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## The Effect of Bacterial Strain and Aging on the Secondary Volatile Metabolites Produced during Malolactic Fermentation of Tannat Red Wine

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During malolactic fermentation (MLF), lactic acid bacteria influence aroma and flavor of wines by the production of volatile metabolites and the modification of aroma compounds derived from grapes and yeasts. In an effort to isolate these bacteria properties as advantages for winemaking, this study aimed to assess the relative contribution of two aspects: the effects of lactic acid bacteria activity on the volatiles compounds in Tannat wines and the consequences of aging in bottle on aroma compounds produced during MLF. To our knowledge, this is the first report related to the effect of wine aging in bottle on the aroma chemical compounds produced by MLF. Solid phase extraction complemented with chromatographic techniques was used to study the wine aroma compounds. A sensory evaluation of the wines was also performed through descriptive methods. We demonstrated modifications in the concentration of acetates, ethyl esters, and other secondary metabolites during MLF. Major sensorial differences between wines that had undergone MLF were also noted. In addition, some modifications detected in the composition of Tannat wines as a consequence of the aging in bottle contributed to the change in differences between wines with and without MLF and furthermore between strains. These changes probably influence its fruity character.

**KEYWORDS:** Malolactic fermentation; *Oenococcus oeni*; Tannat wine; aroma; aging

### INTRODUCTION

In wines, malolactic fermentation (MLF) results in the bio-conversion of malic acid to lactic acid and carbon dioxide by lactic acid bacteria. Certain strains of *Oenococcus oeni* commonly predominate due to their tolerance and adaptation to high acid and alcohol contents in wine (1–3). MLF is a desirable step for red wine aging, and also for certain white wines, as it results in benefits such as deacidification and improving the biological stability (1, 2). The commercial introduction of bacterial cultures for wine inoculation has improved the control of MLF, and their use has become popular among winemakers for the prevention of problems associated with spontaneous MLF (4, 5).

Malolactic bacteria may also influence wine aroma and flavor by various mechanisms, including the production of volatile secondary metabolites and the modification of grape and yeast derived metabolites (1, 6). The aromatic impact of bacterial activity can be variable depending on the wine type (7–9).

The flavor attributes can also vary depending on the particular strain of malolactic organism employed (6, 10, 11). Therefore, it is important to continue to study new starter cultures to not only improve their performance but also to evaluate their influence on wine quality.

Of the various reaction products produced during MLF, only those with lactic or butter-like odors, such as diacetyl and other carbonyl compounds, have been well studied (12, 13). The nature and quantity of other compounds associated with lactic acid bacteria activity in wine remain largely unknown. In recent years, the use of gas chromatography (GC), gas chromatography–mass spectrometry (GC-MS), gas chromatography olfactometry (GCO), and multidimensional gas chromatography (MDGC) have made possible the study of variations on the volatile fraction of wines, produced as a consequence of MLF (8, 14–17). However, the overall effects of MLF on volatile secondary metabolites of wine are not fully understood as a consequence of the use of different base wines and MLF strains. In addition, the evolution of these changes during aging has not been evaluated.

The present research complements previous experiments carried out on *Vitis vinifera* cv. Tannat, the most important red wine in Uruguayan viticulture. Strategies to improve Tannat wine quality using advanced viticultural and wine-making technology (18) started with the analytical characterization of its complex flavor profile and polyphenol composition (7, 19–22). This variety is one of the richest in polyphenol content of red *Vitis vinifera* but with moderate intensity aromas which are described as raspberry, plum, quince, and small-berry-like scents (7, 23). Understanding the effect of MLF on this variety may allow

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improved management of this process for increasing the intensities of certain desirable aromas.

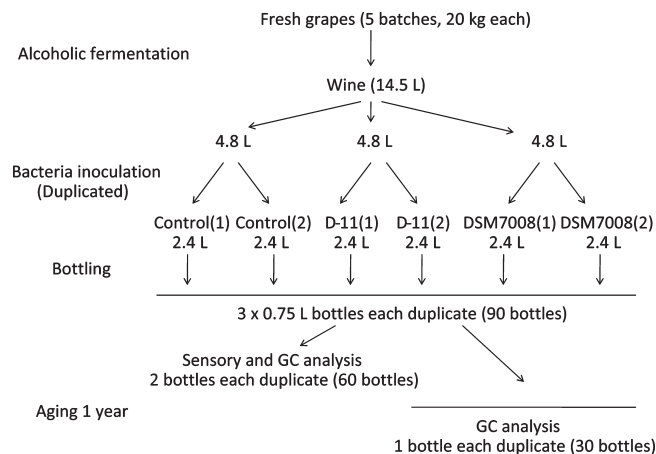
We studied the influence of MLF on the minor volatile compounds and on the final wine aroma profile. In particular, we considered the influence of two different commercial *Oenococcus oeni* strains and the aroma modifications produced during wine aging. To our knowledge, this is the first report related to the effect of wine aging in bottle on the aroma chemical compounds produced by MLF.

## MATERIALS AND METHODS

**Wine Making.** Fresh grapes ripeness (*Vitis vinifera* L. cv. Tannat) were sourced from local vineyards (Canelones, Uruguay) and delivered in the morning to winemaking facilities. Five batches from different vineyards (20 kg each), of the 2005 vintage, were microvinified. Grapes were destemmed and crushed. A subsample of each batch was analyzed for sugar content, total acidity, and pH. Sulfite was added to the must (50 mg/L), which was then inoculated with reactivated dry yeast (*Saccharomyces cerevisiae*, strain CIVC 8130; Gist Brocades, Chile). Fermentation was carried out at 22–25 °C, and then wine was separated from the skins and pressed (maximum pressure, 1.2 bar). The free run wine and press wine were combined (approximately total volume per batch, 14.5 L). Upon completion of alcoholic fermentation (sugar content below 2 g/L), each batch was divided into three equal portions (4.8 L each) without filtration. Two of the three portions were inoculated in duplicate (2.4 L each) with two different *Oenococcus oeni* strains for MLF (D-11, Malolactine O, Groupe Oeno-France, France, and DSM 7008, Viniflora oenos, Chr. Hansen's, Horsholm, Denmark). To the third portion, 50 mg/L of sulfite was added to suppress MLF while it was kept under conditions similar to those applied to the inoculated samples. A 200 mL aliquot of wine was kept as a control (no sulfite addition) to monitor that MLF did not occur spontaneously. All samples were held at 18 °C during the inoculated samples were finished MLF. This was followed by malic and lactic acid concentrations by thin-layer chromatography (24). Plating of samples on malo lactic basal (MLB) medium (25), solidified with agar (30 g/L), and supplemented with cycloheximide (100 mg/L) to inhibit yeast growth was performed during MLF in order to verify if any additional microorganisms were present. For each wine sample, 50 mg/L of sulfite were added immediately upon completion of MLF. All samples were stabilized at 4 °C for 20 days, and free sulfite content was corrected to 35 mg/L. Samples were finally sterile-filtered (0.45 µm cellulose acetate membrane) and bottled using conventional glass bottles of 0.75 L, stopped with natural cork (45 mm × 24 mm). After bottling, part of the wine was analyzed, and the other part of the bottles was conserved in a horizontal position at 18 °C and 75% humidity, without direct light, to be analyzed after one year of aging. For clarification of the experimental plan, Figure 1 presents a flowchart of the process including the volumes of wine for each step. The entire design included 90 bottles; 60 of them were submitted to sensory and GC analysis after bottling, while 30 were aged and analyzed by GC after one year.

