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Wine Volatile and Amino Acid Composition after Malolactic Fermentation: Effect of *Oenococcus oeni* and *Lactobacillus plantarum* Starter Cultures

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Red wine amino acids and volatile compounds were analyzed before and after malolactic fermentation carried out by four different starter cultures of the species *Oenococcus oeni* and *Lactobacillus plantarum.* The purpose of this study was to determine whether differences can be attributed to the lactic acid bacteria strain used in this important step of the wine-making process. The malolactic cultures selected for this study were indigenous wine lactic acid bacteria strains. The data were evaluated using different multivariate analysis techniques. Results showed different malolactic behaviors for *O. oeni* and *L. plantarum* and significant metabolic differences between both species. A degree of diversity was found within each lactic acid bacteria group, since wines presented specific characteristics depending on the lactic acid bacteria strain used. In all cases, malolactic fermentation seemed to modify the amino acid and volatile composition of the wine.

KEYWORDS: ***Oenococcus oeni; Lactobacillus plantarum*; wine; malolactic fermentation; amino acids; volatile compounds**

INTRODUCTION

Lactic acid bacteria (LAB) are widely used in food biotechnology, and efficient control of these microbiological processes requires an increase in the knowledge of bacterial behavior and metabolisms. LAB perform malolactic fermentation (MLF), one of the main phases of wine making. Different genera of LAB, including *Lactobacillus*, *Pediococcus*, and *Oenococcus*, are involved in this so-called secondary wine fermentation (*1*). Among them, *Oenococcus oeni* is recognized as the most tolerant to wine conditions (low pH and high ethanol concentration) and is the major bacteria species found in wines during MLF. Currently, a small number of commercial starter cultures of this species have been shown to successfully perform MLF, and further research into new starter cultures with defined technological and flavoring abilities is required. It has, recently, been demonstrated that *Lactobacillus plantarum* strains have resistance mechanisms that enable them to survive and proliferate in wine, and therefore, like *O. oeni*, they could also be used as starters for inducing MLF (*2*).

Wine deacidification is the main trigger for MLF and consists of the conversion of L-malic acid to L-lactic acid, resulting in a decrease in titratable acidity and a small increase in pH. MLF also leads to enhanced microbial stability and is usually believed to improve the complexity of the wine aroma (*3*−*5*). However, except for diacetyl, which is one of the main and most studied aroma byproducts of MLF (for a review see ref *6*), the influence of LAB on wine chemical and organoleptic properties is not yet fully understood.

Different studies have focused on the biosynthesis of aroma compounds during MLF and the concomitant organoleptic consequences (*7*, *8*). Maicas et al. (*9*) demonstrated that MLF noticeably changes major and minor volatile compounds that are beneficial to wine flavor during MLF. In contrast, Sauvageot and Vivier (*10*) conducted sensory analysis on Chardonnay and Pinot Noir wines with and without MLF and concluded that wine tasters perceived only slight sensory differences between the two types of wine. On the other hand, the influence of the LAB strain used for MLF on wine aroma composition and complexity is not well-known. Aroma/flavor attributes can vary according to the strain used for inducing MLF (*11*−*14*), whereas according to de Revel et al. (*15*) and Delaquis et al. (*16*), individual effects of the malolactic culture used to induce MLF are difficult to establish.

Different studies have shown that the levels of volatile compounds in wine are related to the yeast amino acid metabolism (*17*, *18*) and that the dynamics of indigenous and inoculated yeast populations have an effect on both sensory

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evaluation and the volatile composition of wines (*19*, *20*). On the other hand, other studies focused on *O. oeni* amino acid requirements for growth and MLF in several growth media (*21*). However, to date little attention has been paid to the influence of MLF on the whole amino acid composition of wine (*22*), and in particular, the effect of the LAB strain involved in MLF on this important wine nitrogenous fraction is still unknown.

The purpose of this work was to investigate the changes in wine volatile compounds and amino acids due to the MLF starter that triggered this important fermentation step and to determine whether differences can arise from the LAB species and/or LAB strain involved. Tempranillo is one of the most important Spanish red grape cultivars and is the principal variety of Rioja Appellation of Origin. MLF was induced in Tempranillo red wine with different malolactic cultures of indigenous LAB strains, and this was compared with wines elaborated with *O. oeni* and with *L. plantarum* selected strains.

