried out by orthonasal tests, using odor comparison profiles (ISO 13299: 2003) to compare the aroma profiles of wines both with and without MLF. A list of four descriptive terms was previously designated in the laboratory: simple descriptors were chosen to simplify the evaluation and to keep the panel's attention. The terms chosen were based on the fruity aroma (fruity), bacterial activity (lactic), and overall aroma potentially having an impact on the fruity aroma (vegetal and smoked/toasted). The panelists evaluated the intensity of the four aromatic attributes on a scale from 1 to 7. All of the panelists are part of the Faculty of Oenology's sensory panel (University of Bordeaux) and familiar with wine tasting. The panel is made up of permanent workers of the institute (researchers, assistant) who regularly work on general descriptors and mastering slight differences of intensity on these descriptors (fruity, vegetal, lactic, etc.).

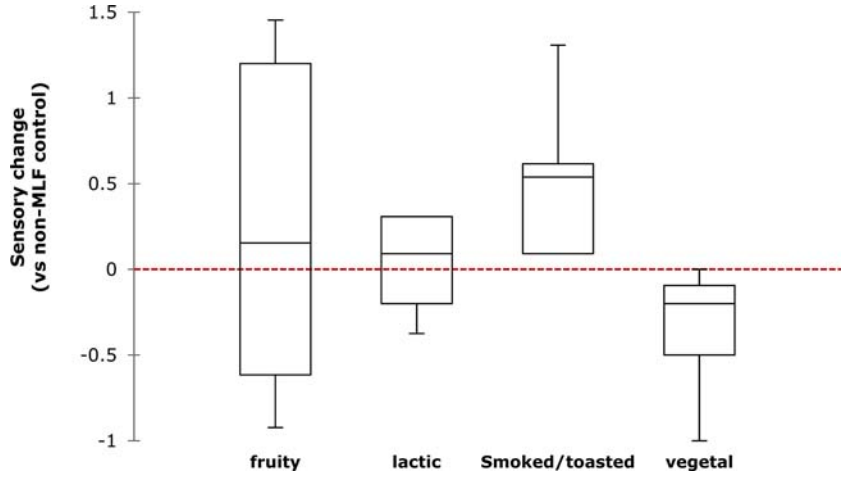

Figure 2. Box-plot graph displaying the variability of sensory changes perceived by the panels between the wines with MLF and the non-MLF controls (without MLF) with minimum and maximum values, median, and quartiles. For each attribute the means of sensory ratings modifications were used to draw the graph.

The number of panelists (n) varied following the tests (n = 11−16) with 39−54% women, depending on personal availability.

All of the tasting sessions took place in a dedicated room (ISO 8589) equipped with individual booths. The samples (50 mL) were presented with "normal daylight" illumination in normalized dark glasses (ISO 3591: 1977) identified with three-digit random codes and covered with half of a Petri dish.

For technical reasons it was not possible to perform sensory evaluation on 48 of the wines (limitation due to volume of the samples and organoleptic spoiling due to the use of small volumes for MLF in 5 L glass tanks). Finally, five Merlot wines were evaluated by sensory analysis: M1a, M1b, and M1c from the same Bordeaux wine (2009) with MLF carried out in laboratory conditions with three different starter cultures (three different strains), M2 from Switzerland (2008) with MLF carried out spontaneously in winery conditions, and M3 from Bordeaux (2009) with MLF carried out in winery conditions after inoculation with another bacterial strain.

Statistical Analysis. A one-way analysis of variance and a Student's t test were performed to find significant differences between the concentrations of the aromatic markers before and after MLF. To display the distribution of the variations in volatile composition measured during MLF, characteristic values of the box-plot graphs were calculated (minimum, maximum, median, quartiles). Linear regressions were performed to establish some correlations. For the strain and matrix effect studies, figures were drawn using box-plot graphs. Pearson's type principal component analyses (PCA) were also carried out on the values of the concentration variations measured for certain compounds in 48 wines.

For the sensory analysis, a two-way analysis of variance (product and judge) and a Student's t test were carried out to find significant differences in the intensity of the attributes noted by the panel members. A box-plot graph was also drawn to display the variations of the attribute intensities during MLF.

Variance analyses, a Student's t test, and linear regressions were carried out using Excel software (Microsoft Corp., Redmond, WA, USA), whereas the box-plot graph and PCA were performed using XL-stat software (Addinsoft, Paris, France).

RESULTS AND DISCUSSION

Modification of the Red Wine Aroma by MLF. The variations in the intensity of the evaluated descriptors between wines tested before and after MLF are represented in Figure 2. Although these results confirm the modification of the fruity aroma of red wines during MLF, they also emphasize the fact that the fruity note is the attribute that is the most altered by LAB activity. The variations perceived on this descriptor by the panel between wines with and without MLF were stronger than for the lactic, vegetal, and smoked/toasted descriptors. However, no clear trends exist, which was confirmed by some detailed results for the Merlot wines (Figure 3) showing that MLF could decrease (M1a), increase (M2, M3), or have no effect at all on the fruity aroma of red wines (M1c). Most of these variations were significant without a judge effect and were in accordance with the lack of consensus found in the literature. On the other hand, these results partially refute the empirical data, which tend to show a decrease of the fruity notes further to an intensification of the lactic aroma. Figures 2 and 3 even show that MLF weakly affected the lactic note in the studied red wines. This lack of alterations, contrary to well-established data showing that MLF enhances lactic aroma,[2,19] is probably due to the reactivity of diacetyl in the wine medium, as suggested by Bartowsky et al.[19] The development of lactic or buttery notes in dairy products such as wine is mainly due to the presence of diacetyl.[20] In wine, most of the diacetyl is produced during MLF by LAB, which metabolize citric acid.[3] Table 2 confirms the production of diacetyl during most of the MLF studied in this work. However, the perception of diacetyl depends not only on the total diacetyl concentration but also on numerous factors such as the chemical composition of the wines and the presence of sulfur dioxide, which interacts with diacetyl and strongly decreases its volatility.[19] It is more difficult to perceive lactic notes in very young wines that have a higher concentration of sulfur dioxide than more aged wines because the interactions between diacetyl and sulfur dioxide are reversible.[21] Thus, although the interactions between the lactic and fruity aroma seem weak in young red wines, a stronger longer term impact cannot be excluded.