**Strains, Media, and Cultivation.** *Oenococcus oeni* isolates used in this study were strain DSM 7008 (Viniflora oenos, Chr. Hansen) and strain D-11 (Malolactine O, Groupe Oeno-France). Dried bacterial preparations were inoculated into 5 mL of malo lactic basal (MLB) medium (25) and incubated for 4–5 days at 25 °C. Culture growth was monitored spectrophotometrically (600 nm) and expressed as dry weight using a calibration curve relating both parameters. These cultures formed the inoculum for subsequent experiments starting with an initial population of 10<sup>4</sup> CFU/mL.

**Wine Analysis.** All wine samples and controls were analyzed simultaneously after completion of MLF (malic acid content below 0.1 g/L). Total acidity and pH were measured according to usual methods (26). MLF development was followed during fermentation by thin layer chromatography (TLC) as previously reported by Boido et al. (24). For quantification purposes, malic and lactic acids were analyzed by reversed-phase HPLC using a technique adapted by us from a previous reported one for white wines (27) using equipment composed of a LC-10AT pump and a SPD-6AV UV-vis detector (Shimadzu Corp., Kyoto, Japan). Peak integration and quantitative calculations were performed on a C-R3A integrator using a calibration curve obtained with standards for each acid. The column was a Beckman Ultrasphere ODS C<sub>18</sub> (150 mm × 4.6 mm i.d.,



**Figure 1.** Experimental design to investigate the effect of MLF and aging in bottle on the aroma compounds. The design consisted in five vinifications of Tannat grape batches, each one submitted to MLF treatment (control without MLF and MLF with two different strains, D-11 and DSM7008) by duplicate. Wine aroma analyses were performed after bottling and one year aging. A flowchart of the process, including the wines volumes for each step, is shown.

particle size 5 µm), the mobile phase was 0.005 M sulfuric acid, the flow rate 0.6 mL/min, the injection volume 20 µL, and column temperature 20 °C. Detection was by UV absorbance at 214 nm.

**Isolation of Volatiles.** The isolation of volatile compounds was performed by adsorption and elution from a Isolute ENV+ cartridge (IST Ltd., Mid Glamorgan, UK) packed with 1 g of highly cross-linked SDVB (styrene-divinyl benzene) polymer (40–140 µm, cod. no. 915-0100-C) as previously reported (20).

**GC Analysis.** Each sample was analyzed by GC on a Shimadzu GC 14 B gas chromatograph equipped with FID and a Shimadzu data processor with EZ-Chrom software, using a Carbowax 20 M (Ohio Valley, USA) bonded fused-silica capillary column (25 m × 0.32 mm i.d.), coated with polyethylene glycol (0.25 µm phase thickness). The experimental conditions were: column temperature, 40 °C for 8 min, rising to 180 at 3 °C/min, then to 230 at 20 °C/min; injector temperature, 250 °C; detector temperature, 250 °C; injection mode, split; split ratio, 1:30; volume injected, 1.0 µL. Carrier gas was hydrogen at 30 kPa.

**GC-MS Analysis.** GC-MS analyses were conducted using a Shimadzu QP 5050 mass spectrometer equipped with reference libraries (28, 29), using a Carbowax 20 M capillary column (Ohio Valley, USA) as described above. The experimental conditions were: injector temperature, 250 °C; injection mode, split; split ratio, 1:40; volume injected, 1.0 µL. Carrier gas was He at 92.6 kPa (55.9 cm/s); interface temperature, 250 °C; energy, 70 eV; acquisition mass range 40–400 amu.

**Identification and Quantification.** The wine aroma components were identified by comparison of their linear retention indices with pure standards supplied by Aldrich (Milwaukee, WI) and Fluka (Buchs, Switzerland) or data reported in the literature. Comparison of mass spectral fragmentation patterns with those stored on databases (28, 29) was also performed. In those cases where the pure reference compounds were not used, the identification was indicated as tentative. GC-FID and GC-MS instrumental procedures using an internal standard (1-heptanol) were applied for quantitative purposes as previously described (30) and evaluated by Carlin (31).

**Sensory Analysis.** A vocabulary of descriptive terms for Tannat wine was devised by a group of three oenologists who selected 27 tertiary tier descriptors from the Wine Aroma Wheel of Noble et al. (32). Eight judges from the sensory panel of the Faculty of Chemistry, with no previous experience in wine evaluation, were trained to recognize that descriptors as follows: (1) recognition of simple aromas prepared either by using Le Nez du Vin aroma standards (33), or by soaking the different components in a 12% alcohol solution (7), (2) recognition of simple aromas in a neutral red wine with added standards (7), and (3) recognition and evaluation of aroma intensity using a 10 point structured scale (0 = none; 1 = threshold;

9 = extreme). This was achieved using commercially available Tannat wines plus experimental wines.

In our experimental design, wine samples without conservation were evaluated in duplicate by each of the eight panelists, followed by a balanced complete block (7). The wines were presented in individual testing booths with "normal daylight" illumination. Samples of 60 mL were served at  $20 \pm 1$  °C in clear, tulip-shaped wine glasses (approximately 250 mL), identified with two-digit random codes and covered with a watch glass. Two samples were evaluated for aroma characteristics during each session. Panelists were required to rate tertiary tier terms using the 10 point structured scale (0 = none; 1 = threshold; 9 = extreme).

**Statistical Analysis.** For free volatile components, variance analyses were performed with the model: wine (five vinifications), MLF (control and MLF with different strains) and aging time (after MLF and one year of aging), with interaction MLF  $\times$  aging time, wine  $\times$  MLF and wine  $\times$  aging time. Principal component analyses (PCA) were carried out on the value for each free volatile compound of the 30 wines. The analyses were conducted using the correlation matrix with varimax rotation.

For the sensory analysis, mean rating and least-significant differences for each term were calculated from an analysis of variance with the model: replicates (two), judges (eight), wines (five vinifications), and MLF (three treatments, control and MLF with different strains).

Variance analyses, least-significant differences test, and PCA were performed using the software Statistica 7.1 (StatSoft, Tulsa, OK, 1984–2005).

## RESULTS AND DISCUSSION

**Chemical Composition of Wines.** All the inoculated samples completed malolactic fermentation. The duration of MLF was shorter for the samples inoculated with DSM 7008 strain (12–22 days) compared with the samples inoculated with D-11 strain (21–36 days) (Table 1). Chemical compositions of Tannat wines from different treatment showed the expected differences (1, 2) in acidity, pH, and malic and lactic acids contents (Table 1).

**Modification of Free Volatile Compounds by MLF: Effect of the *O. oeni* Strain.** Thirty-seven volatile components, including alcohols, esters, carbonyl compounds, and acids, were identified and quantified. Analysis of variance (ANOVA) of the free aroma compounds showed that the five different Tannat wines included in this study presented variations in the level of most of the compounds measured. The ANOVA showed significant differences in the MLF effect for 17 compounds at a significance level of  $p < 0.001$  and eight with minor significance level (Table 2). The mean values and standard deviation, for the volatile compounds in the wine samples (control and with MLF) and for the selected strains, are presented in Table 3. The absence of a wine  $\times$  MLF interaction for all the compounds studied (data not shown), eliminated the possible existence of winemaking artifacts, derived from oxidative effects or produced by other microorganisms, which might be the origin of the observed differences. Moreover, colony and cell morphology observed in the MLB plated samples confirmed the absence of additional microorganisms in the fermented samples.

After MLF, an increase in ethyl lactate was found for both *O. oeni* strains (Table 3) as was expected (2, 6, 15). Moreover, this increase was higher for the D-11 strain (Table 3), indicating a different behavior of strains in contrast to previous reports (10).