MATERIAL AND METHODS

Wine Production. Red wine was elaborated from cv. Tempranillo red grapes from local vineyards of the northern Spanish region of La Rioja. Fermentations were carried out in 15 000-L wooden tanks. Alcoholic fermentation was performed in the presence of grape skins, seeds, and stalks, after SO_2 addition and until the residual reducing sugar content was 0.65 g/L. At this end point of alcoholic fermentation, wine was drawn off from the yeast lees and was filtered through diatomaceous earth and a 0.45 μm filtering cartridge (Durapore TP, Millipore) to eliminate the endogenous microbiota. Wine turbidity was measured (Turbidimeter 2100N, Hatch Co.) and the value obtained after those filtrations was 4 NTU. The bacterial count in this starting wine was 0 cfu/mL. This wine was designated the "initial wine" for the experimental design of this work.

MLF Starter Inoculation in Wine. Two *O. oeni* strains, IS-18 and IS-159, and two *L. plantarum* strains, J-39 and J-51, were selected from the bacteria collection of the Department of Food and Agriculture of the University of La Rioja. These four strains were selected for being autochthonous; i.e., they were isolated from red wines of Rioja Appellation of Origin during their spontaneous MLF, had been fully characterized, and presented appropriate characteristics for wine production (Lopez et al., unpublished data). Strains were grown in MRS broth (Scharlau Chemie S. A, Barcelona. Spain) at 30 °C to obtain the appropriate biomass (4 10^7 cfu/mL) and were adapted to wine conditions by growing them as "pied de cuve" in wine diluted with water (1:1 vol/vol). When L-malic acid concentration decreased to 90%, they were added to 25-L stainless steel tanks and filled to 25 L with the initial wine. MLF was followed by measuring wine L-malic content (L-malic acid enzymatic kit, Boehringer-Mannheim). Wine samples of 10 mL were collected at different times and subjected to microbiological analysis. After 33 days, wine tanks inoculated with *L. plantarum* starters had not yet begun MLF, and L-malic acid (Sigma-Aldrich, Madrid, Spain) was added up to a final concentration of 2 g/L to activate MLF. When MLF had finished (L-malic acid concentration < 0.02 g/L), wines were sulfited and samples were taken for amino acid and volatile compound analysis.

All experiments were done in triplicate in independent 25-L stainless steel tanks, and a total of 13 wines were studied. Results reported here are the average values of three independent experiments.

Bacterial Count and Identification. Serial decimal dilutions were prepared in sterile saline solution, and appropriate volumes were spread in duplicate onto MRS agar (Scharlau Chemie S. A, Barcelona, Spain) fresh plates with 200 μg of nystatin/mL (Acofarma, S. Coop. Terrassa, Spain) for LAB counts. Samples were incubated at 30 °C under strict anaerobic conditions (GasPak. Oxoid Ltd., Basingstoke, England) for at least 5 days, and viable counts were obtained as the number of cfu/mL. Five colonies from each wine sample were selected for reisolation and identification. Strain species were identified by their morphology, Gram staining, and species-specific PCR analyses for *O. oeni* (*23*) and *L. plantarum* (*24*). Clonal characterization of strains was carried out by pulsed field gel electrophoresis (PFGE), as previously described (*2*). Briefly, bacterial cells from fresh cultures were recovered by centrifugation and immobilized in 1% agarose (pulse field certified agarose, Bio-Rad, Hercules, CA) in 0.5× TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.0). Agarose blocks were incubated with proteinase K (1 mg/mL) (Sigma-Aldrich, Madrid, Spain) in digestion buffer. After these enzyme treatments, agarose blocks were cut (slices 1−2 mm) and digested with *Sfi* I restriction enzyme (Biolabs, Beverly, MA) following the manufacturer's instructions. Gel blocks were loaded onto 1% (w/v) agarose D-5 (Pronadisa, Madrid, Spain) gels. DNA fragments were separated in 0.5× TBE buffer in a PFGE in a CHEF DR II system (Bio-Rad Laboratories, Hercules, CA).

Chemical Analysis of the Wines. Alcohol degree, total acidity, volatile acidity, pH, free and total SO_2, and reducing sugars were measured according to EC official methods (*25*).