The smoked and toasted attributes were more affected than the lactic aroma. Whereas, overall, MLF seemed to enhance the intensity of this descriptor according to Figure 2, on the other hand, the detailed results were not as clear (Figure 3). The development of smoked and toasted notes during MLF has already been observed in a previous study.[22] These odors could be associated with certain notes of reduction that often develop during MLF and might be linked to a decrease in the fruity aroma in some cases. It might be the case for wine M1a, and to a lesser extent M1b (not significant), in which the decrease of fruity intensity is linked to an increase of smoked/toasted notes

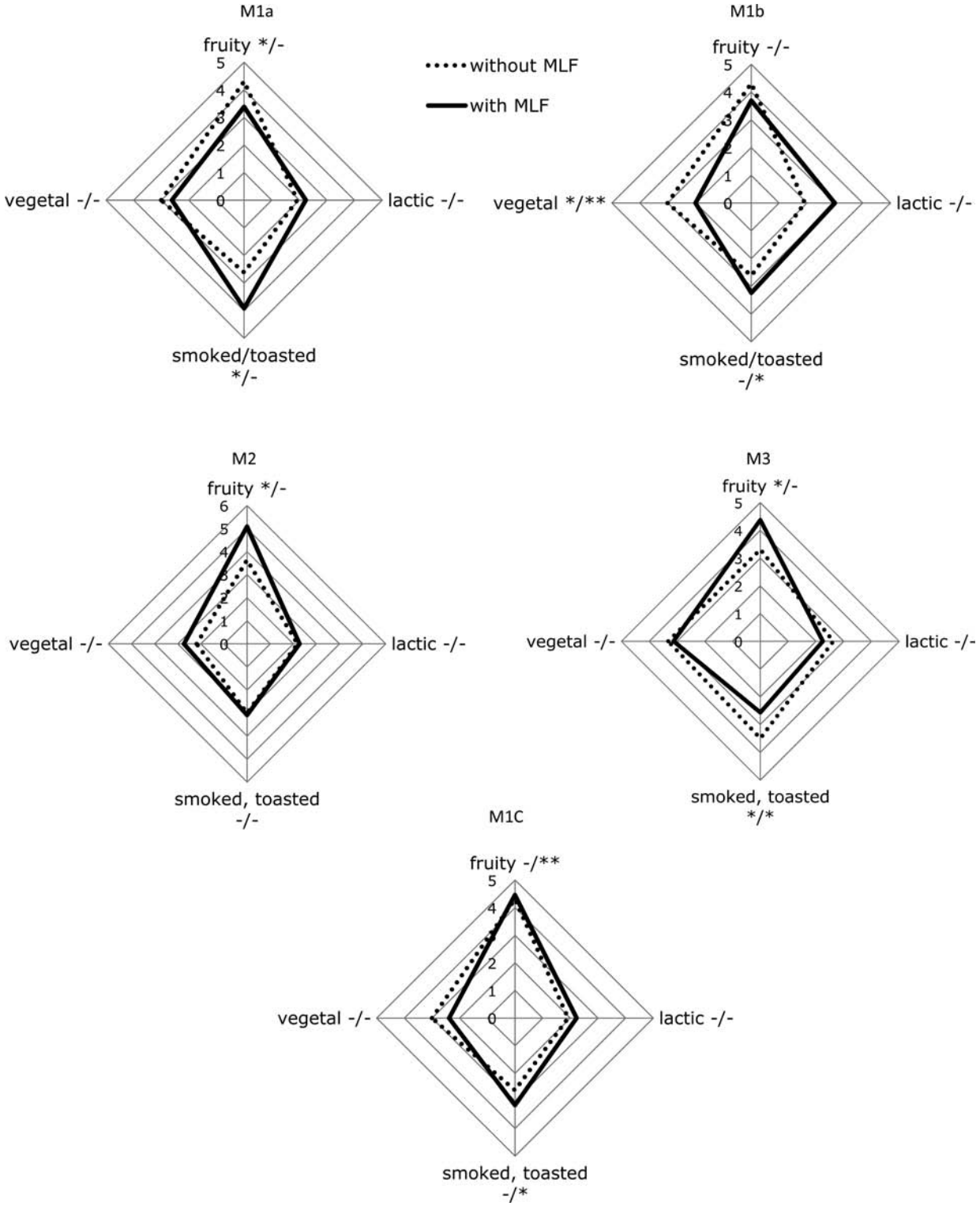

Figure 3. Mean sensory descriptor values for five Merlot wines with MLF (solid line) and without MLF (dotted line). Significant differences are indicated with asterisks (wine/judge).

after MLF. At the same time, the concentration of some markers of fruity notes, such as esters, increased, which indicates a likely olfactory mask of smoked notes over the fruity aroma (Table 3). These smoked/toasted notes were difficult to evaluate according to the judge effect (Figure 3). The reduction off-flavor notes are often associated with the production of H_2S.[23] In M1a and M1b wines, the MLF led to the production of H_2S at levels close to the perception threshold (1.7−10 μg/L)[24,25] (Table 3). Toasted flavors can also be imparted by some volatile phenols known to be produced by certain strains of LAB.[8,33] However, volatile phenols were not measured in our studies, and additional studies will have to prove the link between smoked aroma enhancement after MLF and bacterial production of volatile phenols.

Table 3. Concentrations in Compounds Quantified in the Wines after AF (Control) and after MLF Analyzed by Sensory Evaluation