In our experimental conditions, the production of other esters (e.g., isobutyl, isoamyl, hexyl, and 2-phenylethyl acetate, and ethyl hexanoate) which contribute for pleasant fruity notes, showed significant differences for MLF effect, and these changes are strain dependent. The hexyl acetate contents decreased after MLF for the two strains studied (Table 3). Contradictory results have been reported for the concentration of this compound, Davis et al. (2) reported a decrease while an increase was reported by others (8). Of the other identified esters, isoamyl, isobutyl, and 2-phenylethyl acetate had different behaviors related to the strain being considered, with increased values for strain D-11, and

**Table 1.** Chemical Analysis of Wines Produced from Tannat Grapes after Malolactic Fermentation<sup>a</sup>

wine <sup>b</sup>	treatment <sup>c</sup>	total acidity (g of H <sub>2</sub> SO <sub>4</sub> /L)	pH	malic acid (g/L)	lactic acid (g/L)	duration of MLF (day)
1	control	4.6	3.34	3.0	0.3	NA <sup>d</sup>
	D-11	3.7	3.45	<0.1	2.3	25
	DSM 7008	3.4	3.47	<0.1	2.5	20
2	control	5.2	3.28	2.0	0.2	NA
	D-11	4.4	3.38	<0.1	1.5	36
	DSM 7008	4.3	3.37	<0.1	1.2	22
3	control	5.5	3.42	2.7	0.1	NA
	D-11	4.6	3.58	<0.1	2.1	28
	DSM 7008	4.5	3.52	<0.1	1.8	12
4	control	4.5	3.38	2.5	0.2	NA
	D-11	3.6	3.48	<0.1	1.9	30
	DSM 7008	3.7	3.45	<0.1	2.0	15
5	control	4.8	3.45	2.3	0.1	NA
	D-11	3.9	3.57	<0.1	1.6	21
	DSM 7008	3.7	3.59	<0.1	1.4	13

<sup>a</sup> Values are the mean of two replicates. <sup>b</sup> Wine produced from each grape batch.

<sup>c</sup> Control, wine sample without MLF; D-11 and DSM 7008, wine sample with MLF (strain D-11 or DSM 7008). <sup>d</sup> NA, not applicable.

decreases in the case of isobutyl acetate for DSM 7008 (Table 3). For ethyl hexanoate, strain D-11 showed a significant decrease ( $p < 0.01$ ), but the differences by the MLF effect were not significant for ethyl octanoate and decanoate (Table 2). However, the ethyl octanoate and octanoic acid levels, the first without significant differences, in the wines inoculated with strain D-11, were higher than in the other wines (Table 3), suggesting a possible metabolic characteristic of this strain. The different *O. oeni* responses found in our experimental conditions for the production of acetates and aliphatic ethyl esters could help to explain conflicting information previously reported (2, 8, 10, 17).

Significant increases of diethyl succinate and  $\gamma$ -butyrolactone contents were observed after MLF only when strain D-11 was used (Table 3). An increment in  $\gamma$ -butyrolactone was previously reported by Maicas et al. (34) in four *O. oeni* strains and one *Lactobacillus*. Differences in these compounds, produced through the  $\alpha$ -ketoglutarate metabolism of lactic acid bacteria by a specific  $\alpha$ -ketoglutaric decarboxylase and resulting in the corresponding ethyl esters as end products (35), could indicate differences in the intensity of this metabolic pathway for the different strains studied.

After MLF, several alcohols presented higher concentration values than in the initial wine (Table 3) as reported by Pozo-Bayón et al. (36), but in our experimental conditions, the sum of alcohols content did not show significant differences. Increments in the concentration of 2-phenylethanol and 3-methylthio-1-propanol observed in the samples inoculated with the D-11 strain, with significance level  $p < 0.001$  for the second compound, could also indicate metabolic differences between strains. This result was in agreement with previous reports where significant differences for several alcohols, depending on the strain of *Oenococcus oeni* used, have been observed when using synthetic media (37) and wine (15).

For C<sub>6</sub> compounds, only 1-hexanol differed significantly ( $p < 0.05$ ) with an increase in concentrations observed for D-11 strain. Total concentration of the C<sub>6</sub> alcohols, associated with the green aroma perception in wine, did not significantly change after MLF.

For the volatile phenols 4-vinyl guaiacol and 4-vinyl phenol, significant increases in the concentration were observed only for



**Table 2.** Significance Level in the Analysis of Variance for the Different Compounds<sup>a</sup>

	identity assignment <sup>b</sup>	wine <sup>c</sup>	MLF	time	MLF×time
Acetates					
isobutyl acetate	A	NS	***	***	*
isoamyl acetate	A	**	***	***	*
hexyl acetate	A	NS	***	**	***
2-phenylethyl acetate	A	***	*	***	NS
<b>acetates sum</b>	<b>A</b>	<b>**</b>	<b>***</b>	<b>***</b>	<b>NS</b>
Esters					
ethyl hexanoate	A	*	***	NS	NS
ethyl octanoate	A	**	NS	*	NS
ethyl decanoate	A	**	NS	***	NS
<b>ethyl esters sum</b>		<b>***</b>	<b>NS</b>	<b>*</b>	<b>NS</b>
ethyl lactate	A	NS	***	***	***
ethyl 3-hydroxybutyrate	A	***	*	*	NS
ethyl 4-hydroxybutyrate	B	**	*	***	NS
diethyl succinate	A	NS	***	***	**
ethyl succinate	B	NS	NS	***	NS
diethyl malate	A	NS	***	***	***
Alcohols					
2-methyl-1-propanol	A	***	**	NS	NS
1-butanol	A	**	NS	***	NS
2- and 3-methyl-1-butanol	A	***	NS	***	NS
4-methyl-1-pentanol	B	**	***	*	NS
3-methyl-1-pentanol	B	***	NS	***	NS
benzyl alcohol	A	**	NS	NS	NS
2-phenylethanol	A	***	*	**	NS
<b>alcohols sum</b>		<b>***</b>	<b>NS</b>	<b>***</b>	<b>NS</b>
C6 Compounds					
1-hexanol	A	***	*	**	NS
<i>trans</i> -3-hexen-1-ol	A	NS	NS	NS	NS
<i>cis</i> -3-hexen-1-ol	A	***	NS	NS	NS
<i>trans</i> -2-hexen-1-ol	B	**	NS	***	NS
<b>C6 compounds sum</b>		<b>***</b>	<b>NS</b>	<b>**</b>	<b>NS</b>
Acids					
2-methylpropanoic (isobutyric) acid	B	***	***	NS	NS
2 and 3-methylbutanoic (isovaleric) acids	B	***	NS	***	NS
butanoic acid	B	*	***	NS	NS
hexanoic acid	A	**	**	**	NS
octanoic acid	A	**	**	**	NS
decanoic acid	B	*	NS	**	NS
<b>acids sum</b>		<b>**</b>	<b>***</b>	<b>*</b>	<b>NS</b>
Other Compounds					
diethyl 2-hydroxyglutarate	B	*	NS	***	NS
4-carboethoxy- $\gamma$ -butyrolactone	B	NS	NS	**	NS
$\gamma$ -butyrolactone	A	***	***	***	*
3-methylthio-1-propanol	B	***	***	NS	NS
2-hydroxy-3,3-dimethyl- $\gamma$ -butyrolactone (pantolactone)	B	NS	***	NS	NS
4-vinylguaiacol	A	NS	***	NS	NS
4-vinylphenol	A	NS	***	NS	NS

<sup>a</sup> Wine, MLF, and time of conservation effect, with interaction, on the volatile compounds of the samples. <sup>b</sup> A, identities confirmed by comparing mass spectra and retention time with those of authentic standards; B, identities tentatively assigned by comparing mass spectra with those obtained from literature (28, 29). <sup>c</sup> \*, \*\*, \*\*\* indicate significance at  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ , respectively; NS, nonsignificant differences.

the DSM 7008 strain (Table 3). These vinyl phenols can be released by  $\beta$ -glycosidase activity (19) and formed through

enzymatic decarboxylation of phenolic acid precursors, namely *p*-coumaric acid for 4-vinylphenol and ferulic acid for 4-vinylguaiacol (38). Moreover, occurrence of an inducible cinnamate decarboxylase potentially responsible for this transformation has been reported in *O. oeni* by Cavin et al. (39). These authors, state that permeabilized cells of *O. oeni* growing in synthetic media with phenolic acids were able to carry out the decarboxylation of these acids. Finally, other authors (38) working with wines from *V. vinifera* Aglianico found the release of both vinylphenols by some strains of *O. oeni*.