Amino Acid Analysis. Amino acids were analyzed in duplicate by reverse-phase HPLC using a liquid chromatograph consisting of a Waters 600 Controller programmable solvent module (Waters, Milford, MA), a WISP 710B autosampler (Waters, Milford, MA), and a HP 1046-A fluorescence detector (Hewlett-Packard). Samples were submitted to automatic precolumn derivatization with OPA in the presence of 2-mercaptoethanol. Solvents and gradient conditions were as described by Moreno-Arribas et al. (*26*). Separations were performed on a Waters Nova-Pak C18 (150 × 3.9 mm i.d., 60 Å, 4 μm) column and the same type of precolumn. Detection was performed by fluorescence (λ excitation = 340, λ emission = 425), and chromatographic data were collected and analyzed with a Millenium32 system (Waters, Milford, MA).

Volatile Compounds. Analysis of the major volatile compounds was performed by direct injection on a Hewlett-Packard (Palo Alto, CA) 5890 series II gas chromatograph under the following conditions: Carbowax 20M fused-silica capillary column (30 m × 0.25 mm i.d.), coated with a stationary phase of 0.25-μm thickness (Quadrex, New Haven, CT); split/splitless injector; FID detector; injector and detector temperature, 220 °C; initial oven temperature, 40 °C (10 min hold); temperature gradient, 7 °C/min to 150 °C, 30 °C/min to 210° (2 min hold); carrier gas, helium (12.5 psi, split 1/15). A total of 50 μL of 3-pentanol (6 mg/mL 10% ethanol) was added as internal standard and 1 μL of wine with the internal standard was injected in split mode. The compounds determined by this method were acetaldehyde, ethyl formiate, ethyl acetate, methanol, 1-propanol, isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and ethyl lactate. A ChemStation data system (HP 3365 series II, v.A.03.21) was used for data processing.

Minor volatile analysis was carried out by headspace solid-phase microextraction (HS-SPME) using a 100-μm poly(dimethylsiloxane) (PDMS) coated fused silica fiber (Supelco, Bellefonte, PA), under the conditions described by Pozo-Bayón et al. (*27*). The compounds determined by this method were 1-hexanol, *cis*-3-hexen-1-ol, isobutyl acetate, isopentyl acetate, hexyl acetate, butyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, diethyl succinate, 2-phenylethyl acetate, hexanoic acid, octanoic acid, decanoic acid, γ-butyrolactone, and β-ionone.

Peak identities were assigned by comparing the relative retention times of the internal standard with those of the analytical standards (more than 99% purity) from Sigma-Aldrich (St. Louis, MO) and Merck KGaA (Darmstadt, Germany).

Statistical Analysis. Statistical methods used for data analysis were principal component analysis (from standardized variables), to examine the relationship among the analyzed variables; cluster analysis, to discover natural groupings of the wine samples of the study; one-way analysis of variance (ANOVA), to test the effect of the studied factor (MLF culture); and the Student−Newman−Keuls test for mean comparisons. The STATISTICA program for Windows, release 5.1 (*28*), was used for data processing.

RESULTS AND DISCUSSION

Development of MLF. Figure 1 shows average values of cell concentration and L-malic acid content of triplicates along MLF. Wines inoculated with IS-18 and IS-159 strains of *O. oeni* carried out MLF in 24 and 18 days, respectively, while

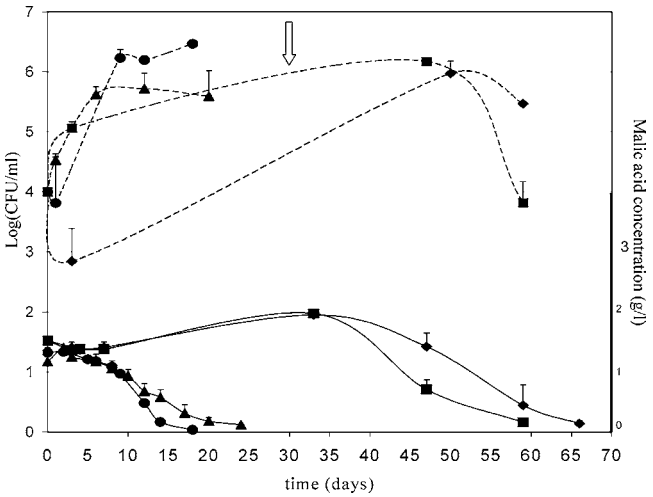

Figure 1. Mean values and standard deviation of cell concentration and L-malic acid concentration in wines ($n = 3$) during MLF. Dotted line: log (cfu/mL) of viable cells. Continuous line: malic acid concentration (g/L). Wines were inoculated with (▲) *O. oeni* IS-18, (●) *O. oeni* IS-159, (◆) *L. plantarum* J-39, and (■) *L. plantarum* J-51. ⟹ indicates L-malic acid addition to 2 g/L final concentration.