	wine									
	M1a		M1b		M2		M3		M1c	
compound	control	MLF	control	MLF	control	MLF	control	MLF	control	MLF
diacetyl[a]	2.2	8.6	2.2	11.7	4.1	4.6	1.1	3.2	2.2	9.3
linalool	2.7	3.5	2.7	3.1	3.3	3.4	5.5	4.6	2.7	3
β-damascone	0.018	0.011	0.018	0.016	0.014	0.027	0.013	0.007	0.018	0.013
β-damascenone	1.72	1.4	1.72	1.61	2.56	2.54	2.6	2.47	1.72	1.58
β-ionone	0.16	0.15	0.16	0.14	0.07	0.06	0.07	0.08	0.16	0.15
α-ionone	0.17	0.13	0.17	0.11	0.12	0.09	0.12	0.12	0.17	0.14
total NI	2.068	1.691	2.068	1.876	2.764	2.717	2.803	2.677	2.068	1.883
γ-octalactone	1.84	1.8	1.84	1.85	1.13	0.88	nq[b]	nq	1.84	1.92
γ-nonalactone	23.9	22.7	23.9	23.3	3.6	3.8	3.6	3.25	23.9	21.9
γ-decalactone	0.85	0.75	0.85	0.76	0.51	0.53	0.43	0.51	0.85	0.8
γ-undecalactone	0.12	0.09	0.12	0.11	0.06	0.08	0.05	0.04	0.12	0.09
γ-dodecalactone	0.13	0.09	0.13	0.12	0.12	0.12	0.04	0.04	0.13	0.12
δ-decalactone	4.24	3.68	4.24	3.16	2.4	2.23	1.47	1.41	4.24	3.71
total lactones	31.08	29.11	31.08	29.3	7.82	7.64	5.59	5.25	31.08	28.54
3-sulfanylhexanol	na[c]	na	na	na	5.3	4.1	7	10.1	na	na
dimethyl sulfide	3.7	5.8	3.7	4	13.4	17.6	3.1	2.9	3.7	4.7
hydrogen sulfide	2.7	6.3	2.7	3.7	4.5	2.8	3.6	2.4	2.7	2.7
ethyl fatty acid esters										
ethyl propanoate	145	123	146	127	57	57	69	60	146	121
ethyl butyrate	91	96	91	85	186	196	373	370	91	92
ethyl valerate	1.6	1.3	1.6	1.3	0.5	0.4	0.5	0.5	1.6	1.3
ethyl hexanoate	281	283	281	281	324	344	663	688	281	259
ethyl heptanoate	2.05	2.19	2.05	2.19	0.4	0.55	0.65	0.64	2.05	2.13
ehyl octanoate	343	426	343	452	402	460	735	820	343	420
ethyl nonanoate	1.59	2.04	1.59	2.39	0.36	0.8	0.58	0.53	1.59	1.89
ethyl decanoate	168	196	168	221	64	156	253	314	168	173
ethyl dodecanoate	15.3	15.8	15.3	21.5	2.5	5.4	11.6	10.4	15.3	14.6
total	1049	1145	1050	1193	1037	1220	2106	2263	1050	1085
acetates										
ethyl acetate[a]	7	16.8	7	26.3	27.7	20.4	20.6	20.2	7	21.8
propyl acetate	7.1	8	7.1	6.8	16.7	15.8	10	10	7.1	6.4
isobutyl acetate	24	24	24	24	120	110	120	90	24	26
isoamyl acetate	181	187	181	174	1854	1575	876	846	181	169
hexyl acetate	0.74	0.64	0.74	0.54	9.43	8.43	1.79	1.82	0.74	0.63
phenylethyl acetate	27	24	27	26	83	71	40	38	27	25
total (− ethyl acetate)	240	244	240	231	2083	1780	1048	986	240	227
ethyl branched acid esters										
ethyl isobutyrate	48	47	48	39	59	51	50	44	48	49
ethyl 2-methylbutyrate	9.1	11	9.1	8.5	7.9	5.7	7.2	6.5	9.1	11.2
ethyl isovalerate	14.6	14.7	14.6	13.2	12.7	13.1	14.3	14.5	14.6	14.5
ethyl phenylacetate	8	1.25	8	1.09	2.18	0.78	2.56	2.31	8	1.06
total	79.7	73.95	79.7	61.79	81.78	70.58	74.06	67.31	79.7	75.76
cinnamates										
ethyl cinnamate	1.81	1.8	1.81	1.92	1.4	1.15	0.52	0.57	1.81	1.96
ethyl dihydrocinnamate	0.61	0.57	0.61	0.7	1.27	1.49	0.45	0.47	0.61	0.59
methyl fatty acid esters										
methyl hexanoate	0.95	0.9	0.95	0.85	0.83	0.89	3.19	3.16	0.95	0.85
methyl octanoate	0.74	0.87	0.74	0.93	0.84	1.05	3.24	3.67	0.74	0.83
methyl decanoate	0.23	0.26	0.23	0.29	0.5	0.43	0.5	0.45	1.56	1.3
isoamyl esters of fatty acid										
isoamyl butyrate	0.2	0.19	0.2	0.24	0.42	0.57	0.94	0.9	0.2	0.18
isoamyl hexanoate	0.49	0.47	0.49	0.53	1.02	1.21	2.02	2.08	0.49	0.44
isoamyl octanoate	1	1.35	1	1.45	1.54	2.64	1.54	1.64	3.02	3.37
minor polar esters										
ethyl 3-hydroxybutyrate	72	81	72	76	142	143	580	561	72	65
ethyl levulinate	1.23	1.01	1.23	1.09	0.31	0.28	0.66	0.54	1.23	0.89
ethyl 3-hydroxyhexanoate	6.1	17.2	6.1	5.3	0.6	1.2	0.6	1.1	6.1	11

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Table 3. continued

	wine									
	M1a		M1b		M2		M3		M1c	
compound	control	MLF	control	MLF	control	MLF	control	MLF	control	MLF
ethyl 6-hydroxyhexanoate	4.8	4.9	4.8	5.3	nq	nq	1.31	1.97	4.8	4.3
major polar esters										
ethyl lactate[a]	7	46.9	7	39.6	21.3	49.8	6.1	33.4	6.9	40
diethyl succinate	868	3222	868	710	376	985	416	782	868	2225
monoethyl succinate[a]	35.6	120.4	35.6	41.5	9.2	11.8	28.7	93.2	35.6	53.2
miscellaneous esters										
ethyl *trans*-2-hexenoate	1.25	1.25	1.25	1.09	0.64	0.78	2.37	2.31	1.25	1.06
isobutyl hexanoate	0.07	0.08	0.07	0.08	0.18	0.21	0.37	0.34	0.07	0.07
methyl geranate	0.13	0.13	0.13	0.15	0.08	0.15	0.2	0.21	0.13	0.13
total esters	1460	1575	1461	1582	3353	3226	3826	3898	1464	1480

[a]Milligrams per liter. [b]nq, nonquantifiable. [c]na, not analyzed.

A decrease in intensity was observed for the vegetal attribute (Figure 2), confirming what has already been observed by several authors.[5,6] However, in terms of the limit values for the variations (Figure 2), the vegetal aroma was only slightly affected by MLF, and an impact on fruity notes is unlikely.

Finally, bacterial variations of the fruity aroma in red wines could be mostly due to the modulation of metabolites directly involved in fruity aroma perception. Thereby, the lack of consensus concerning this issue reflects the complexity of wine metabolome modulation by LAB.

Bacterial Impact on Varietal Markers Derived from Glycosides and Lactones. Some aglycones known to contribute to the fruity aroma of red wines were quantified. The measurement of the linalool concentrations showed that MLF significantly increased its contents, although the level of variations was very low (Table 2). These results are in accordance with the numerous data presented in the literature, demonstrating the presence of β-glycosidase activities in wine LAB that lead to the release of terpenols.[26−28] From a sensorial point of view, the variations observed during MLF were too low overall to be perceived. Merlot and Cabernet Sauvignon are grape varieties that are too poor in terpenol for a sensorial modification. A more relevant sensory impact could be considered with linalool-rich red grape varieties such as the Portuguese cultivar Touriga Nacional.[29]

On the other hand, C13-norisoprenoids seem to contribute substantially to the fruity aroma of red wines.[30] However, MLF did not greatly affect the C13-norisoprenoid contents with regard to the low variations measured (Table 2). Overall, no releases of this kind of aglycones have occurred during the studied MLF. Only α-ionone seemed to be a little more influenced by LAB activity but did not show any particular trends, and with regard to its perception threshold (400 ng/L), a sensory impact is unlikely.[31] Although β-damascenone and β-ionone are more odorant, their perception threshold is very dependent on the matrix, and in red wines, the levels of bacterial variations observed (Table 2) were largely too low to affect the fruity aroma.[27,29,32] The capacity of LAB to hydrolyze glycosidic precursors of C13-norisoprenoids has already been proven.[26,27] However, other authors found differences in the balance between bound and free volatiles such as C13-norisoprenoids.[28,33] Moreover, Boido et al.[26] reported adsorption and occlusion phenomena between polysaccharides released by LAB and C13-norisoprenoids, limiting the release of free C13-norisoprenoids during MLF. Similar phenomena could also be thought to occur in this study.