4-Ethyl guaiacol and 4-ethyl phenol were not detected in any of the different samples studied.

Of the acids, butanoic and isobutyric acids had significant differences ( $p < 0.01$ ), with an increase in the samples inoculated with the D-11 strain.

The D-11 strain also showed a significant increase in panto-lactone production.

**Modification of the Free Volatile Compounds as Consequence of MLF and Aging Effects.** A large number of compounds showed variations following bottle storage, most of them with significance level  $p < 0.001$ , as reported in Table 2. However, the absence of a wine×aging time interaction for all the compounds studied (data not shown) allowed us to demonstrate the absence of variations due to artifacts produced, for example, by the bottle closure.

In our experimental conditions, we observed a marked increase, during aging, in the ethyl lactate level. This increase showed a strong interaction between the factors MLF and conservation time ( $p < 0.001$ ). The difference between the control wine and the wines that had undergone MLF is more pronounced one year after the completion of the MLF, with very high levels for wines with MLF (Table 2 and Table 3).

Table 4 presents the minimal and maximal odor activity values for the five wines in each treatment. Only those compounds with values greater or equal to 1, at least in one wine for any of the treatments, are presented. The odor activity value for ethyl lactate was superior to one for samples with MLF (both strain) after 1 year of aging in bottle (Table 4). Although the rise in ethyl lactate was also detected after one year in wines of other grape varieties subjected to chemical esterification (40), the higher concentration of lactic acid obtained as a consequence of the role of MLF provoked an increase of ethyl lactate not previously discussed by other authors.

On the other hand, a significant increase for diethyl malate after one year of aging was found in control wines but not in MLF wines (significant interaction between MLF and conservation time,  $p < 0.001$ ), resulting in an increase in the chemical differences between control and MLF wines (Table 2 and Table 3). This behavior was not surprising because of the presence of malic acid and ethanol in the medium.

The level of other esters contributing to wine odor, such as the aliphatic ethyl esters and acetates, decreased after one year of aging in bottle (Table 3), as was previously reported for white wines (40, 41). The changes for some acetate were higher in the control wine than in the wine with MLF, as it is shown in the odor activity values of Table 4. Furthermore, Table 2 shows significant levels of the interaction between MLF and aging time in the variance analysis. These changes, probably as a consequence of higher acidity in control wines, determined that some significant differences in the concentration found for these compounds could disappear during aging in bottle.

Although the ester content decreases during aging, Table 3 shows that alcohol and acid levels in the samples after one year of bottle storage did not show an increase.

Finally, during aging of all samples, both control and post MLF, the levels of  $\gamma$ -butyrolactone and diethyl succinate in-

**Table 3.** Mean Concentration of the Five Wines with Two Replicates for Each Treatment ( $\pm$  Standard Deviation) in  $\mu\text{g/L}$  Equivalents of *n*-Heptanol of Free Volatile Compounds<sup>a</sup>