wines inoculated with *L. plantarum* starters had not initiated MLF 33 days after inoculation. The addition of L-malic acid (up to 2 g/L final concentration) triggered activation of *L. plantarum* strains J-39 and J-51, and MLF took 26 and 33 days, respectively (Figure 1). Microbiological identification of isolates at initial and full MLF revealed that the inoculated *L. plantarum* strains were responsible for MLF (79% of the isolates were *L. plantarum* species). Implantation control by PFGE was necessary to differentiate among indigenous strains and the inoculated *O. oeni* strains IS-18 and IS-159. PFGE patterns revealed 80−100% implantation, and therefore, the inoculated *O. oeni* strains were responsible for MLF in the corresponding fermentation tanks. At full MLF, bacterial populations reached around 10^6 cfu/mL in all wine tanks (Figure 1). *L. plantarum* starters were much more sensitive to L-malic acid concentration, and their cell population started to decrease after full MLF, when the L-malic acid concentration fell below 0.5 g/L, whereas *O.oeni* starters maintained the cell population after finishing MLF and the L-malic concentration dropped below 0.1 g/L. Similarly, the *L. plantarum* starters were not active until L-malic acid concentration had increased to 2 g/L. These observations agree with results reported before that showed that *L. plantarum* fermentation rate was improved when L-malate was in the 2−10 mM range (*29*) and that *L. plantarum* growth rates and apparent biomass increased with malate addition (*30*). These results clearly indicate a different behavior in MLF performance for *O. oeni* and *L. plantarum* and that the malolactic activity of *L. plantarum* strains in wine was less efficient than that of *O. oeni* strains.

Effect of MLF on Wine Chemical Composition. Before MLF, the wine (initial wine) had an alcohol content of 12.4%, 0.4 g of acetic acid/L of volatile acidity, 5.8 g of tartaric acid/L total acidity, and a pH of 3.4. During MLF, pH values increased slightly (0.01−0.09 units) as well as volatile acidity (0.05−0.32 g/L), whereas, as expected, total acidity decreased (0.46−0.91 g/L).

Principal component analysis was applied to establish relationships among wine amino acid and volatile compound concentrations. Taking into account that ethyl lactate is directly related to the initial L-malic acid concentration, which was not the same in the two different fermentations (with *O. oeni* and *L. plantarum*), the value of ethyl lactate was not considered to perform PCA analysis. Four principal components were obtained, and these explained 78.2% of the total data variance. The first principal component, which explains 33.1% of the total variance, was strongly correlated with GABA (−0.92), valine (−0.87), α-alanine (−0.86), tryptophan (0.85), decanoic acid (0.83), isoleucine (−0.81), methionine (0.83), and ethyl decanoate (0.80). The second principal component, which explains 20.7% of the total variance, was mainly correlated with the variables serine (0.96), phenylalanine (0.91), lysine (0.87), and aspartic acid (0.81). In Figure 2, the 13 samples of wines are plotted on the plane defined by these two first principal components, as well as the corresponding 95% confidence ellipses for each of the four groups. The initial wine (No-MLF wine in Figure 2) is plotted on the right side and is clearly separated from the MLF wines. A high similarity between *O. oeni* wines (IS-18 and IS-159 samples) is shown in Figure 2, as well as between *L. plantarum* wines (J-39 and J-51 samples). Moreover, a degree of dispersion among strains was also observed. Figure 2 shows that the first principal component differentiates the initial wine from the wines that have performed MLF. The initial wine and *O. oeni* wines showed lower concentrations of GABA, α-alanine, valine, and ethyl lactate and higher values of ethyl decanoate and tryptophan than *L. plantarum* wines. Similarity between wines is best estimated in the dendrogram obtained by cluster analysis of standardized variable data (Figure 3), without using the ethyl lactate value. The squared Euclidean distance was taken as a measure of proximity between two samples and Ward's method was used as a linkage rule. In this dendrogram, two main groups can be observed, one of them formed by *L. plantarum* wines and the other containing *O. oeni* wines and the initial wine. In this second group, the initial wine is clearly separated from *O. oeni* wines. Thus, from the results of PCA and cluster analysis, it was possible to differentiate between wines before and after MLF, as well as between wines obtained with *O. oeni* and *L. plantarum* species.