Like C13-norsisoprenoids, lactone concentrations slightly vary during MLF, confirming previous works.[28,34] An aromatic impact is unlikely with respect to the perception threshold (30 μg/L)[35] and the variations in the γ-nonalactone concentration, which is the main lactone contributing to red wine aroma (Table 2). Formation pathways for lactones in wine are still not well established, but they seem to be mainly synthesized by the transesterification of hydroxylated fatty acids or esters present in wine via either an enzymatic or chemical pathway.[36,37] Enzymatic transesterification can be performed by yeast, but LAB do not seem able to do it,[38] confirming the low variations observed in our study. As lactones are mainly synthesized during wine aging,[39] a late synthesis of lactones due to bacterial β-glycosidase and oxidase activities might be possible according to some studies.[37,40] However, additional work will have to prove this by monitoring the release of lactone precursors during MLF.

In summary, bacterial β-glycosidase activities are weakly involved in the modulation of the fruity aroma in red wines during MLF. A more relevant impact of LAB β-glycosidase and oxidase activities could be indirectly possible during wine aging.

Influence of MLF on the Concentration of Some Sulfur-Containing Compounds. Sulfur-containing compounds known to contribute to the fruity aroma in red wines, such as some 3-sulfanylhexanol and DMS, were studied. Dihydrogen sulfide was also measured because this compound contributes to reduction off-flavors, potentially masking fruity aroma. As the analysis of thiols is particularly long and difficult in red wines, the number of analyzed samples was limited (n = 20).

Although it was not significant, MLF seemed rather to decrease the concentration of 3-sulfanylhexanol (Table 2). A few studies have shown the capacity of LAB to reduce the level of 3-sulfanylhexanol.[41,42] However, for the first time, increases of 3-sulfanylhexanol concentration after MLF were measured in some cases (Table 2). With respect to the levels of these variations, it is possible that there might be an impact on wine aroma. Indeed, with a perception threshold at 60 ng/L,[43] 3-sulfanylhexanol is a very odorant compound. Whereas in red wines its perception threshold is certainly higher, the level of measured variations could probably affect the fruity aroma at least via a synergisitic effect. This type of impact was observed in the case of wine M3 (Figure 3), in which a concentration increase of 3-sulfanylhexanol associated with a content increase of ethyl fatty acid esters led to a significant increase in the fruity aroma (Table 3). Thus, in some cases, the bacterial modulation

of the 3-sulfanylhexanol concentration can influence the fruity aroma in red wines either by reducing or increasing the intensity of this note. The catabolism of 3-sulfanylhexanol by LAB is not still known, and, on the contrary, LAB usually tend to synthesize sulfur-containing compounds from amino acids such as methionine. However, the capacity of bacterial lees to retain 3-sulfanylhexanol might explain the decreases observed for the contents of this thiol as well as for yeast.[44] Conversely, the lower proportion (30%) of 3-sulfanylhexanol biosynthesis by LAB observed might be due to cystathionine lyase activities. 3-Sulfanylhexanol is partly derived from nonodorous cysteinylated precursors present in grapes[45] and cleaved during alcoholic fermentation by the cystathionine β-lyase activity of yeast.[46] This enzymatic activity has recently been characterized in *O. oeni*.[47] Cystathionine β-lyase should exhibit a lower affinity toward cysteinylated 3-sulfanylhexanol than cystathionine, as has already been demonstrated in the case of methionine metabolism in dairy LAB.[48] This low affinity could explain the lower proportion of MLF leading to an increase in the 3-sulfanylhexanol concentration.

For the first time, the influence of wine LAB on DMS concentration was reported. Although the impact of MLF on volatile sulfur compounds (VSC) was not significant, on the other hand, LAB showed a clear trend for the synthesis of DMS. More than 75% of the studied wines led to increases of the DMS contents during MLF, although the levels were low overall (Table 2). However, in certain cases, the variations could potentially affect the fruit aroma of red wines.[11] This is notably the case for wine M2 (Figure 3) in which an increase in the DMS concentration associated with an increase in the ethyl fatty acid ester contents probably contributes to enhancing the fruity notes of wine after MLF (Table 3). DMS is mainly produced during wine aging by *S*-methylmethionine degradation,[49] but a part is synthesized by yeast during AF by the catabolism of amino acid[50] or DMSO reduction.[16] These pathways could also apply to LAB, which are able to catabolize amino acids[3] and to also carry out reductase activities.[2,51]

A bacterial synthesis of H_2S was measured in 55% of MLF, which is clearly lower than for DMS (Table 2). LAB can notably produce H_2S by catabolizing cysteine,[52] but it is already known that in wine, this compound can easily combine with ethanol to form ethanethiol, which contributes to reduction off-flavors[53] (not quantified in our study). The high content of ethanol in a wine medium after AF favors this reactivity and could explain the variability observed regarding the impact of LAB activity on the final content of H_2S in wines.

Modulation of the Ester Composition in Red Wines by LAB Activities. Forty esters were quantified in this study, among which four major esters were weakly odorant: ethyl acetate, ethyl lactate, and monoethyl and diethyl succinate. Some authors suggested that ethyl acetate can contribute at low levels to the fruitiness of wines.[54] However, it has never been scientifically demonstrated, and on the contrary ethyl acetate is much more often associated with solvent off-flavor notes. The variations measured (<30 mg/L) were probably too low to affect wine aroma. The contents of the other major esters significantly increased during MLF (Table 2), as has already been shown in the literature.[2,54] However, with regard to their perception thresholds,[2,9] the bacterial modulations of these esters measured in this study probably do not affect the wine aroma.

The other studied esters are more odorant, and they are much more involved in the perception of a fruity aroma in wines. The modifications of the total odorant ester concentrations during MLF were highly variable (Figure 4). The

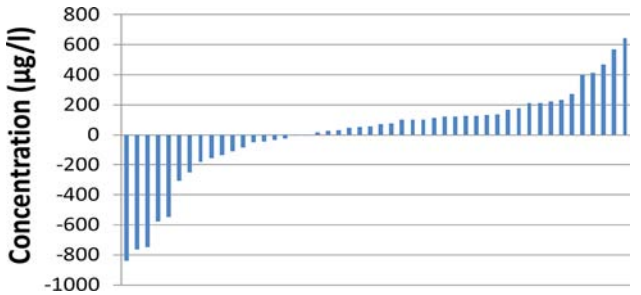

Figure 4. Variations in odorant ester concentrations during MLF in the 48 studied wines.

variations depend on the group of esters and even on individual esters within the same group. These results confirm the data in the literature dealing with the capacity of LAB to modulate the ester composition of wine.[54] Of all of the studied metabolites, the variability of bacterial impact on the composition of esters best reflects the variability of red wine fruity aroma alterations by MLF. It is all the more relevant as the overall variations of odorant esters (from −750 to 650 μg/L) can potentially affect wine aroma with regard to the central role played by esters in the perception of red wine fruity notes.[9,10] Indeed, although the measured variations were below the perception threshold for the esters, similar levels of concentrations can be perceived by a sensory additive effect[55] and synergistic effects between esters[10] and between esters and others compounds enhancing the flavor of esters,[9] as was probably the case for the M2 and M3 wines (Figure 3).

Three groups of esters can be discerned in terms of their contribution to the fruity aroma of red wines: ethyl branched acid esters, ethyl fatty acid esters, and higher alcohol acetates.