	control after bottle	MLF DSM7008 after bottle	MLF D-11 after bottle	control 1 year	MLF DSM7008 1 year	MLF D-11 1 year
Acetates						
isobutyl acetate	737 $\pm$ 67 d	482 $\pm$ 106 c	932 $\pm$ 32 e	261 $\pm$ 36 ab	194 $\pm$ 7 a	432 $\pm$ 65 bc
isoamyl acetate	1081 $\pm$ 107 b	1059 $\pm$ 102 b	1603 $\pm$ 197 c	284 $\pm$ 27 a	288 $\pm$ 28 a	451 $\pm$ 74 a
hexyl acetate	43 $\pm$ 9 b	3 $\pm$ 2 a	1.8 $\pm$ 0.4 a	9 $\pm$ 4 a	4.5 $\pm$ 2 a	1.2 $\pm$ 0.3 a
2-phenylethyl acetate	156 $\pm$ 29 b	149 $\pm$ 46 b	245 $\pm$ 73 c	70 $\pm$ 9 a	73 $\pm$ 12 a	120 $\pm$ 18 ab
<b>acetate sum</b>	<b>2017 <math>\pm</math> 204 b</b>	<b>1693 <math>\pm</math> 246 b</b>	<b>2781 <math>\pm</math> 244 c</b>	<b>625 <math>\pm</math> 72 a</b>	<b>559 <math>\pm</math> 42 a</b>	<b>1003 <math>\pm</math> 101 ab</b>
Esters						
ethyl hexanoate	480 $\pm$ 51 c	495 $\pm$ 160 c	166 $\pm$ 38 ab	451 $\pm$ 49 c	374 $\pm$ 30 bc	150 $\pm$ 27 a
ethyl octanoate	187 $\pm$ 21 ab	195 $\pm$ 69 ab	335 $\pm$ 140 b	123 $\pm$ 11 a	130 $\pm$ 8 a	202 $\pm$ 31 ab
ethyl decanoate	83 $\pm$ 10 ab	100 $\pm$ 21 b	91 $\pm$ 33 b	54 $\pm$ 2 ab	51 $\pm$ 3 ab	45 $\pm$ 11 a
<b>ethyl ester sum</b>	<b>750 <math>\pm</math> 82 b</b>	<b>790 <math>\pm</math> 248 b</b>	<b>593 <math>\pm</math> 211 ab</b>	<b>628 <math>\pm</math> 61 ab</b>	<b>555 <math>\pm</math> 40 ab</b>	<b>396 <math>\pm</math> 52 a</b>
ethyl lactate	960 $\pm$ 109 a	3022 $\pm$ 334 b	4892 $\pm$ 436 c	6256 $\pm$ 1093 c	118317 $\pm$ 11178 d	191579 $\pm$ 13491 e
ethyl 3-hydroxybutyrate	195 $\pm$ 26 ab	203 $\pm$ 24 ab	225 $\pm$ 31 b	163 $\pm$ 16 a	182 $\pm$ 17 ab	202 $\pm$ 23 ab
ethyl 4-hydroxybutyrate	3641 $\pm$ 444 c	3832 $\pm$ 671 c	4503 $\pm$ 641 c	873 $\pm$ 185 a	1594 $\pm$ 205 b	1972 $\pm$ 307 b
diethyl succinate	196 $\pm$ 17 a	193 $\pm$ 26 a	266 $\pm$ 41 b	1575 $\pm$ 57 c	1654 $\pm$ 102 c	2245 $\pm$ 145 d
ethyl succinate	14090 $\pm$ 623 a	15411 $\pm$ 1987 a	19226 $\pm$ 3256 a	44543 $\pm$ 14187 b	41050 $\pm$ 4084 b	49937 $\pm$ 2690 b
diethyl malate	152 $\pm$ 27 a	171 $\pm$ 42 a	149 $\pm$ 27 a	1077 $\pm$ 111 b	114 $\pm$ 16 a	103 $\pm$ 4 a
Alcohols						
2-methyl-1-propanol	7855 $\pm$ 816 b	5937 $\pm$ 777 a	6469 $\pm$ 785 ab	6792 $\pm$ 548 ab	5999 $\pm$ 564 a	6557 $\pm$ 558 ab
1-butanol	360 $\pm$ 54 ab	391 $\pm$ 65 ab	469 $\pm$ 131 b	238 $\pm$ 17 a	229 $\pm$ 16 a	252 $\pm$ 53 a
2- and 3-methyl-1-butanol	162170 $\pm$ 19889 b	162509 $\pm$ 24293 b	169553 $\pm$ 36722 b	113338 $\pm$ 10840 a	121159 $\pm$ 10956 a	122903 $\pm$ 18609 a
4-methyl-1-pentanol	42 $\pm$ 10 ab	61 $\pm$ 6 ab	168 $\pm$ 49 c	27 $\pm$ 5 a	33 $\pm$ 5 a	93 $\pm$ 30 b
3-methyl-1-pentanol	199 $\pm$ 30 b	200 $\pm$ 38 b	193 $\pm$ 46 b	120 $\pm$ 19 a	113 $\pm$ 12 a	106 $\pm$ 13 a
benzyl alcohol	62 $\pm$ 15 a	80 $\pm$ 18 ab	81 $\pm$ 19 ab	110 $\pm$ 8 b	74 $\pm$ 9 ab	78 $\pm$ 17 ab
2-phenylethanol	35876 $\pm$ 6026 ab	38159 $\pm$ 8387 ab	46125 $\pm$ 10411 b	23084 $\pm$ 1403 a	28820 $\pm$ 3366 a	34778 $\pm$ 4301 ab
<b>alcohols sum</b>	<b>206564 <math>\pm</math> 26226 b</b>	<b>207336 <math>\pm</math> 32788 b</b>	<b>223059 <math>\pm</math> 47564 b</b>	<b>143708 <math>\pm</math> 12641 a</b>	<b>156426 <math>\pm</math> 14199 a</b>	<b>164768 <math>\pm</math> 22582 a</b>
C6 Compounds						
1-hexanol	1009 $\pm$ 117 b	973 $\pm$ 122 ab	1258 $\pm$ 230 c	806 $\pm$ 68 a	792 $\pm$ 34 a	998 $\pm$ 99 b
<i>trans</i> -3-hexen-1-ol	26 $\pm$ 3	28 $\pm$ 8	22 $\pm$ 7	23 $\pm$ 4	32 $\pm$ 4	25 $\pm$ 4
<i>cis</i> -3-hexen-1-ol	55 $\pm$ 7	48 $\pm$ 7	49 $\pm$ 10	48 $\pm$ 2	53 $\pm$ 2	52 $\pm$ 4
<i>trans</i> -2-hexen-1-ol	57 $\pm$ 7 c	54 $\pm$ 4 bc	55 $\pm$ 8 bc	32 $\pm$ 1 a	39 $\pm$ 6 ab	38 $\pm$ 4 ab
<b>C6 compounds sum</b>	<b>1147 <math>\pm</math> 134 ab</b>	<b>1103 <math>\pm</math> 140 ab</b>	<b>1385 <math>\pm</math> 251 b</b>	<b>909 <math>\pm</math> 75 a</b>	<b>915 <math>\pm</math> 36 a</b>	<b>1113 <math>\pm</math> 103 ab</b>
Acids						
2-methylpropanoic (isobutyric acid)	603 $\pm$ 84 a	662 $\pm$ 121 ab	1044 $\pm$ 193 c	625 $\pm$ 42 a	627 $\pm$ 62 a	984 $\pm$ 95 bc
2 and 3-methylbutanoic (isovaleric acids)	837 $\pm$ 116 b	832 $\pm$ 102 b	879 $\pm$ 100 b	676 $\pm$ 86 a	697 $\pm$ 59 a	742 $\pm$ 66 ab
butanoic acid	380 $\pm$ 40 a	386 $\pm$ 61 a	1065 $\pm$ 182 b	336 $\pm$ 20 a	341 $\pm$ 7 a	928 $\pm$ 31 b
hexanoic acid	1326 $\pm$ 123 ab	1270 $\pm$ 169 ab	1740 $\pm$ 308 c	933 $\pm$ 51 a	1079 $\pm$ 51 ab	1434 $\pm$ 100 bc
octanoic acid	1378 $\pm$ 188 ab	1399 $\pm$ 312 ab	2087 $\pm$ 467 c	856 $\pm$ 34 a	1063 $\pm$ 105 a	1584 $\pm$ 156 b
decanoic acid	90 $\pm$ 31 b	108 $\pm$ 48 b	90 $\pm$ 42 b	39 $\pm$ 10 a	30 $\pm$ 3 a	23 $\pm$ 4 a
<b>acids sum</b>	<b>4614 <math>\pm</math> 531 ab</b>	<b>4657 <math>\pm</math> 760 ab</b>	<b>6904 <math>\pm</math> 1214 c</b>	<b>3466 <math>\pm</math> 201 a</b>	<b>3836 <math>\pm</math> 169 ab</b>	<b>5696 <math>\pm</math> 279 bc</b>
Other Compounds						
diethyl 2-hydroxyglutarate	322 $\pm$ 58 a	372 $\pm$ 107 a	340 $\pm$ 59 a	927 $\pm$ 115 b	806 $\pm$ 161 b	773 $\pm$ 57 b
4-carboethoxy- $\gamma$ -butyrolactone	120 $\pm$ 9 a	229 $\pm$ 66 ab	249 $\pm$ 30 ab	371 $\pm$ 54 c	277 $\pm$ 51 bc	333 $\pm$ 38 bc
$\gamma$ -butyrolactone	506 $\pm$ 94 a	593 $\pm$ 163 a	1374 $\pm$ 301 b	695 $\pm$ 69 a	948 $\pm$ 167 ab	2176 $\pm$ 226 c
3-methylthio-1-propanol	1352 $\pm$ 225 ab	1388 $\pm$ 285 ab	2081 $\pm$ 404 c	1118 $\pm$ 117 a	1270 $\pm$ 123 ab	1924 $\pm$ 176 c
2-hydroxy-3,3-dimethyl- $\gamma$ - butyrolactone (pantolactone)	85 $\pm$ 14 a	102 $\pm$ 30 a	249 $\pm$ 60 b	92 $\pm$ 10 a	122 $\pm$ 11 a	328 $\pm$ 31 b
4-vinylguaiaicol	9 $\pm$ 2 a	39 $\pm$ 18 b	11 $\pm$ 5 a	10 $\pm$ 2 a	61 $\pm$ 10 b	19 $\pm$ 2 a
4-vinylphenol	7 $\pm$ 1 a	63 $\pm$ 15 b	7 $\pm$ 1 a	6 $\pm$ 3 a	84 $\pm$ 11 b	11 $\pm$ 2 a

<sup>a</sup> Significant differences ( $p < 0.05$ ) according to the LSD test are indicated by using different letters.

creased (Table 3), which was also reported in other studies (40). In our experimental conditions, higher contents for these compounds were observed in the samples in which MLF was performed, a behavior that could be explained by the presence of high levels of products from the  $\alpha$ -ketoglutarate metabolism.

This was the case for ethyl 4-hydroxybutyrate, a product of  $\alpha$ -ketoglutarate metabolism, whose content decreased significantly between samples analyzed after MLF and after aging of 1 year.