Modifications of Amino Acids and Volatile Compounds by *O. oeni* and *L. plantarum*. One-way analysis of variance was used to obtain more information about variations in wine composition as a result of MLF and to compare wines obtained by inoculation of *O. oeni* and *L. plantarum* selected strains.

Amino Acids. Amino acid concentration in the initial wine and mean ± standard deviation values of amino acid concentrations in wines after MLF are shown in Table 1. The results of applying the Student−Newman−Keuls test to mean values are also shown in the table. There is a slight increase in amino acid concentration after MLF by *O. oeni* strains. Only the amino acids arginine, β-alanine, and methionine showed decreases in concentration, to a greater or lesser extent, after MLF. From the results of Table 1, it can be deduced that increases in amino acid content were more evident in *O. oeni* wines that in *L. plantarum* wines. If the arginine value that disappeared during MLF is excluded, total amino acid content increases by 22.46 and 32.56 mg/L due to *O. oeni* IS-18 and IS-159 activities respectively, whereas in the case of *L. plantarum* strains, only an increase of 16.46 and 2.12 mg/L was obtained with strains J-39 and J-51, respectively. It has been reported that amino acids are generally present in lower concentrations after MLF as a result of LAB growth (*22*). However, no consistent trends have emerged in the literature for any individual amino acid, except for arginine (*3*, *31*). The increase in total amino acid content observed here is in agreement with repeated previous evidence that showed that some *O. oeni* strains can produce extracellular

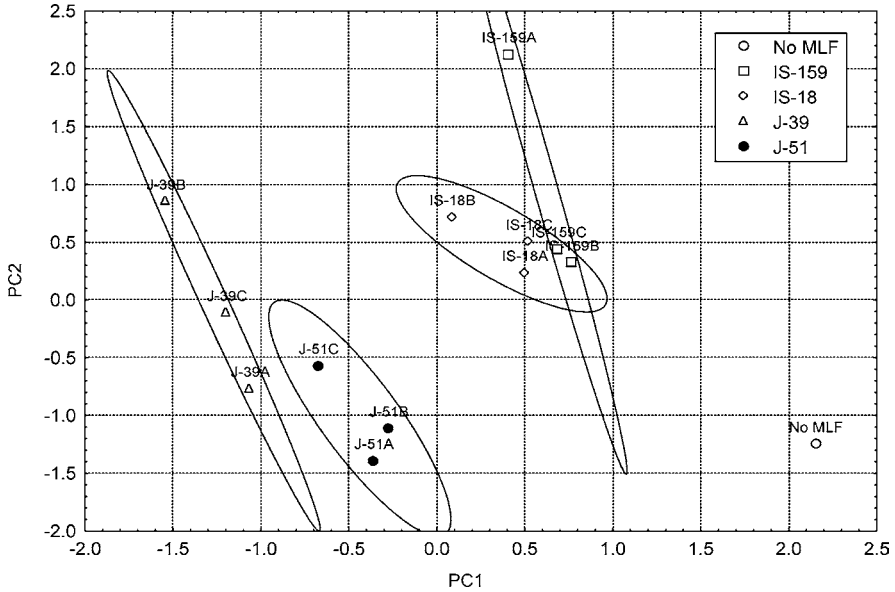

Figure 2. Plot of the 13 samples of wines on the plane defined by the first two principal components from amino acid and volatile compound data. A, B, and C correspond to the individual tanks of each MLF experiment.