In the ethyl branched acid esters group, MLF significantly decreased the levels of ethyl phenylacetate and, instead, led to the synthesis of the three other esters from this group (ethyl isobutyrate, ethyl 2-methylbutyrate, ethyl isovalerate), although this was not significant and decreases in the concentrations were also measured (Table 2). Ethyl phenylacetate bacterial degradations probably did not affect the fruity notes with regard to its perception threshold (73 μg/L).[56] On the other hand, in some cases, bacterial modulations of the three other ethyl branched acid esters could potentially have an impact on the fruity aroma according to Pineau et al.[10] These esters are derived from the catabolism of amino acids such as valine, leucine, isoleucine, and phenylalanine. However, branched acid ethyl esters are mainly synthesized during wine aging by esterification with ethanol from the corresponding branched acids.[57] The quantification of these acids revealed a trend of increase during MLF in certain branched acids, especially for 2-methylbutyric acid with a significant bacterial synthesis demonstrated for the first time (Table 2). This regular synthesis of 2-methylbutyric acid did not necessarily lead to the formation of the corresponding ester. Thereby, esterification by esterase activities of LAB could be the limiting factor of ethyl branched acid ester biosynthesis during MLF. Furthermore, these different trends have the effect of reducing the ester/acid ratio, promoting a stronger synthesis of ethyl branched acid esters during wine aging and thus, later, the development of fruity notes.

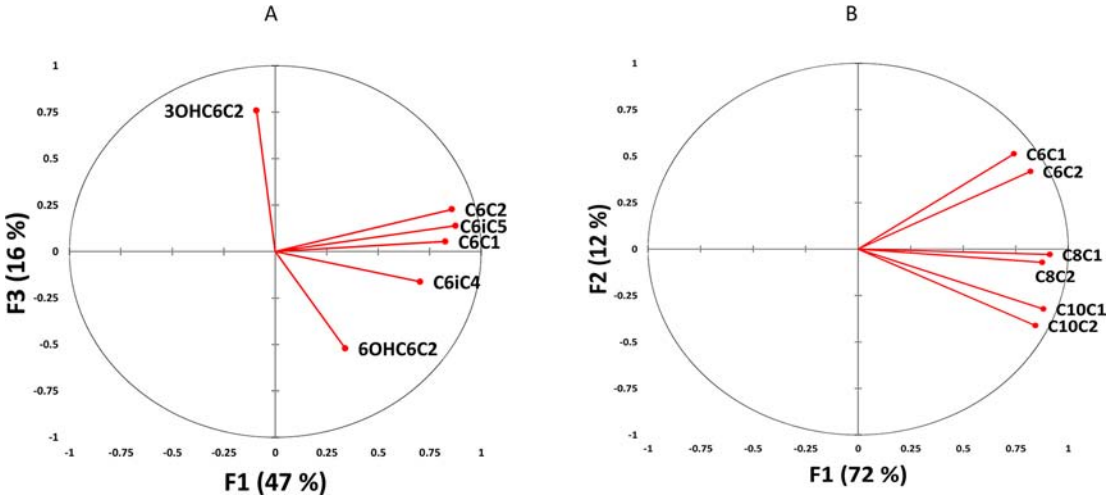

Figure 5. Principal component analysis on the variations for the hexanoic acid esters in 45 wines (A) and variations for the methyl and ethyl fatty acid esters in 48 wines (B). The 2D projections are performed on the (3 × 1) plane for PCA-A and on the principal plane for PCA-B. C6C2, ethyl hexanoate; C6C1, methyl hexanoate; C6iC5, isoamyl hexanoate; C6iC4, isobutyl hexanoate; 3OHC6C2, ethyl 3-hydroxyhexanoate; 6OHC6C2, ethyl 6-hydroxyhexanoate; C8C2, ethyl octanoate; C8C1, methyl octanoate; C10C2, ethyl decanoate; C10C1, methyl decanoate.

Fatty acid ethyl esters and higher alcohol acetates are fermentative compounds mainly produced by yeast activity, but they may also be metabolized by LAB. They have a central role in the perception of wine fruity aroma, but fatty acid ethyl esters seem to contribute more than higher alcohol acetates to aromatic bacterial modifications in red wines. In the cases of wines M2 and M3, the enhancement of fruity notes was linked to increases in ethyl fatty acid ester contents, whereas higher alcohol acetate concentrations either strongly decrease or stabilize ethyl fatty acid ester contents (Figure 3 and Table 3).

All of the trends were observed for higher alcohol acetate variations during MLF except for phenylethyl acetate, which was weakly affected by LAB activities (Table 2). These results are not in accordance with a recent study displaying a trend to hydrolysis[58] but confirm other previous studies.[54,59]

On the other hand, although all of the trends were also measured for ethyl fatty acid esters, MLF seems instead to increase the concentration of this group overall, which is in agreement with the latest studies.[58,59] However, a PCA used to examine the variations in the different esters during MLF revealed that bacterial alterations of wine ester composition depended not only on the type of acid but also on the length of its carbon chain (Figure 5). In the case of PCA-A, the projection of the 2D (1 × 3) plane, representing 63% of the total variance, separated the ethyl hexanoic acid ester group from the hydroxylated hexanoic acid ester group, indicating the importance of the nature of the acid in the bacterial modulation of esters. Conversely, in the case of PCA-B, the projection of the 2D principal plane, displaying 84% of the total variance, separated the ethyl and methyl fatty acid esters on the basis of the length of the carbon chain of the corresponding acids. Furthermore, medium-chain fatty acid esters are more greatly affected by bacterial activity than short-chain fatty acids. Indeed, greater variations were observed during MLF in the ethyl octanoate and ethyl decanoate concentrations than in the concentrations of ethyl propionate, ethyl butyrate, and even ethyl hexanoate (Table 2). Moreover, unlike short-chain fatty acid esters, wine LAB tend to synthesize medium-chain fatty acid esters (Table 2). These trends were observed not only for fatty acid ethyl esters with even and odd numbers of carbon atoms but also for methyl fatty acid esters and isoamyl fatty acid esters (Table 2).

These results contradict some data available in the literature. Wine LAB esterase activities have a stronger affinity for short-chain substrates (acetate and butyrate)[60] with a regulation system probably similar to that of yeast.[60−62] In this case, the limiting factor is the availability of the substrate.[62] This hypothesis implies that the preferential substrates for ester synthesis by wine LAB are probably not simple fatty acids. Indeed, with respect to the concentration of fatty acids and ethyl fatty acids esters in wine,[63] the stronger affinity of LAB for short-chain substrates[60] would result in the bacterial synthesis of short-chain fatty acid esters, which contradicts the previously observed results. Other authors suggested that fatty acid ethyl esters might be synthesized in wine by LAB via alcoholysis with glycerides and without cofactors, as in most dairy LAB.[64] This is particularly relevant to wine, as the aqueous environment and high availability of ethanol increase transferase activity.[61] Moreover, the presence of glycerides in wine has already been reported by several authors.[65,66] The higher levels of glycerides with a longer carbon chain in wines increase their availability compared to shorter carbon chain substrates, which is in agreement with the glycerides hypothesis.[67]