Other compounds, such as ethyl succinate and diethyl 2-hydroxyglutarate, also showed increments during aging

**Table 4.** Minimal and Maximal Odor Activity Values for the Five Wines in Each Treatment<sup>a</sup>

	threshold ( $\mu\text{g/L}$ )	reference <sup>a</sup>	descriptor	control after bottle	MLF DSM7008 after bottle	MLF D-11 after bottle	control 1 year	MLF DSM7008 1 year	MLF D-11 1 year
isobutyl acetate	73	1	fruit, banana, apple, pear	7–12	4–10	4–10	2–4	2–2	3–7
isoamyl acetate	30	2	banana, pear	29–46	27–45	27–63	5–11	6–11	6–19
hexyl acetate	20	1	berries, pears, fruity	1–3	0	0	0	0	0
2-phenylethyl acetate	250	3	rose, honey, tobacco	0	0–1	0–1	0	0	0
ethyl hexanoate	14	2	apple, fruit	27–45	13–65	6–19	25–42	18–30	5–15
ethyl octanoate	5	2	fruit	27–49	16–75	16–92	19–31	20–29	20–55
ethyl lactate	60000	4	strawberry, raspberry	0	0	0	0	1–2	2–3
3-methyl-1-butanol	30000	2	whiskey, malt, burned	4–7	3–7	3–8	2–4	2–5	2–5
2-phenylethanol	14000	2	honey, spices, rose	1–3	1–4	1–5	1–1	1–2	1–3
2-methylpropanoic (isobutyric) acid	50	5	rancid, butter, cheese	8–17	7–20	11–31	10–14	10–17	17–27
2 and 3-methylbutanoic (isovaleric) acids	33	2	sweat, acid, rancid	18–35	18–33	20–35	11–26	15–26	15–27
butanoic acid	173	2	rancid, cheese, sweat	1–2	1–2	3–8	1–2	1–2	4–5
hexanoic acid	420	2	sweat, acid, rancid	2–3	2–3	2–5	1–2	2–2	2–4
octanoic acid	500	2	sweat, cheese	2–3	1–4	2–6	1–1	1–2	2–3
3-methylthio-1- propanol	1000	2	sweet, potato	0–1	0–2	1–3	0–1	0–1	1–2
4-vinylguaiacol	40	3	clove, curry	0	0–2	0	0	0–2	0

<sup>a</sup> Only those compounds with values greater or equal to 1, at least in one wine for any of the treatments, are presented. <sup>a</sup> References: 1, the threshold determination was performed in water (43); 2, the matrix was an 11% water/ethanol solution containing 7 g/L glycerol, 5 g/L tartaric acid, pH adjusted to 3.4 with 1 M NaOH (44); 3, the mixture was a 10% solution in ethanol (45); 4, determinate in red wine (16); 5, the matrix was water (46).

(Table 3) for both control and post MLF samples, as described for other wine varieties (40, 41).

As an overview of the results, the PCAs of aroma compounds rated the 30 samples as presented in Figure 2. The first three PCs explain 77.3% of the variance (46.9%, 18.3%, and 12.1%, for PC1, PC2, and PC3, respectively). As shown in Figure 2, these PC's separated the samples with different treatments. Samples without aging and aged for one year were separated by a diagonal between the two first PCs, contrasting diethyl succinate, ethyl succinate, diethyl 2-hydroxyglutarate, 4-carboethoxy- $\gamma$ -butyrolactone, and ethyl lactate, with ethyl esters and acetates. The control samples, without MLF, were positioned at negative PC2 values (hexyl acetate and ethyl hexanoate presenting higher loading over this axis with negative values). After aging, these control samples were located at a position similar to that of the loading of diethyl malate. Samples where MLF was performed with D-11 strain and particularly those aged, were displaced over the PC3 axis ( $\gamma$ -butyrolactone and butanoic and 2-methylpropanoic acids presented the higher loading on this axis).

In summary, the PCA analysis allowed to demonstrate the role of aroma compounds in the separation of the different groups of wine samples studied.

**Sensory Modification of the Samples by MLF Fermentation.** The descriptors with significant differences in the sensory analysis of the samples, control and post MLF (without bottle aging), are shown in Figure 3. The replicate and wine effects were not significant for all the sensory descriptors, while the judge effect showed significant differences for some of these descriptors (Figure 3). The samples inoculated with DSM 7008 strain resulted in significant decreases of “raspberry”, “black currant”, “apricot”, “green pepper”, and “cut grass” descriptors, and increases of “quince”, “butter”, “coffee”, and “musk” ones. The samples inoculated with D-11 strain resulted in decreases of “cherry”,

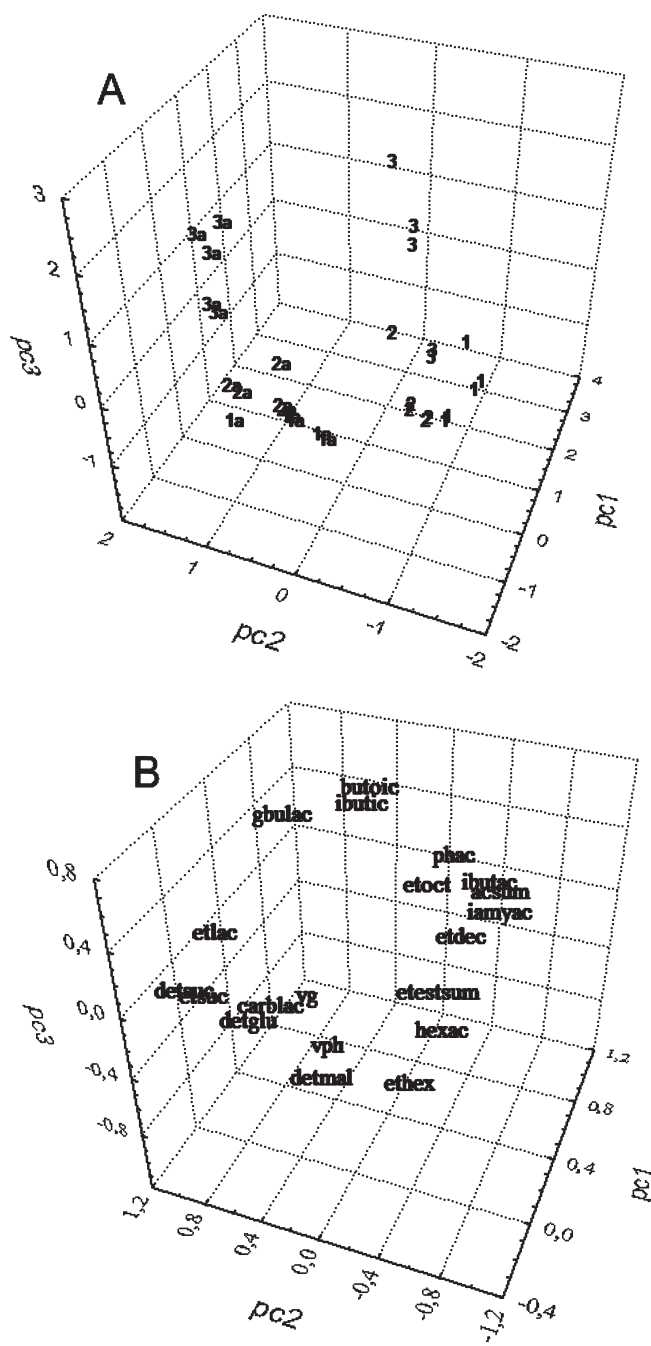
“dried fig”, and “plum”, and increases of “apricot”, “cut grass”, “butter”, and “yeasty” descriptors.