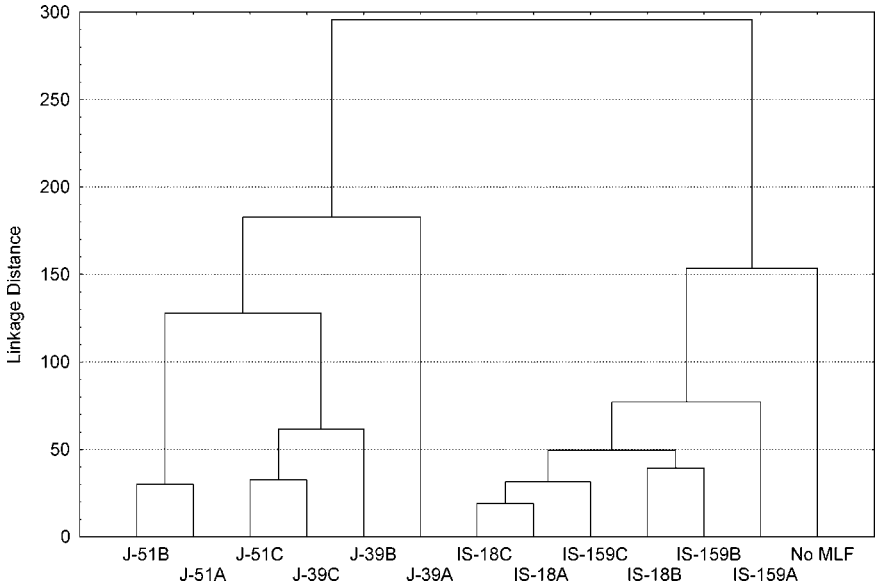

Figure 3. Dendrogram of the 13 samples of wine according to amino acid and volatile compound data. A, B, and C correspond to individual tanks of each MLF experiment.

peptidases or proteases that metabolize peptides and proteins to release detectable concentrations of amino acids (*32*−*34*).

From Table 1 it can be seen that the most important reduction in concentration observed during MLF corresponds to arginine, which, as expected, was quantitatively the major amino acid in the wine before MLF. The arginine concentration decreased to nondetectable levels, both in *O. oeni* and *L. plantarum* experiments, and was accompanied by an increase in ornithine levels. These findings suggest that these strains can degrade arginine-forming ornithine via the arginine deiminase pathway, as reported for other heterofermentative LAB (*35*, *36*).

With the exception of biogenic amine formation and arginine catabolism to ethyl carbamate, amino acid metabolism during MLF is not well-known. Recently, it has been shown that some wine LAB, similar to dairy LAB, can metabolize methionine into different sulfonated volatile compounds that affect wine aroma complexity (*37*). Results from Table 1 indicate a significantly different evolution of methionine in *O. oeni* and *L. plantarum* wines, probably due to the different capacities to catabolize methionine in these two LAB. In addition to methionine, significant differences were also found in our *O. oeni* and *L. plantarum* wines for tryptophan and threonine. *L. plantarum* degraded these amino acids, while *O. oeni* did not. Moreover, concentration values of seven of the 21 amino acids determined in our wines, especially those of glutamine, glycine, β-alanine, α-alanine, GABA, valine, and lysine, varied significantly, depending on the LAB strain performing MLF. It is well-known that some of these amino acids can contribute to specific tastes in foods (*30*) and could, therefore, also affect wine flavor.

Volatile Compounds. Table 2 lists concentrations of the volatile compounds determined in the initial wine (before MLF) and mean ± standard deviation values for the contents of these compounds in wines after MLF. Letters indicate significant differences among the mean values obtained for each of the LAB strains that performed MLF. Table 2 shows that MLF caused changes in the volatile composition of Tempranillo wines. This outcome agrees with previous studies on other red grape varieties (*9*, *22*). Most of the alcohols were at higher

Table 1. Amino Acid Content in Wine before MLF and Mean ± SD Values of the Amino Acids (mg/L) in the Wines after MLF[a]