However, in the case of bacterial acetate synthesis, acid substrates are unlikely to be glycerides because glycerin acetates are artificial compounds not present in wines. On the other hand, alcohol transferase activity from acetyl-CoA might be possible. Acetyl-CoA is a key metabolic compound in microorganisms such as LAB, which are already known to contribute to the biosynthesis of several metabolites such as fatty acid and lipids.[1] Furthermore, this pathway has also been documented for esters in other microorganisms such as yeast.[54]

All of these results show that bacterial modulations of the composition of wine esters depend not only on esterase activities linked to the strain of LAB but also on the composition in wine substrates after AF. To determine which is the most influential factor, a comparison was carried out between the variability in the concentrations variations measured for three strain effect studies and two matrix effect studies. The strain effect studies were carried out by inoculating the same wine with different strains of LAB, whereas the matrix

effect studies consisted of inoculating different wines with the same strain. Although LAB strains certainly contribute to the tremendous variability of MLF impact on wine ester composition, the major impacting factor is the composition of wines post-AF (Figure 6). These results confirm recent

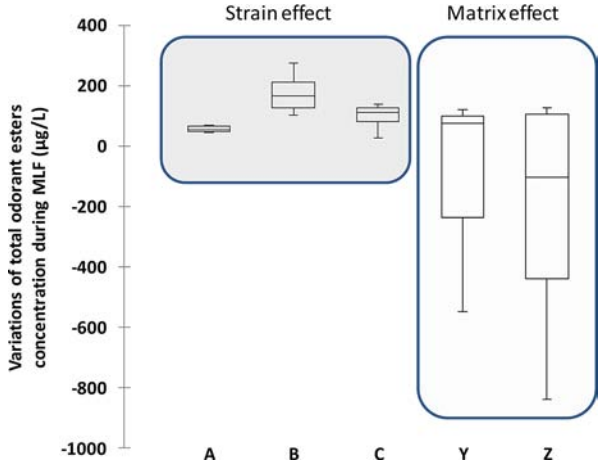

Figure 6. Comparison of variations in odorant ester concentrations during MLF in strain-effect studies (A, B, C) and matrix-effect studies (Y, Z). Studies A, B, and C were, respectively, carried out with five, six, and five starters. Studies Y and Z were, respectively, performed in three and four wines.

studies emphasizing the importance of the chemical parameters of wines on the modulation of metabolites during MLF.[59,68,69] The post-AF wine composition partly depends on the substrates released by the yeast. Thus, the interactions between yeast and LAB play a key role in the modification of ester composition and aroma in wine during MLF. This was confirmed by recent studies concerning the impact of the timing of inoculation with LAB on secondary metabolites and wine aroma.[58,68,70]

In summary, our study shows that the fruity aroma of red wines is widely affected by MLF, in the short term as well as in the longer term, but that all trends are possible. This variability reflects the complexity of bacterial metabolic activities that occurred during MLF. It was suggested that MLF produces an olfactive mask that affects the fruity notes in red wines, but, contrary to the empirical theories, it is not a "lactic mask" but probably rather a smoked/toasted reduction-like mask note that will have to be characterized by further experiments. However, the modification of the composition of the fruity note markers in red wines by LAB seems to be predominant. Whereas β-glycosidase activities of LAB are weakly involved in these variations, conversely, esterase seems to play a key role with, in some cases, other activities, which have been revealed for the first time, having an impact on sulfur-containing compounds such as thiols and DMS at a lower level. A quantification of numerous esters also allowed us to highlight new data on the metabolism of this group of metabolites in LAB present in wines and, thus, opens new directions of research on bacterial activities that occur during MLF and have a wide impact on the fruity aroma. Finally, the importance of the composition of wines after AF on the bacterial variations in metabolites was used to emphasize the key role of the interactions between yeast and LAB on the aromatic alterations produced by MLF. In the future, overall metabolomic approaches dealing with these complex interactions should lead to a better understanding of these phenomena at the roots of the sensorial and biochemical modifications of wine aroma during MLF. Besides, other bacterial enzymatic activities not discussed in the present study, such as tannase, could have an indirect role in the aromatic modification of red wines during MLF. Different studies respectively reported the presence of tannase enzymes in wine LAB and the importance of the nonvolatile matrix in the aromatic perception of red wines.[71,72] The impact of LAB on the nonvolatile matrix will also have to be investigated to establish links with the studies focusing on the interactions between the volatile and nonvolatile matrices of wines.

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REFERENCES

(1) Ribéreau-Gayon, P.; Dubourdieu, D.; Donèche, B.; Lonvaud, A. In *Handbook of Enology. Microbiology of Wine and Vinification*, 5th ed.; Wiley: Chichester, UK, 2000; Vol. 2.

(2) de Revel, G. *Le diacétyle, les composés dicarbonylés et leurs produits de réactions dans les vins*. Thesis 190, University Bordeaux II, 1992.

(3) Pripis-Nicolau, L.; de Revel, G.; Bertrand, A.; Lonvaud-Funel, A. Methionine catabolism and production of volatile sulphur compounds by *Oenococcus œni*. *J. Appl. Microbiol.* **2004**, *96*, 1176−1184.

(4) McDaniel, M.; Henderson, L. A.; Watson, B. T.; Heatherbell, D. Sensory panel training and screening for descriptive analysis of the aroma of pinot noir wine fermented by several strains of malolactic bacteria. *J. Sens. Stud.* **1987**, *2*, 149−167.

(5) Henick-Kling, T.; Acree, T. E.; Krieger, S.; Laurent, A.; Edinger, W. D. Modification of wine flavor by malolactic fermentation. *Wine East* **1994**, *4*, 8−15. and 29−30.

(6) Laurent, A.; Hening-Kling, T.; Acree, T. E. Changes in the aroma and odour of chardonnay wine due to malolactic fermentation. *Vitic. Enol. Sci.* **1994**, *49*, 3−10.

(7) Sauvageot, F.; Vivier, P. Effects of malolactic fermentation on sensory properties of four Burgundy wines. *Am. J. Enol. Vitic.* **1997**, *48*, 187−192.

(8) Boido, E.; Medina, K.; Farina, L.; Carrau, F.; Versini, G.; Dellacassa, E. The effect of bacterial strain and aging on the secondary volatile metabolites produced during malolactic fermentation of tannat red wine. *J. Agric. Food Chem.* **2009**, *57*, 6271−6278.

(9) Escudero, A.; Campo, E.; Farina, 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.* **2007**, *55*, 4501−4510.

(10) 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.* **2009**, *57*, 3702−3708.

(11) 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 micro-extraction-gas chromatography-mass spectrometry. *Food Chem.* **2010**, *121*, 1236−1245.