The differences of some fruity descriptors between the control and MLF samples could be correlated with the decrease in the odor activity values of some esters (Table 4). This is the case of the decrease for the “raspberry” descriptor in the treatment with strain DSM 7008 which correlated with isoamyl acetate, ethyl hexanoate, and ethyl ester sum (correlation coefficient 0.82, 0.83, and 0.85, respectively). The decrease for the “cherry” and “dried fig” descriptors in the treatment with strain D-11, which correlated with ethyl ester sum (correlation coefficient 0.83 and 0.84, respectively). Additionally, the differences found for the descriptor “apricot” in wines, produced by different strains, could be explained by the variations observed in the concentration of compounds related to fruit notes, such as isoamyl and hexyl acetates, ethyl octanoate and acetates sum (correlation coefficients 0.82, 0.88, 0.87, and 0.84 respectively). Because of the decrease of the odor activity values for acetates in the wines after conservation in bottle (Table 4), it is possible that sensory perception of fruit aroma decrease in these samples and some differences between control and MLF wines disappear.

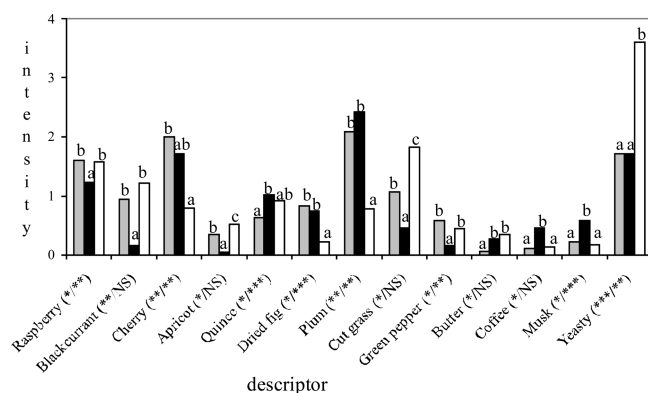
The increase in herbaceous notes such as “cut grass” in samples inoculated with D-11 strain could be explained as a consequence of the increase of pantolactone, acids (butanoic and 2-methylpropanoic), methionol, and  $\gamma$ -butyrolactone as reported for sherry wine (42).

The increase in the “butter” descriptor after MLF has been reported by numerous authors (12, 13) and assigned to the increase in diacetyl concentration, which was not considered in this work.

The “musk” notes, which showed an increase in the samples inoculated with DSM 7008 strain, could be a consequence of the higher vinylphenolic compounds contents produced by this strain



**Figure 2.** Principal component analysis of volatile compounds in five Tannat wines with different MLF treatments after bottling and one year aging. Plot representation of the samples, average of two replications, on the first three principal components (A) and factor loading (B). For a better comprehension, only those compounds with higher factor loadings were included. 1, samples control; 2, samples with malolactic fermentation (strain DSM 7008); 3, samples with malolactic fermentation (strain D-11); 1a, samples control, aging one year; 2a, samples with malolactic fermentation (strain DSM 7008), aging one year; 3a, samples with malolactic fermentation (strain D-11), aging one year. Abbreviations: ibutac, isobutyl acetate; iamyac, isoamyl acetate; phac, 2-phenylethyl acetate; hexac, hexyl acetate; acsum, acetates sum; ethex, ethyl hexanoate; etoct, ethyl octanoate; etdec, ethyl decanoate; etestsum, ethyl esters sum; butoic, butanoic acid; ibutic, isobutyric acid; gbulac,  $\gamma$ -butyrolactone; detmal, diethyl malate; etsuc, ethyl succinate; detsuc, diethyl succinate; etlac, ethyl lactate; vph, 4-vinylphenol; vg, 4-vinylguaiacol; carblac, 4-carboethoxy- $\gamma$ -butyrolactone; detglu, diethyl 2-hydroxyglutarate.



**Figure 3.** Mean of the sensory descriptors values with significant differences in the analysis of the wines with different MLF treatments, before aging. Control (gray filled square), MLF with DSM 7008 (black filled square) and D-11 (open square) strain. Treatments with significant differences in MLF effect are indicated with different letters. Code: NS, \*, \*\*, \*\*\* indicate not significant and significance at  $p < 0.05$ ,  $< 0.01$ ,  $< 0.001$ , respectively. The first superscript in the label of X axis refers to the significance of the differences in the MLF effect, the second to the differences in the judge effect.

(correlation coefficient 0.71). The increase in the “yeasty” descriptor was not related to increases in the odor activity values of the compounds studied.

Summarizing this work, we analyzed the concentration changes of volatile metabolites in Tannat wines produced during MLF using two different lactic acid bacterial strains. Some of these compounds are important for fruity aroma notes, which could explain the results obtained in the sensorial analysis, for example differences in “raspberry”, “cherry”, “apricot”, and “dried fig” descriptors in the different treatment wines. We also demonstrated the role of red wine aging on the aroma changes, in samples with and without MLF, which affect the aromatic compounds differently. The results showed a different decrease in some acetates and ethyl esters in wines after storage in bottle, influencing some fruity aromas. Finally, our results indicated that an aging period, after vinification, contributed to the change in differences between wines with and without MLF and furthermore between strains.

#### ABBREVIATIONS USED

HPLC, high performance liquid chromatography; GC, gas chromatography; FID, flame ionization detector; MS, mass spectrometry; MLF, malolactic fermentation; TLC, thin-layer chromatography.

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#### LITERATURE CITED

- Henick-Kling, T., Malolactic fermentation. In *Wine, Microbiology and Biotechnology*, Fleet, G. H., Ed.; Harwood Academic Publishers: Chichester, U.K., 1993; pp 289–326.
- Davis, C.; Wibowo, D.; Eschenbruch, R. E.; Lee, T. H.; Fleet, G. H. Practical implications of malolactic fermentation: a review. *Am. J. Enol. Vitic.* **1985**, *36*, 290–301.