		after MLF (n = 3 tanks)			
		O. oeni		*L. plantarum*	
amino acids	before MLF: initial wine	IS-18	IS-159	J-39	J-51
Asp	9.18	9.47a ± 0.39	9.76a ± 0.74	9.88a ± 0.31	8.98a ± 0.20
Glu	13.81	17.35a ± 0.53	18.57a ± 2.07	19.17a ± 1.74	16.82a ± 1.12
Asn	11.48	13.12a ± 0.69	13.43a ± 1.26	14.15a ± 1.14	12.75a ± 0.79
Ser	5.79	6.62a ± 0.17	6.73a ± 0.50	6.61a ± 0.38	6.10a ± 0.18
Gln	8.32	9.50ab ± 0.46	8.34a ± 0.89	10.41b ± 0.46	9.27ab ± 0.26
His	nd	nd	nd	nd	nd
Gly	7.63	10.49a ± 0.76	9.18a ± 1.06	15.99b ± 2.20	8.27a ± 4.44
Thr	6.55	7.36b ± 0.10	8.55c ± 0.70	0.00a ± 0.00	0.00a ± 0.00
Arg	16.29	nd	nd	nd	nd
β-Ala	3.97	0.00a ± 0.00	4.13b ± 0.05	0.00a ± 0.00	0.00a ± 0.00
α-Ala	12.95	15.10a ± 0.42	15.57a ± 1.65	19.34a ± 2.91	17.02a ± 1.32
GABA[b]	8.66	10.27a ± 0.44	10.26a ± 0.91	13.76b ± 1.95	12.53ab ± 0.77
Tyr	8.39	8.77a ± 0.02	8.78a ± 0.28	6.03a ± 5.23	7.30a ± 2.06
Met	5.34	5.18b ± 0.21	4.78b ± 1.36	0.00a ± 0.00	0.00a ± 0.00
Val	5.71	6.73a ± 0.24	7.13a ± 0.54	8.20b ± 0.47	7.22a ± 0.37
Trp	7.91	6.86b ± 0.98	8.09c ± 0.20	0.00a ± 0.00	0.00a ± 0.00
Phe	8.82	9.70a ± 0.39	9.87a ± 0.18	9.74a ± 0.49	9.36a ± 0.25
Ile	5.21	5.78a ± 0.03	5.92a ± 0.39	6.31a ± 0.46	5.94a ± 0.10
Leu	5.79	7.08a ± 0.07	7.48a ± 0.40	7.75a ± 0.60	7.00a ± 0.21
Orn	5.44	12.19a ± 7.84	14.79a ± 1.02	8.23a ± 7.22	9.27a ± 8.27
Lys	8.29	10.10b ± 0.24	10.40b ± 0.69	10.16b ± 0.02	9.25a ± 0.24
sum of amino acids	165.53	171.70ab ± 1.30	181.84b ± 3.21	165.70ab ± 2.50	147.12a ± 2.87

[a] Means within rows without a common letter are significantly different ($P < 0.05$); nd, not detected. [b] GABA, gamma aminobutyric acid.

Table 2. Volatile Compounds Content in the Wine before MLF and Mean ± SD Values of the Volatile Compounds (mg/L) in the Wines after MLF[a]

		after MLF ($n = 3$ tanks)			
		O. oeni		*L. plantarum*	
	before MLF: initial wine	IS-18	IS-159	J-39	J-51
Alcohols					
1-propanol	36.18	36.90a ± 0.77	38.56a ± 1.83	37.72a ± 0.49	39.19a ± 2.31
isobutanol	79.04	80.37a ± 2.33	79.66a ± 2.62	79.18a ± 6.03	82.22a ± 3.86
2 + 3-methyl-1-butanol	273.82	279.40a ± 8.47	279.28a ± 6.67	254.68a ± 52.36	28340a ± 14.07
1-hexanol	1.11	2.75a ± 0.61	2.94a ± 1.89	3.22a ± 0.18	3.89a ± 0.86
methanol	144.45	159.60a ± 6.95	162.21a ± 16.92	173.00a ± 6.15	180.13a ± 14.24
cis-3-hexen-1-ol	<0.20	<0.20	<0.20	<0.20	<0.20
Esters					
ethyl formiate	14.94	14.68a ± 0.39	14.26a ± 0.19	15.71a ± 1.90	16.03a ± 0.63
ethyl acetate	66.03	82.82a ± 1.52	78.51a ± 6.32	90.60a ± 5.16	82.32a ± 12.03
isobutyl acetate	nd	nd	nd	nd	nd
isopentyl acetate	0.85	0.93a ± 0.09	0.87a ± 0.01	0.84a ± 0.10	0.85a ± 0.06
hexyl acetate	<0.02	<0.02	<0.02	<0.02	<0.02
butyl acetate	0.27	0.27a ± 0.01	0.26a ± 0.01	0.31b ± 0.03	0.27a ± 0.01
ethyl butyrate	nd	nd	nd	nd	nd
ethyl octanoate	0.63	0.45a ± 0.06	0.60a ± 0.02	0.39a ± 0.08	0.47a ± 0.05
ethyl decanoate	0.85	0.43ab ± 0.17	0.67b ± 0.09	0.22a ± 0.10	0.41ab ± 0.07
ethyl lactate	36.96	168.5ab ± 17.78	137.1a ± 4.29	235.04c ± 29.90	189.77b ± 10.44
ethyl hexanoate	0.75	0.73a ± 0.03	0.73a ± 0.02	0.66a ± 0.08	0.69a ± 0.06
diethyl succinate	3.94	8.79a ± 0.39	7.77a ± 1.22	6.99a ± 0.45	7.72a ± 1.39
2-phenylethyl acetate	<0.06	<0.06	<0.06	<0.06	<0.06
Fatty Acids					
hexanoic acid	5.53	5.56a ± 0.62	5.10a ± 0.26	5.77a ± 0.14	6.00a ± 0.38
octanoic acid	3.58	4.00b ± 0.11	3.66ab ± 0.23	3.10a ± 0.10	3.40ab ± 0.55
decanoic acid	1.66	1.16b ± 0.10	1.28b ± 0.22	0.70a ± 0.15	0.57a ± 0.18
Other Compounds					
acetaldehyde	24.58	18.37a ± 0.58	17.80a ± 0.37	22.73a ± 6.24	31.12b ± 0.61
γ-butyrolactone	3.49	8.65b ± 1.92	7.98b ± 4.39	3.68a ± 2.02	4.78a ± 0.40
β-ionone	0.10	0.16a ± 0.03	0.12a ± 0.04	0.15a ± 0.01	0.18a ± 0.03