(12) Antalick, G. *Biochemical and Sensorial Modifications of the Fruity Aroma of Red Wines during Malolactic Fermentation: Specific Role of Esters*. Thesis 1792, University Bordeaux II, 2010; http://www.theses.fr/2010BOR21792.
(13) Gindreau, E.; Joyeux, A.; de Revel, G.; Claisse, O.; Lonvaud-Funel, A. Evaluation de l'établissement des levains malolactiques au sein de la microflore bactérienne indigène. *J. Int. Sci. Vigne Vin* **1997**, *31*, 197−202.
(14) International methods of analysis of wines and must OIV (International Organisation of Vine and Wine); http://www.oiv.int/oiv/info/enmethodesinternationalesvin?lang=en.
(15) de Revel, G.; Pipris-Nicolau, L.; Barbe, J. C.; Bertrand, A. The detection of α-dicarbonyl compounds in wine by formation of quinoxaline derivates. *J. Sci. Food Agric.* **2000**, *80*, 102−108.
(16) Anocibar Beloqui, A.; Kotseridis, Y.; Bertrand, A. Détermination de la teneur en sulfure de diméthyle dans quelques vins rouges. *J. Int. Sci. Vigne Vin* **1996**, *30*, 167−170.
(17) Mateo-Vivaracho, L.; Cacho, J.; Ferreira, V. Improved solid-phase extraction procedure for the isolation and in-sorbent pentafluorobenzyl alkylation of polyfunctional mercaptans. *J. Chromatogr. A* **2008**, *1185*, 9−18.
(18) Rodríguez-Bencomo, J. J.; Schneider, R.; Lepoutre, J. P.; Rigou, P. Improved method to quantitatively determine powerful odorant volatile thiols in wine by headspace solid-phase microextraction after derivatization. *J. Chromatogr. A* **2009**, *1216*, 5640−5646.
(19) Bartowsky, E. J.; Henschke, P. A. The buttery attribute of wine-diacetyl-desirability, spoilage and beyond. *Int. J. Food Microbiol.* **2004**, *96*, 235−252.
(20) Rankine, B. C.; Fornachon, J. C. M.; Bridson, D. A. Diacetyl in Australian dry red wines and its significance in wine quality. *Vitis* **1969**, *8*, 129−134.
(21) Nielsen, J. C.; Richelieu, M. Control of flavor development in wine during and after malolactic fermentation by *Oenococcus oeni*. *Appl. Environ. Microbiol.* **1999**, *65*, 740−745.
(22) Keim, H.; de Revel, G.; Bertrand, A. Instrumental and sensory evaluation of malolactic fermentation wine. In*Flavour research at the dawn of the Twenty-first Century*; Proceedings of the 10th Weurman Flavour Research Symposium, Beaune, France, 2002; Tec & Doc: Cachan, 2003, pp 401−404.
(23) Ugliano, M.; Kolouchova, R.; Henschke, P. A. Occurrence of hydrogen sulfide in wine and in fermentation: influence of yeast strain and supplementation of yeast available nitrogen. *Aust. J. Grape Wine Res.* **2011**, *38*, 423−429.
(24) Siebert, T.; Bramley, B.; Solomon, M. Hydrogen sulfide: aroma detection threshold study in white and red wines. *Technol. Rev.* **2009**, *183*, 14−16.
(25) Swiegers, J. H.; Bartowsky, E. J.; Henschke, P. A.; Pretorius, I. S. Yeast and bacterial modulation of wine aroma and flavour. *Austr. J. Grape Wine Res.* **2005**, *11*, 139−173.
(26) Boido, E.; Medina, K.; Carrau, F.; 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.* **2002**, *50*, 2344−2349.
(27) Ugliano, M.; Genovese, A.; Moio, L. Hydrolysis of wine aroma precursors during malolactic fermentation with four commercial starter cultures of *Oenococcus oeni*. *J. Agric. Food Chem.* **2003**, *51*, 5073−5078.
(28) Hernandez-Orte, P.; Cersosimo, M.; Loscos, N.; Cacho, J.; Garcia-Moruno, E.; Ferreira, V. Aroma development from non-floral grape precursors by wine lactic acid bacteria. *Food Res. Int.* **2009**, *42*, 773−781.
(29) Silva Ferreira, A. C.; Falqué, E.; Castro, M.; Oliveura e Silva, H.; Machado, B.; Guedes de Pinho, P. Identification of key odorants related with high quality Touriga Nacional wine. *Dev. Food Sci.* **2006**, *43*, 217−220.
(30) Kotseridis, Y. *Etude de l'arôme des vins de Merlot et Cabernet-Sauvignon de la région bordelaise*. Thesis 652, University Bordeaux II, 1999.
(31) Fazzalari, F. A., Ed. *Compilation of Odor and Taste Threshold Values Data*; American Society for Testing and Materials: Philadelphia, PA, 1978.
(32) Pineau, B.; Barbe, J. C.; Van Leeuwen, C.; Dubourdieu, D. Which impact for β-damascenone on red wines aroma? *J. Agric. Food Chem.* **2007**, *55*, 4103−4108.
(33) Ugliano, M.; Moio, L. The influence of malolactic fermentation and *Oenococcus oeni* strain on glycosidic aroma precursors and related volatile compounds of red wine. *J. Sci. Food Agric.* **2006**, *86*, 2468−2476.
(34) Ugliano, M.; Moio, L. Changes in the concentration of yeast-derived volatile compounds of red wine during malolactic fermentation with four commercial starter cultures of *Oenococcus oeni*. *J. Agric. Food Chem.* **2005**, *53*, 10134−10139.
(35) Nakamura, S.; Crowell, E. A.; Ough, C. S.; Totsuka, A. Quantitative analysis of γ-nonalactone in wines and its threshold determination. *Food Sci.* **1988**, *53*, 1243−1244.
(36) 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.* **2007**, *55*, 6674−6684.
(37) Segurel, M. A.; Baumes, R.; Langlois, D.; Riou, C.; Razungles, A. Role of glycosidic aroma precursors on the odorant profiles of Grenache noir and Syrah wines from the Rhone valley. Part 2: Characterisation of derived compounds. *J. Int. Sci. Vigne Vin* **2009**, *43*, 213−223.
(38) Abeijón Mukdsi, M. C.; Medina, R. B.; Alvarez, M. F.; González, S. N. Ester synthesis by lactic acid bacteria isolated from goat's and ewe's milk and cheeses. *Food Chem.* **2009**, *117*, 241−247.
(39) López, R.; Ezpeleta, E.; Sánchez, I.; Cacho, J.; 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.* **2004**, *88*, 95−103.
(40) Wanikawa, A.; Hosoi, K.; 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.* **2000**, *58*, 51−56.
(41) Murat, M. L. *Recherches sur la Vinification des Vins Rosés et Clairets de Bordeaux*; Université Bordeaux 2: Bordeaux, France, 2001.
(42) de Revel, G. Les composés aromatiques produits par les bactéries lactiques: impact sur l'arôme des vins. *Personal communication in Proceedings of the 6th Scientific Technical Day of Bordeaux Wine Council,* Saint-Germain-de-la riviére, France, 2005.
(43) Tominaga, T.; Niclass, Y.; Frérot, E.; Dubourdieu, D. Stereoisomeric distribution of 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate in dry and sweet white wines made from *Vitis vinifera* (var. Sauvignon blanc and Semillon). *J. Agric. Food Chem.* **2006**, *54*, 7251−7255.
(44) Blanchard, L. *Recherche sur la contribution de certains thiols volatils à l'arôme des vins rouges. Etude de leur genèse et de leur stabilité*. Thesis, Université Bordeaux II, 2000.
(45) Tominaga, T.; Peyrot des Gachons, C.; Dubourdieu, D. A new type of flavor precursors in *Vitis vinifera* L. cv. Sauvignon blanc: *S*-cysteine conjugates. *J. Agric. Food Chem.* **1998**, *46*, 5215−5219.
(46) Thibon, C.; Marullo, P.; Claisse, O.; Cullin, C.; Dubourdieu, D.; Tominaga, T. Nitrogen catabolic repression controls the release of volatile thiols by *Saccharomyces cerevisae* during wine fermentation. *FEMS Yeast Res.* **2008**, *8*, 1076−1086.
(47) Knoll, C.; Du Toit, M.; Schnell, S.; Rauhut, D.; Irmler, S. Cloning and characterisation of a cystathionine β/γ-lyase from two *Oenococcus oeni* oenological strains. *Appl. Microbiol. Biotechnol.* **2011**, *89*, 1051−1060.
(48) Alting, A. C.; Engels, W. J. M.; van Schalkwijk, S.; Exterkate, F. A. Purification and characterization of cystathionine β-lyase from *Lactococcus lactis* subsp. *cremoris* B78 and its possible role in flavor development in cheese. *Appl. Environ. Microbiol.* **1995**, *61*, 4037−4042.
(49) Segurel, M. A.; Razungles, A.; Riou, C.; Salles, M.; Baumes, R. Ability of possible DMS precursors to release DMS during wine aging