- (3) Henschke, P. A. An overview of malolactic fermentation research. *Aus. N.Z. Wine Ind. J.* **1993**, *8*, 69–79.
- (4) Henick-Kling, T.; Park, Y. H. Considerations for the use of yeast and bacterial starter cultures: SO<sub>2</sub> and timing of inoculation. *Am. J. Enol. Vitic.* **1994**, *45*, 464–469.
- (5) Nielsen, J. C.; Prahl, C.; Lonvaud-Funel, A. Malolactic fermentation in wine by direct inoculation with freeze-dried *Leuconostoc oenos* cultures. *Am. J. Enol. Vitic.* **1996**, *47*, 42–48.
- (6) Bartowsky, E.; Henschke, P. Malolactic fermentation and wine flavour. *Aust. Grapegrower Winemaker* **1995**, *378a*, 83–94.
- (7) Gámbaro, A.; Boido, E.; Zlotajabko, A.; Medina, K.; Lloret, A.; Dellacassa, E.; Carrau, F. Effect of malolactic fermentation on the aroma properties of Tannat wine. *Aust. J. Grape Wine Res.* **2001**, *7*, 27–32.
- (8) Laurent, M.-H.; Henick-Kling, T.; Acree, T. E. Changes in the aroma and odor of Chardonnay wine due to malolactic fermentation. *Vit. Enol. Sci.* **1994**, *49*, 3–10.
- (9) Sauvageot, F.; Vivier, P. Effects of malolactic fermentation on sensory properties of four Burgundy wines. *Am. J. Enol. Vitic.* **1997**, *48*, 187–192.
- (10) Delaquis, P.; Cliff, M.; King, M.; Girard, B.; Hall, J.; 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.* **2000**, *51*, 42–48.
- (11) 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.
- (12) Martineau, B.; Henick-Kling, T. Performance and diacetyl production of commercial strains of malolactic bacteria in wine. *J. Appl. Bacteriol.* **1995**, *78*, 526–536.
- (13) Bartowsky, E. J.; Henschke, P. A. The “buttery” attribute of wine—diacetyl—desirability, spoilage and beyond. *Int. J. Food Microbiol.* **2004**, *96*, 235–252.
- (14) De Revel, G.; Martin, N.; Pripis-Nicolau, L.; Lonvaud-Funel, A.; Bertrand, A. Contribution to the knowledge of malolactic fermentation influence on wine aroma. *J. Agric. Food Chem.* **1999**, *47*, 4003–4008.
- (15) Fernandes, L.; Relva, A. M.; Gomes Da Silva, M. D. R.; Costa Freitas, A. M. Different multidimensional chromatographic approaches applied to the study of wine malolactic fermentation. *J. Chromatogr., A* **2003**, *995*, 161–169.
- (16) Lloret, A.; Boido, E.; Lorenzo, D.; Medina, K.; Carrau, F.; Dellacassa, E.; Versini, G. Aroma variation in Tannat wines: effect of malolactic fermentation on ethyl lactate level and its enantiomeric distribution. *Ital. J. Food Sci.* **2002**, *14*, 175–180.
- (17) 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.
- (18) Carrau, F. The emergence of a new Uruguayan wine industry. *J. Wine Res.* **1997**, *8*, 179–185.
- (19) Boido, E.; Lloret, A.; Medina, K.; Carrau, F.; Dellacassa, E. Effect of  $\beta$ -glycosidase activity of *Oenococcus oeni* on the glycosylated flavor precursors of Tannat wine during malolactic fermentation. *J. Agric. Food Chem.* **2002**, *50*, 2344–2349.
- (20) Boido, E.; Lloret, A.; Medina, K.; Fariña, L.; Carrau, F.; Versini, G.; Dellacassa, E. Aroma composition of *Vitis vinifera* cv. Tannat: the typical red wine from Uruguay. *J. Agric. Food Chem.* **2003**, *51*, 5408–5413.
- (21) Alcalde-Eon, C.; Boido, E.; Carrau, F.; Dellacassa, E.; Rivas-Gonzalo, J. Pigment profiles in monovarietal wines produced in Uruguay. *Am. J. Enol. Vitic.* **2006**, *57*, 449–459.
- (22) Boido, E.; Alcalde-Eon, C.; Carrau, F.; Dellacassa, E.; Rivas-Gonzalo, J. Aging effect on the pigment composition and color of *Vitis vinifera* L. Cv. Tannat wines. Contribution of the main pigment families to wine color. *J. Agric. Food Chem.* **2006**, *54*, 6692–6704.
- (23) Gámbaro, A.; Varela, P.; Boido, E.; Gimenez, A.; Medina, K.; Carrau, F. Aroma characterization of commercial red wines of Uruguay. *J. Sens. Stud.* **2003**, *18*, 353–366.
- (24) Boido, E.; Dellacassa, E.; Carrau, F.; Moyna, P. An improved means of monitoring malolactic fermentation in wines by TLC- densitometry. *J. Planar Chromatogr.—Modern TLC* **1999**, *12*, 269–271.
- (25) Cox, D. J.; Henick-Kling, T. Protonmotive force and ATP generation during malolactic fermentation. *Am. J. Enol. Vitic.* **1995**, *46*, 319–323.
- (26) Zoecklein, B., *Wine Analysis and Production*; Chapman & Hall: New York, 1995.
- (27) Benassi, M. T.; Cecchi, H. M. Method development for the simultaneous determination of carboxylic acids, phenolic compound and sorbic acid in white wines. *J. Liq. Chromatogr. Relat. Technol.* **1998**, *21*, 491–501.
- (28) McLafferty, F. W.; Stauffer, D. B., *The Wiley/NBS Registry of Mass Spectral Data*, 5th ed.; Wiley and Sons: New York, 1991.
- (29) Adams, R. P., *Identification of Essential Oils Components by Gas Chromatography/Mass Spectroscopy*, 4th ed.; Allured Publishing: Carol Stream, IL, 2007.
- (30) Versini, G.; Orriols, I.; Dalla Serra, A. Aroma components of Galician Albariño, Loureira and Godello wines. *Vitis* **1994**, *33*, 165–170.
- (31) Carlin, S. Metodi di arricchimento di composti dell’aroma per una possibile tipicizzazione di vini Traminer. Bachelor’s Thesis. Università degli Studi di Udine: Udine, Italy, **1998**.
- (32) Noble, A. C.; Arnold, R. A.; Buechsenstein, J.; Leach, E. J.; Schmidt, J. O.; Stern, P. M. Modification of a standardized system of wine aroma terminology. *Am. J. Enol. Vitic.* **1987**, *38*, 143–146.
- (33) Lenoir, J., *Le Nez du Vin*; Editions Jean Lenoir: Carnoux en Provence, France, 1990.
- (34) Maicas, S.; Gil, J. V.; Pardo, I.; Ferrer, S. Improvement of volatile composition of wines by controlled addition of malolactic bacteria. *Food Res. Int.* **1999**, *32*, 491–496.
- (35) Radler, F.; Bröhl, K. The metabolism of several carboxylic acids by lactic acid bacteria. *Z. Lebensm. Unters. Forsch. A* **1984**, *179*, 228–231.
- (36) Pozo-Bayón, M. A.; Alegría, E. G.; Polo, M. C.; Tenorio, C.; Martín-Alvarez, P. J.; Calvo De La Banda, M. T.; Ruiz-Larrea, F.; Moreno-Arribas, M. V. Wine volatile and amino acid composition after malolactic fermentation: Effect of *Oenococcus oeni* and *Lactobacillus plantarum* starter cultures. *J. Agric. Food Chem.* **2005**, *53*, 8729–8735.
- (37) Edwards, C. G.; Peterson, J. C. Sorbent extraction and analysis of volatile metabolites synthesized by lactic acid bacteria in synthetic medium. *J. Food Sci.* **1994**, *55*, 192–196.
- (38) 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.
- (39) Chatonnet, P.; Dubourdieu, D.; Boidron, J. N. The influence of *Brettanomyces/Dekkera* sp. yeasts and lactic acid bacteria on the ethylphenol content of red wines. *Am. J. Enol. Vitic.* **1995**, *46*, 463–468.
- (40) Pérez-Coello, M. S.; Martín-Álvarez, P. J.; Cabezo, M. D. Prediction of the storage time in bottles of Spanish white wines using multivariate statistical analysis. *Z. Lebensm. Unters. Forsch. A* **1999**, *208*, 408–412.
- (41) Pérez-Prieto, L. J.; López-Roca, J. M.; Gómez-Plaza, E. Differences in major volatile composition and quality of dry white table wines. *J. Food Compos. Anal.* **2003**, *16*, 697–705.
- (42) Peinado, R. A.; Moreno, J.; Medina, M.; Mauricio, J. C. Changes in volatile compounds and aromatic series in sherry wine with high gluconic acid levels subjected to aging by submerged flor yeast cultures. *Biotechnol. Lett.* **2004**, *26*, 757–762.
- (43) Segal, G. M.; Lewis, M. J.; Woskow, A. In *Evaluation of Beer Flavour Compounds*; American Society of Brewing Chemists: St. Paul, MN, 1967; pp 156–164.
- (44) Ferreira, V.; Lopez, R.; Cacho, J. Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agric.* **2000**, *80*, 1659–1667.
- (45) Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027–3032.
- (46) Van Gemert, L. J. *Compilations of Odour Threshold Values in Air, Water and Other Media*; Oliemans Punter & Partners BV: Utrecht, The Netherlands, 2003.

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