[a] Means within rows without a common letter are significantly different ($P < 0.05$); nd, not detected.

concentrations after MLF than in the initial wine. Also, levels of short-chain esters, such as ethyl acetate, ethyl lactate, and diethyl succinate, increased during MLF, as expected. Formation and hydrolysis of esters during MLF is probably due to the action of LAB esterases responsible for the synthesis and degradation of these compounds. However, to date there are no studies that demonstrate these changes. Ethyl lactate, one of the most important byproducts of LAB metabolism (*3*), increased more than 4 times in the wines after MLF, reaching different concentrations depending on the LAB strain. In contrast, a drop in the ethyl decanoate concentration, an ester of a long-chain acid, was observed, with final values depending

on the malolactic culture used. The butyl acetate content was also significantly influenced by the LAB strain. On the other hand, no differences were observed in the contents of fruity esters, hexyl acetate, and 2-phenylethyl acetate. For the fatty acids, significant differences were found for octanoic and decanoic acids, depending on the malolactic culture. It is worth noting that total fatty acid concentration was not above 20 mg/L in any case. Higher concentrations of these compounds have been reported to impair the final aroma (*39*, *40*). The acetaldehyde concentration changed during MLF, and significant differences were found depending on the different malolactic cultures used. This decreased in wines fermented by *O. oeni*, but not in *L. plantarum* wines, probably indicating a partial degradation of this compound by *O. oeni*, in agreement with previous results (*41*). The γ-butyrolactone concentration was higher in wines after MLF, and significantly higher increments were shown for *O. oeni* strains compared with *L. plantarum* (Table 2). These findings are in accordance with those of Maicas et al. (*9*), who also detected higher levels of this compound in wines due to MLF.

Individual strains of LAB have been found to produce distinctive flavors (*5*). Other studies have revealed differences in volatile metabolites among LAB species and, more specifically, strains of *O. oeni*, when these bacteria were cultivated in specific and complex media (*11*). In the present work, these latter observations were confirmed when other selected strains were individually used to induce MLF in wine. In summary, the concentrations of seven (butyl acetate, ethyl acetate, ethyl decanoate, octanoic acid, decanoic acid, acetaldehyde, and γ-butyrolactone) of the 25 volatile compounds analyzed in this study significantly differed in relation to the type of LAB used for MLF. Moreover, the concentrations of some of these compounds appear to be influenced by the LAB species, while for other compounds significant differences depended on the strain used, reflecting a degree of diversity among strains of the same species.

In conclusion, our results reveal a different malolactic behavior for *O. oeni* and *L. plantarum*. Lactobacilli malolactic activity seems to be more dependent on the amount of L-malic acid available in the medium, and a minimal threshold of L-malic acid concentration is required to trigger MLF. They are, therefore, less efficient than oenococci at inducing MLF. It can also be concluded that MLF by both species may contribute to wine quality by modifying the concentration of some of the amino acids and aroma compounds of wine. Moreover, the application of a variety of statistical techniques to analyze wine amino acid and volatile compound data has revealed significant metabolic differences between *O. oeni* and *L. plantarum* species. Results also suggest a degree of diversity in both LAB groups, since wines also showed specific characteristics depending on the LAB strain. These conclusions justify inducing MLF with selected strains that produce beneficial sensory attributes and no defects in wines.

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