and in the conditions of heat-alkaline treatment. *J. Agric. Food Chem.* **2005**, *53*, 2637−2645.
(50) de Mora, S. J.; Eschenbruch, R.; Knowles, S. J.; Spedding, D. J. The formation of dimethyl sulphide during fermentation using a wine yeast. *Food Microbiol.* **1986**, *3*, 27−32.
(51) Osborne, J. P.; Mira de Orduña, R.; Pilone, G. J.; Liu, S. Q. Acetaldehyde metabolism by wine lactic acid bacteria. *FEMS Microbiol. Lett.* **2000**, *191*, 51−55.
(52) Pipris-Nicolau, L. *Volatile sulfur compounds in wine and metabolism of lactic acid bacteria*. Thesis, University Bordeaux II, 2002.
(53) Landaud, S.; Helinck, S.; Bonnarme, P. Formation of volatile sulfur compounds and metabolism of methionine and other sulfur compounds in fermented food. *Appl. Microbiol. Biotechnol.* **2008**, *77*, 1191−1205.
(54) Sumby, K. M.; Grbin, P. R.; Jiranek, V. Microbial modulation of aromatic esters in wine: current knowledge and future prospects. *Food Chem.* **2010**, *121*, 1−16.
(55) Cometto-Muniz, J. E.; Cain, W. S.; Abraham, M. H. Odour detection of single chemicals and binary mixtures. *Behav. Brain Res.* **2005**, *156*, 115−123.
(56) Tat, L.; Battistutta, F.; Comuzzo, P.; Zironi, R. Ethyl Phenylacetate as the probable responsible of honey-like character in Aglianico del Vulture wine. *Acta Hortic.* **2007**, *754*, 557−562.
(57) Diaz-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.* **2005**, *53*, 3503−3509.
(58) Bartowsky, E. J.; Costello, P. J.; McCarthy, J. MLF-adding an 'extra dimension' to wine flavour and quality. *Aust. N. Z. Grapegrower Winemaker* **2008**, *533a*, 60−65.
(59) Knoll, C.; Fritsch, S.; Scnell, S.; Grossmann, M.; Rauhut, D.; Du Toit, M. Influence of pH and ethanol on malolactic fermentation and volatile aroma compound composition in white wines. *LWT−Food Sci. Technol.* **2011**, *44*, 2077−2086.
(60) Matthews, A.; Grbin, P. R.; Jiranek, V. Biochemical characterisation of the esterase activities of wine lactic acid bacteria. *Appl. Microbiol. Biotechnol.* **2007**, *77*, 329−337.
(61) Holland, R.; Liu, S. Q.; Crow, V. L.; Delabre, M. L.; Lubbers, M.; Bennett, M.; Norris, G. Esterases of lactic acid bacteria and cheese flavour: milk fat hydrolysis, alcoholysis and esterification. *Int. Dairy J.* **2005**, *15*, 711−718.
(62) Saerens, S. M. G.; Verstrepen, K. J.; Van Laere, S. D. M.; Voet, A. R. D.; Van Dijck, P.; Delvaux, F. R.; Thevelein, M. The *Saccharomyces cerevisiae* EHT1 and EEB1 genes encode novel enzymes with medium-chain fatty acid ethyl ester synthesis and hydrolysis capacity. *J. Biol. Chem.* **2006**, *281*, 4446−4456.
(63) Torres-Allegre, V. M. *Formation des acides gras et autres produits secondaires au cours de la vinification: interprétation statistique des résultats*. Thesis, Université Bordeaux II, 1982.
(64) Liu, S. Q.; Holland, R.; Crow, V. Ester synthesis in an aqueous environment by *Streptococcus thermophilus* and other dairy lactic acid bacteria. *Appl. Microbiol. Biotechnol.* **2003**, *63*, 81−88.
(65) Gallender, J. F.; Peng, A. C. Lipid and fatty acid compositions of different grape types. *Am. J. Enol. Vitic.* **1980**, *31*, 24−27.
(66) Pueyo, E.; Martinez-Rodriguez, A.; Polo, M. C.; Santa-Maria, G.; Bartolomé, B. Release of lipids during yeast autolysis in a model wine system. *J. Agric. Food Chem.* **2000**, *48*, 116−122.
(67) Trotton, D.; Charpentier, M.; Robillard, B.; Calvayrac, R.; Duteurtre, B. Evolution of the lipid contents of Champagne wine during the second fermentation of *Saccharomyces cerevisae*. *Am. J. Enol. Vitic.* **1989**, *40*, 175−182.
(68) Abrahamse, C. E.; Bartowsky, E. J. Timing of malolactic fermentation inoculation in Shiraz grape must and wine: influence on chemical composition. *World J. Microbiol. Biotechnol.* **2011**, *28*, 255−265.
(69) 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 strain *Oenococcus oeni* strain and wine matrix composition. *Austr. J. Grape Wine Res.* **2012**, *18*, 287−301.
(70) Antalick, G.; Perello, M. C.; de Revel, G. Changes in wine secondary metabolites composition by the timing of inoculation with lactic acid bacteria: impact on wine aroma. In *Proceedings of the 3rd International Symposium MACROWINE 2010 on Macromolecules and Secondary Metabolites in Grapevine and Wines*; Universita di Torin: Torino, Italy, 2010; pp 143−148.
(71) Matthews, A.; Grbin, P. R.; Jiranek, V. A survey of lactic acid bacteria for enzymes of interest to oenology. *Aust. J. Grape Wine Res.* **2006**, *12*, 235−244.
(72) Sáenz-Navajas, M. P.; Fernández-Zurbano, P.; Ferreira, V. Contribution of non-volatile matrix to wine flavour. *Food Rev. Int.* **2012**, *28*, 389−411.